1. Precool a small pestle and mortar with liquid nitrogen, and snap-freeze 200–400 mg of plant tissue in liquid nitrogen in a polypropylene tube. Make sure there is a pool of liquid nitrogen in the pestle, then hit the tube containing the sample against the bench to fragment the tissue, pour it into the liquid nitrogen in the pestle, and grind to a fine powder. Keep the powder wet all the time by adding more liquid nitrogen, as appropriate.
2. Mix 0.5 mL guanidinium buffer and 0.5 mL acid phenol:chloroform:isoamyl alcohol (25:24:1) in an 11-mL polypropylene push-cap tube.
3. Let the nitrogen evaporate from the pestle (wait until the tissue changes from dark green to light green), then pour the powder straight into the extraction buffer. Vortex to mix.
4. Centrifuge (10 min, 1000*g*) in a bench top centrifuge.
5. Transfer the aqueous (top) phase to a 1.5-mL Eppendorf tube containing 0.5 mL phenol:chloroform:isoamyl alcohol. Mix, spin, and repeat until the interface is clear (usually three or four times).
6. Collect the aqueous phase, add 0.2 vol of 1*M* acetic acid and 0.7 vol of cold 96% ethanol. Mix and incubate overnight at –20°C.
7. Pellet by spinning at maximum speed (12,000*g*) in a microcentrifuge at 4ºC for 10 min. Drain the pellet, add 400 µL 3*M* sodium acetate, pH 5.5, and vortex. Respin for 10 min at 4ºC. Repeat once more (low-mol-wt RNA and contaminating polysaccharides will redissolve).
8. Remove the salt with a final wash with 70% ethanol, spin 10 min and redissolve the dried pellet in 20–30 µL sterile ddH_2O. If hard to dissolve, add more water and/or heat to 95ºC for 2 min and quench on ice.

For hints and comments on recalcitrant tissue, *see* **Note 7**.

3.2. Design of Primers

Primer design has been discussed extensively in various books (e.g., **refs. *5*** and ***6***), and there are a variety of computer programs that aim to automate the process. In our experience, these programs may be useful, but are not prerequisites to good primer design. There is still too little known about the interactions between short oligonucleotides to be able to produce good predictions on whether a particular primer pair is a good one. In general, the trick is to test several sets of primers, rather than to rely on one set and try to optimize conditions for that pair. With that in mind, the following guidelines are offered:

1. There are no definite rules to guarantee the success of a primer pair. Because some primer pairs are 100- to 1000-fold more sensitive than others for elusive reasons ***(7)***, several sets of primers should be tried out.
2. Design the primers by visual inspection of the nucleic acid sequence. Then, if available, use a primer design program (e.g., primer from the Whitehead Institute) to check for some of the features mentioned below.