An efficient method for isolation of RNA from tissue cultured plant cells

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Submitted June 18, 1991

Total cellular RNA has commonly been prepared from plant material utilizing methods involving CTAB (cetyltrimethylammonium bromide), SDS (sodium dodecyl sulfate), phenol/chloroform, or guanidine hydrochloride (Logemann *et al.*, 1987). Although very effective in the preparation of intact RNA from leaf tissue, when applied to cultured plant cells these methods all result in a low yield and a substantial contamination by polysaccharides.

The procedure outlined in this report is a substantial modification of a published method developed for minipreparation of plant leaf RNA (Verwoerd *et al.*, 1989). Our method is specifically designed to reduce the amount of insoluble polysaccharides associated with cultured cell material and to maintain an efficient yield of intact RNA suitable for poly(A)$^+$ enrichment and analysis of less abundant transcripts.

Homogenization step. Tissues (100 mg to 15 g) are frozen in liquid nitrogen and ground to a fine powder using mortar and pestle or a coffee mill. The extract is then homogenized further utilizing a polytron by the addition of 3–5 volumes of hot extraction buffer. Extraction buffer contains phenol 0.1 M LiCl, 0.1 M Tris–HCl, 0.01 M EDTA, 1% SDS at 80°C. Following a 5 min incubation in an 80°C water bath, a half volume chloroform/isoamyl alcohol (24:1 v:v) is added, the homogenate is thoroughly mixed, and centrifuged at 10,000 *g* (4°C) for 15 minutes.

Cold phenol extraction and washing step. The upper aqueous phase is extracted once more with one volume of cold phenol/chloroform/isoamyl alcohol (24:23:1 v:v:v), and centrifuged for 15 minutes at 10,000 *g*. The aqueous phase is then mixed with one volume of 4 M LiCl and RNA is allowed to precipitate for at least three hours at −20°C. Subsequently, RNA is pelleted by centrifugation for 30 minutes at 12,000 *g*. In our experience, at this point the RNA pellet still appears impure and requires at least one additional wash (thoroughly resuspended) in 2 M LiCl and 0.05 M EDTA and a subsequent centrifugation for 30 minutes at 12,000 *g*.

Removal of polysaccharides and RNA precipitation. The soluble polysaccharides can be simply removed by addition of 0.1 volume ethanol or 3 M sodium acetate at pH 5.2 (Logemann *et al.*, 1987). If the residual polysaccharide is not a problem, the RNA can be precipitated by adding 0.1 volume of 5 M NaCl and 2.5 volumes of ethanol.

Results. The isolation procedure described here was performed using 18 different *Medicago sativa* callus cultures (Figure 1) as well as over two dozen alfalfa and bean leaf samples (Table I). Figure 1A shows the high quality of callus culture RNA that is isolated using this method. Specific RNA species were further examined by Northern blot analysis using various chloroplast and nuclear genes. Figure 1B shows an example of these analyses using the plasmid-encoding, psbA. We have also successfully used RNA prepared by this procedure for selection of poly(A)$^+$ RNA and construction of complementary DNA (cDNA) library. The cloning efficiency of the cDNA was equally high (over 1×10^5 pfu/μg) regardless of the tissue type used to prepare the RNA.

In summary, the modified hot phenol method outlined in this report is an efficient technique for large scale preparation of RNA from both plant and cultured cell material and it is particularly suitable for obtaining RNA from tissues with high polysaccharide contents.

REFERENCES

1. Logemann,J., Schell,J. and Willmitzer,L. (1987) *Anal. Biochem.* **163**, 16–29.
2. Verwoerd,C., Dekker,B.M. and Hoekema,A. (1989) *Nucleic Acids Res.* **17**, 2362.

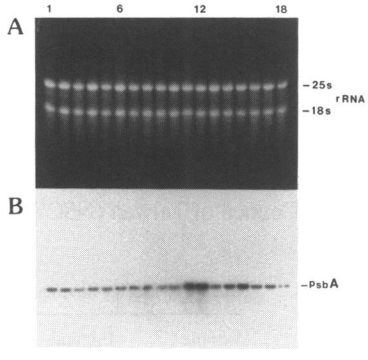

Figure 1. Gel electrophoresis and Northern blot analysis of callus culture RNA.

Table I. RNA yield using various extraction methods

	Yield of RNA (μg/g tissue)			% Poly(A)$^+$ RNA from Total RNA		
	Guanidine	Phenol/SDS	Hot phenol	Guanidine	Phenol/SDS	Hot phenol
Leaf	500	550	550	1.0	1.3	1.5
Callus	40	30	140	N.A.	N.A.	1.2

N.A. Not available (due to low yield of total RNA).