

to start with, or when particularly noxious inhibitors exist, then some kind of extra purification step is needed. Unfortunately, many inhibitors appear to copurify with nucleic acids, and separation can be difficult. There are two main approaches to getting rid of inhibitors: a chemical approach, in which the nucleic acids and inhibitors, e.g., polyphenolics, are separated by differential extraction; and a biochemical approach, in which the target is specifically trapped and washed to get rid of the contaminants. This latter approach is particularly useful for viruses, because specific capture columns can be made using antibodies to the viral CP, before rupture of the particles for cDNA synthesis and/or PCR on the now pure nucleic acid (12,13). Various resins and matrixes are available commercially that have been reported to be sufficiently effective at removing particular inhibitors from samples to allow RT-PCR amplification (e.g., Magic/Wizard [Promega], Elutip-d columns [Schleicher and Schuell, Germany], Genereleaser [Cambio, UK]). These methods add considerable cost to sample preparation, and purification through Sephadex G-50 columns can be equally effective for removal of some impurities. Copurification of polysaccharides is a common problem when isolating nucleic acids, and the inhibitory effect some have on *Taq* polymerase can be overcome by including Tween-20 (0.5%), DMSO (5%), or polyethylene glycol 400 (5%) in the PCR buffer (14). Alternatives are to clean the nucleic acid with a 2M sodium chloride precipitation (15), or to use tissue extraction buffers containing substances such as sodium chloride and cetyltrimethylammonium bromide (CTAB) to reduce copurification of complex carbohydrates, or the cation-exchange resin Chelex-100 (Bio-Rad) at 20–80% (w/v) (16). Inclusion of 0.2–1% (v/v)  $\beta$ -mercaptoethanol and/or 1% (w/v) polyvinyl pyrrolidone is effective at inhibiting oxidation and removing polyphenols. Methods have also been developed using triisopropyl naphthalene sulfonic acid and *p*-aminosalicylic acid (NaTINS-pAS) (17); hot borate at alkaline pH, in combination with PVP and deoxycholate (18); or taking advantage of the differential solubilities of nucleic acids and contaminants in solvents such as 2-butoxyethanol (19). A favored method in our laboratory for DNA extraction from difficult plant species is that of Lodhi et al. (20), which combines CTAB, sodium chloride, polyvinyl pyrrolidone, and  $\beta$ -mercaptoethanol, with the modification that the concentration of EDTA in the extraction buffer is reduced to 10 mM. The method would also be suitable for RNA extraction, but the incubation step at 60°C should be reduced to 5–10 min to reduce RNA degradation. Poulson (21) reports that CTAB extractions do not generally completely remove polysaccharides and sugar phosphates, which can be achieved by dissolving RNA in 25 mM Tris-HCl, pH 8.0, 25 mM sodium chloride, and then adding equal volumes of 2.5M potassium phosphate, pH 8.0, and 2-methoxyethanol. The mixture is shaken vigorously for 2 min, centrifuged 5 min, and the upper organic phase transferred to a fresh tube. An equal volume of 1% CTAB is added, the sample incubated on ice for 5 min, and then centrifuged (5000g, 5 min). The RNA pellet is then washed three times with 70% ethanol containing 0.2M sodium acetate to convert it to the water-soluble sodium salt.