

A Miniprep for Extraction of RNA from Potato

Safety tip: This procedure uses hot solutions containing phenol and chloroform. Always wear appropriate skin, eye, and hand protection. Work in a fume hood. Dispose of wastes properly!

- Harvest approximately 100 mg of leaf tissue from culture or plant, put in microcentrifuge tube, freeze in liquid nitrogen (LN₂), store at -80°C.

Tuber samples can be used, but are usually not handled as minipreps -- poor yield.

From here on, work quickly and be careful. Start with one tube; extract it and start centrifugation. While it is spinning, extract a second tube. When the first tube is done, transfer the supernatant and add LiCl (see below). Place on ice and finish tube two. Then start a new pair.

- Allow the tube containing frozen tissue to warm slightly but not thaw.
- Mix 1 volume pH neutralized phenol + 1 volume Extraction Buffer... Vortex well, place 550 µl aliquots in individual microfuge tubes in 80°C water bath.

Extraction Buffer: 100 mM LiCl, 100 mM Tris pH 8, 10 mM EDTA pH8, 1% SDS

- Remove tube with leaves from liquid nitrogen, place in rack, slowly add liquid nitrogen to leaf until tube is ³/₄ full. Grind leaf tissue to powder with plastic pestle (Kontes) that has been pre-cooled in LN₂
- Vortex a tube of hot phenol/buffer, add 500 µl to frozen leaf powder. Vortex 30-60 seconds; at least until powder is thawed.

*Make sure all powder is in suspension before proceeding! That means-- mix it furiously but do not take more **time** to resuspend! If necessary, use less powder in subsequent samples.*

- Add 250 µl of CHCl₃ and vortex 30 seconds. Spin in microfuge for 5 minutes

Make sure Chloroform is accurately pipetted. The vapor pressure of chloroform causes it to drip. Up-and-down pipetting before the actual uptake of 250 µl coupled with a quick, short-distance transfer from reagent to sample is your best bet.

- Remove top phase to microfuge tube, add an equal volume of 4M LiCl, precipitate at least 1 hours at -20°C.

Avoid interface even if you have to sacrifice sample. Spin a little longer if interface is not compact enough to avoid. Pull LiCl precipitations out of freezer just before/as they freeze solid. In our experience, nothing is gained by leaving frozen-solid precipitations in freezer longer. Nothing is lost either.

- Spin samples 10 minutes, remove all of supernatant, resuspend in 200 µl DEPC treated water.

Complete removal of supe is important to get rid of as much DNA and protein as possible. "Thirsty sticks" are ideal for last droplets.

- For green tissues, centrifuge the suspension for 5 minutes. Transfer the supernatant to a clean tube. (This step removes green insoluble material.)
- Precipitate RNA with $\frac{1}{10}$ volume DEPC treated Na-Acetate and 2.2 volumes EtOH. Spin 5-10 minutes, wash pellets with 70% EtOH and dry briefly (over-dried RNA difficult to dissolve).
- Determine concentration by absorbance at 260 nM. Dilute original tube to between 1 and 2 µg/µl.
- For Northern blot analysis, suspend total RNA samples (10-20 µg/lane) in a constant volume. Add an equal volume of 2X RNA Loading Buffer (0.02% [w/v] xylene cyanol, 0.02% [w/v] bromophenol blue, 50% [w/v] glycerol). Denature by heating at 80°C for 5 minutes. Transfer to ice bath and load gel immediately.
- For routine analysis, it isn't necessary to use either glyoxyl or formaldehyde gels. Samples can be fractionated on thick (~6 mm) 0.8 to 1.2% agarose and then transferred to charged nylon membranes by capillary transfer (up or down) in 1X SSC.

For optimal results, samples should be glyoxylated[1].

1. Sambrook, J., E.F. Fritsch, and T. Maniatus, *Molecular Cloning A Laboratory Manual*. 2nd ed. 1989, Cold Springs Harbor, New York: Cold Spring Harbor Laboratory Press.

Typical Glyoxylation after Sambrook *et al.* p. 200

1. Ethanol precipitate the RNA if it isn't concentrated enough. For 10 µg/lane, must have ≥ 2.7 µg/µl.
2. Pour a 0.8% agarose gel with 10mM phosphate buffer pH 7. [1.2 grams agarose, 3 ml 0.5M Pi to 150 ml with water.]
3. Set up gel box with pump and make 1.5 - 2 liters of 10mM Pi buffer.
4. Glyoxylation reaction

	<u>MW Markers</u>	<u>1 Lane¹</u>	<u>2Lanes¹</u>	<u>3 Lanes¹</u>	<u>4 Lanes¹</u>
6M Glyoxal	3.7 µl	3.24 µl	6.48 µl	9.7 µl	12.96 µl
DMSO	11.0 µl	9.6	19.2 µl	28.8	38.4 µl
0.1M NaPO ₄	2.2 µl	1.92 µl	3.84 µl	5.76 µl	7.68 µl
RNA (10µg)	5.0 µl	4.44 µl	8.88 µl	13.32 µl	17.76 µl
Incubate at 50°C for 15 minutes, put on ice and add sample buffer:					
	5.5 µl	4.8 µl	9.6 µl	14.4 µl	19.2µl

Load the gel immediately. 20 µl 20 µl 20 µl 20 µl
 Note 1: Formula is for excess. Load 20 µl per lane! Based on Maniatus + **20%**.

Sample buffer = 50% glycerol; 0.01M NaPi pH7; 0.4% BPB.

5. Turn pump on after samples have run into the gel. Pump from + to -. Run gel for 2-3 hours at 80 volts. Run BPB a little over half way. Can be stained with either 30 µg/ml acridine orange or ethidium bromide.
8. Remove the GS+ filter from the stack. Reverse the glyoxal reaction by placing the filter in 50mM NaOH for 15 seconds, then transferring to 1X SSC-0.2M Tris HCl pH 7.5 for 30 seconds.
9. Air dry. Ready to hybridize. No baking required.