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Research Note

Rapid purification of grapevine RNA by a simple electrophoretic method

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Several methods have already been published to isolate RNA from various plant species and organs. The existing techniques involve repeated precipitation or washing steps (Shirzadegan *et al.* 1991; Levi *et al.* 1992; López-Gómez and Gómez-Lim 1992) or ultracentrifugation (Taylor and Powell 1982; Logeman *et al.* 1987; Baker *et al.* 1990; John 1992) to remove DNA and contaminating polysaccharides and phenolic compounds frequently present in plant samples including grapevine. These steps make the protocol labour and time consuming or require expensive laboratory equipment. To overcome these difficulties we have developed a simple electroelution protocol, already used in several laboratories to isolate plasmids (Le Brun *et al.* 1988) and DNA fragments from agarose gels (He *et al.* 1988; Shia 1990; Pai and Bird 1991; Peloquin and Platzer 1991), for total plant RNA isolation. As compared to the previous methods, we basically modified the protocol so that the deproteinized crude extract was first size fractionated in agarose gel prior to nucleic acid precipitation to prevent coprecipitation of contaminants.

Crude plant nucleic acid preparations were made basically according to Verwoerdt *et al.* (1991). 2 g of grapevine crown gall or leaf sample was powdered in liquid nitrogen and homogenized in 5 ml of lysis buffer:phenol (1:1) mixture and extracted with 2.5 ml of chloroform. After centrifugation at 5000 rpm for 5 min 1.3 ml of the deproteinized plant extract was mixed with 0.3 ml of loading buffer supplemented with ethidium bomide (Angermuller and Sayavedra-Soto 1990). This preparation was used directly for electrophoretic separation of the RNA fraction.

For RNA purification two large wells with a depth of 8 mm were formed on a single gel tray (70 mm wide, 40 mm long). One (50 x 4 mm) at the cathodal side and the other one (50 x 2 mm) at 2 cm from the first well at the anodal side were prepared with dialysis membrane (approx. cut-off size: 700-800 dalton). The dialysis membrane was fixed with a small piece of adhesive tape to the anodal side of the well comb and moistened with sterile running buffer (1 x TAE) prior to pouring the gel (0.7 % agarose). Adhering agarose, if present, should be carefully removed from the cathodal side of the incorporated dialysis membrane after gelling. The horizontal electrophoresis tank and the anodal well were filled up with running buffer so that it did not cover the gel (Fig. 1) prior to loading the sample. The migration of RNA during electrophoresis was followed by overhead illumination with a small ultraviolet lamp (6 W, 312 nm). When the RNA fraction entered the anodal well the electrophoresis was stopped and the RNA fraction was pipetted out, phenol:chloroform (1:1) extracted and concentrated by ethanol precipitation. Total RNA can be easily purified using this method and it was found to be perfectly suitable for Northern hybridization experiments (Fig. 2). The yield of RNA from 1.3 ml of plant extract (approximately corresponding to 1 g tissue) was 200-300 µg from leaves and 50-100 µg from crown gall tumor tissues. The RNA was found to be free of polysaccharides and other contaminants and could be quickly redissolved in distilled water.

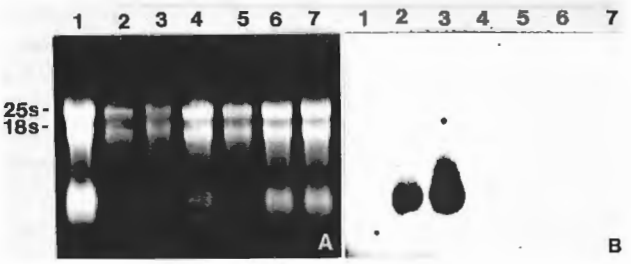

Fig. 1: Side view of agarose gel used for RNA isolation. a: sample well, b: setup of dialysis membrane. The arrow indicates the direction of migration.

Fig. 2: A: Electrophoretically purified RNA separated in 1.2 % (w/v) formaldehyde/agarose gel. Samples were prepared from non-transformed grapevine (cv. Narancsízü) leaves (lane 1, 40 µg), and from sterile crown gall tumors induced on the same variety by *Agrobacterium vitis* strain Tm4 (lane 2, 8 µg), AB3 (lane 3, 9 µg), AT1 (lane 4, 21 µg), AT66 (lane 5, 10 µg), S4 (lane 6, 22 µg) and Sz1 (lane 7, 20 µg). B: RNA was blotted onto Amersham Hybond-C extra membrane according to the instruction of the supplier and hybridized under standard conditions (Sambrook *et al.* 1989) with ^{32}P labelled 1.9 kbp EcoRV fragment of pTiTm4 TA-DNA (Paulus *et al.* 1989) which is specific for *A. vitis* octopine Ti plasmids.

This method was also suitable to isolate bacterial and other plant RNAs (Fig. 3). The electrophoretic RNA purification allows to work with low volumes and the RNA prepared by this way is purer than that obtained after the traditional LiCl precipitation (Fig. 3, lanes 6-8). Some DNA contamination may occur if the gel is overloaded.

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Angermuller, S. A.; Sayavedra-Soto, L. A.; 1990: Rapid visualization of genomic DNA and total RNA in agarose gels. BioTech. **8**, 36-37.
Baker, S. S.; Rugh, C. L.; Kamalay, J. C.; 1990: RNA and DNA isolation from recalcitrant plant tissues. BioTech. **9**, 268-272.

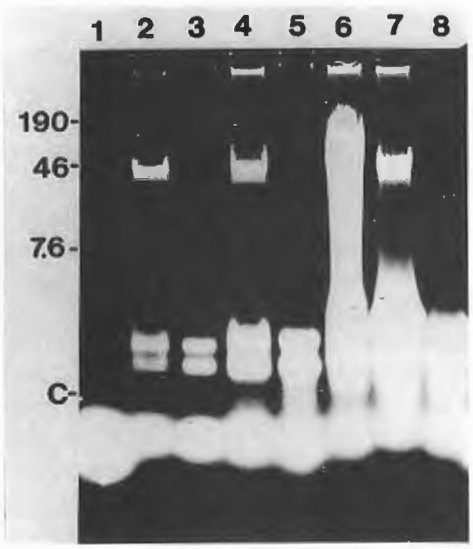

Fig. 3: Isolation of RNA from various sources. Bacterial cells were lysed in 50 mM Tris-HCl, pH = 8.0, 100 mM NaCl, 20 mM Na_2EDTA and 1 % SDS for 10 min at room temperature followed by phenol:chloroform extraction. Lane 1: DNA molecular weight markers (in kilobases), c: front of bromothymolblue; lane 2: phenol:chloroform extracted lysate of *Agrobacterium vitis* AB3; lane 3: purified RNA from the same sample; lane 4: phenol:chloroform extracted lysate of *Escherichia coli* LE392; lane 5: electrophoretically purified *E. coli* RNA; lane 6 shows deproteinized leaf extract of *Helianthus tuberosus*; lane 7: *H. tuberosus* RNA preparation obtained by the LiCl method (VERWOERDT *et al.* 1989); lane 8: RNA obtained from the same (lane 6) sample by electrophoretic purification.

HE, M.; BEECHEY, R. B.; KADERBHAI, M. A; 1988: A procedure for electroelution of DNA from agarose gels. BioTech. **6**, 728-730.

JOHN, M. E.; 1992: An efficient method for isolation of RNA and DNA from plants containing polyphenolics. Nucl. Acid. Res. **20**, 2381.

LE BRUN, J. J.; RENTIER-DELRUE, F.; MERCIER, L.; 1988: A simple method for the preparation of the covalently closed circular form of plasmid DNA. BioTech. **6**, 832-845.

LEVI, A.; GALAU, G. A.; WETZSTEIN, H. Y.; 1992: A rapid procedure for the isolation of RNA from high phenolic containing tissues of pecan. HortScience **27**, 1316-1318.

LOGEMAN, J; SCHELL, J.; WILLMITZER, L.; 1987: Improved method for the isolation of RNA from plant tissues. Analyt. Biochem. **163**, 16-20.

LÓPEZ-GÓMEZ, R.; GÓMEZ-LIM, M. A.; 1992: A method for extracting intact RNA from fruits rich in polysaccharides using ripe mango mesocarp. HortScience **27**, 440-442.

PAI, S. R.; BIRD, R. C.; 1991: Quick and high efficiency electroelution of nucleic acid fragments. Gen. Analyt. Tech. Applic. **8**, 214-216.

PAULUS, F; HUSS, B.; BONNARD, G.; RIDE, M.; SZEGEDI, E.; TEMPÉ, J.; PETIT, A.; OTTEN, L.; 1989: Molecular systematics of *Agrobacterium tumefaciens* biotype III Ti plasmids. Mol. Plant-Microbe Interact. **2**, 64-74.

PELOQUIN, J. J.; PLATZER, E. G.; 1991: A simple, inexpensive electroelution device for the recovery of nucleic acid fragments from agarose gels. BioTech. **10**, 159-160.

SAMBROOK, J.; FRITSCH, E. F.; MANIATIS, T.; 1989: Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SHIA, S.-P.; 1990: A simple and efficient method for recovery of DNA from gels. BioTech. **9**, 565-568.

SHIRZADEGAN, M.; CHRISTIE, P.; SEEMANN, J. R.; 1991: An efficient method for isolation of RNA from tissue cultured plant cells. Nucl. Acid. Res. **19**, 6055.

TAYLOR, B.; POWELL, A.; 1982: Isolation of plant DNA and RNA. Focus **4**, 4-6.

VERWOERDT, T. C.; DEKKER, B. M. M.; HOEKEMA, A.; 1989: A small scale procedure for the rapid isolation of plant RNAs. Nucl. Acid. Res. **17**, 2362.