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A small-scale procedure for the rapid isolation of plant RNAs

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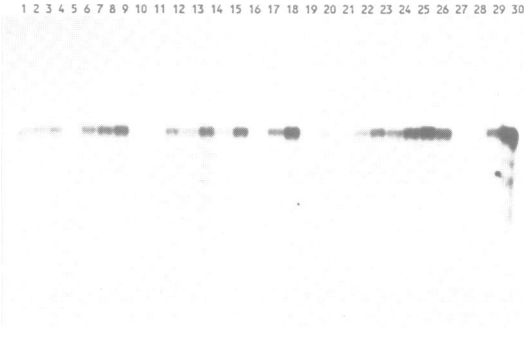
The analysis of RNA levels in large numbers of (transgenic) plants is elaborate and time consuming. In the conventional set up (ref.), RNA is prepared from whole leaves of mature plants by grinding in a mortar in relatively large volumes. This increases the risk of RNase contamination and the number of samples that can be handled is limited.
We adapted this method to allow the rapid isolation of total RNA from as little as 100 mg of leaf material. All steps are performed in eppendorf tubes so many samples can be handled simultaneously.



Northern blot analysis of 30 transgenic Solanum tuberosum cv. Desiree plants using a radiolabeled DNA probe. In each lane 10 μg of total RNA was loaded.

Fresh leaf discs are collected in 2 ml eppendorf tubes, frozen quickly in liquid nitrogen and stored at -80 °C until use. The leaf discs are ground using a steel bar (precooled in liquid nitrogen) that perfectly fits the eppendorf tube. Keeping the plant material frozen allows easy grinding to a fine powder. After grinding 500 μl of hot extraction buffer (80 °C) is added [phenol - 0.1 M LiCl, 100 mM Tris.HCl pH=8.0, 10 mM EDTA, 1% SDS (1:1)]. The mixtures are homogenized by vortex for 30 seconds, and 250 μl chloroform - isoamylalcohol (24:1) is added and vortexed. After centrifugation for 5 minutes, the waterphases are removed and mixed with one volume of 4 M LiCl. RNAs are allowed to precipitate overnight and collected by centrifugation. The pellets are dissolved in 250 μl water, 0.1 volume of 3 M NaOAc pH=5.2 is added and the RNAs are precipitated with 2 volumes of ethanol. After centrifugation the RNA pellets are washed with 70% ethanol and dried.
RNAs were tested in Northern analysis (fig.). Routinely between 25 and 50 μg of total RNA is obtained from 100 mg tobacco, tomato or potato leaf tissue.

Reference: Van Slogteren, C.M.S., Hoge, J.H.C., Hooykaas, P.J.J. and Schilperoort, R.A., (1983) Plant Mol. Biol. 2, 321-333.

