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 (Shirzadeh 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 electrophoretic protocol, basically according to Verwoert (1991). 2 g of grapevine crown gall 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 crude extract was mixed with 0.3 ml of loading buffer supplemented with ethidium bromide (Angermüller 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. cutoff 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. Narancsfüz) 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), Att (lane 4, 21 μg), AT66 (lane 5, 10 μg), S4 (lane 6, 22 μg) and Sz1 (lane 7, 29 μg), B: RNA was blotted onto Amersham Hybond-C extra membrane according to the instructions of the supplier and hybridized under standard conditions (Sambrook et al. 1989) with 32P labelled 1.9 kbp EcoRI fragment of pTITm4 TA-DNA (Paillus 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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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$_2$EDTA 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 bromothymol blue; 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.