2. For isolation of polysomes from tobacco and similar dicots, collect leaves and remove the midribs. Separate into eight batches, each of which consists of 2.5 g of leaf blades (*see* **Subheading 3.4., Note 3** for isolation of polyribosomes from cereals).

3a. For isolation of total (free and membrane-bound) polysomes, immediately grind each batch for 2 min in 25 mL of cold 1X extraction buffer using a prechilled (~4°C) mortar and pestle. Change to a fresh, cold mortar and pestle after two grinds. Perform this procedure in a fume hood, because β-mercaptoethanol is noxious. Pour each of eight homogenates into chilled 50-mL plastic or glass centrifuge tubes that can withstand *g*-forces of up to 25,000*g*. Store on ice prior to centrifugation.

3b. To separate membrane-bound polysomes from the free polysomes ***(17)***, modify the extraction buffer used to grind the tissue by adding only 50 m*M* KCl (final concentration), and eliminate the Triton X-100. Then centrifuge the brei for 5 min at 500*g* in a preparative fixed-angle rotor (in a Sorval SS34 Rotor) to pellet nuclei, chloroplasts, mitochondria, and organelle fragments. Discard the low-speed pellet and transfer the supernatant into eight fresh plastic or glass tubes. Then centrifuge at 20,000*g* in the SS34 rotor to separate the supernatant fraction (free polysomes) from the pellet (membrane-bound polysomes). Save both the supernatant and pellet fractions. Add KCl to 400 m*M* and Triton X-100 to 1% to the supernatant. Resuspend the pellet containing the membrane-bound polysomes in the same volume of extraction buffer (containing 400 m*M* KCl and 1% Triton X-100) as the free polysome supernatant fraction.

4. Centrifuge the samples from **steps 3a** (total polysomes) or **3b** (supernatant = free polysomes; pellet = membrane-bound polysomes) at 20,000*g* for 10 min at 4°C.

5. While the samples in **step 4** are spinning, pipet 5 mL of sucrose pad buffer into 30-mL Beckman polycarbonate bottles. Chill on ice. Remove the cleared supernatant from the cell debris pellet carefully with a 25-mL disposable plastic pipet. Load the supernatant over the sucrose pad, being careful not to disturb the interface.

6. Pellet the polysomes by centrifuging at 315,000*g* in a fixed-angle ultracentrifuge rotor (Beckman Ti60 rotor) for 80 min at 4°C (*see* **Subheading 3.4., Note 4**).

7. Remove and discard the supernatant by aspirating off the green material. Then, rinse the sides of the tubes with 5 mL of ice-cold ddH_2O. Remove the rinse water by aspiration, then repeat the rinsing procedure. Aspirate the sucrose pad off of the pellet and invert the tube to expose the clear polysome pellet. Quickly rinse the pellet with 1 mL of cold H_2O to remove residual sucrose. To do this, turn the tube so that the side containing the pellet is on top. Add 1 mL of H_2O and rotate the tube in a complete circle during a 15-s interval. Resuspend each pellet in 1.25 mL of 1X resuspension buffer by dislodging them with a Pasteur pipet and vortexing. Leave on ice for 15 min. Combine the suspensions, then rinse each tube sequentially with 1.25 mL of 1X resuspension buffer.

8. Measure the optical density at A_{260} to determine the yield (*see* **Subheading 3.4., Note 5**).