Hybridization of Plant Virus ssRNAs Transferred to Hybond N Membrane

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There are various modifications of the Northern blotting technique. Widely used are, *e.g.* transfer of glyoxal/DMSO denaturated RNA from nondenaturating agarose gels to nitrocellulose [1], blotting onto Zeta-Probe membrane under alkaline conditions [2], and/or transferring of RNA from agarose/formaldehyde gels to nitrocellulose or nylon membranes [3].

In this paper we present a protocol for the nondenaturating agarose gel electrophoresis of plant virus ssRNAs, their blotting onto Hybond N membrane, and hybridization with [alfa ^{32}P]dNTP-labelled cDNA probe. The protocol is not pretentious on technical equipment, omits denaturation and neutralization steps and some chemicals required in other modifications.

Viral RNA is denaturated for 10 min at 65 °C in the presence of 8 % formaldehyde, and cooled in ice/water bath. The samples are electrophoresed (3 V cm^{-1}) in submarine 1 % nondenaturating agarose gel using the TBE buffer (0.089 M Tris-borate, 0.089 M boric acid, 0.002 M-EDTA, pH 8.3) system. To avoid pH changes, the buffer is recirculated or regularly (every 10—15 min) mixed by shaking the apparatus [4]. The gel is placed in horizontal gel apparatus onto two sheets of filter paper saturated with transfer 20 × SSPE buffer (3.6 M-NaCl, 0.2 M sodium phosphate, pH 7.7, 0.002 M-Na_2EDTA) and covered with unwetted Hybond N membrane. The absorbent stack (5 cm) is compressed using a glass plate and 1 kg mass. Transfer proceeds overnight at room temperature, however 8 h transfer provides also a good signal. The blot is dried, wrapped in Saran Wrap, and RNAs are fixed by UV light (254 nm, 120 mJ cm^{-2}) for 4 min. The membrane is prehybridized in buffer containing 50 % formamide (Serva, anal. grade, not deionized), 5 × SSPE, 5 × Denhardt's solution (0.1 % Ficoll, 0.1 % poly(vinyl pyrrolidone), 0.2 % bovine serum albumin), and denaturated salmon sperm DNA (250 μg cm^{-3}) for 1—4 h at 42 °C. Hy-

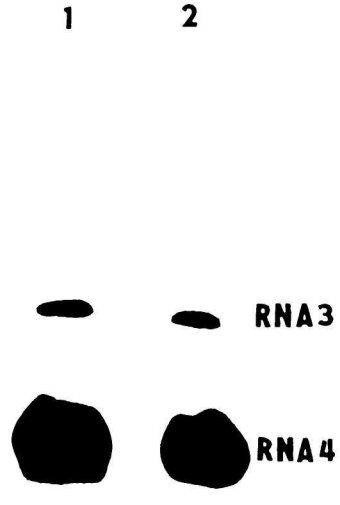

Fig. 1. Hybridization of the alfalfa mosaic virus RNAs. 4 μg of total RNAs (RNA 1—4 with lengths 3640 nt, 2590 nt, 2040 nt, and 964 nt) isolated from strain T6 (lane 1) and 425 (lane 2) were fractionated in 1 % nondenaturating agarose gel (Sea Plaque GTG). The RNA blot was prehybridized for 1 h at 42 °C, and hybridized for 10 h at 42 °C with [alfa ^{32}P]dCTP (110 TBq mmol^{-1}) labelled cDNA probe coding for coat protein of strain 425 (cDNA sequence is localized on genomic RNA3 and subgenomic RNA4). The high stringency conditions for washing were used.

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bridization is performed in buffer of the same composition plus radioactive probe for 10—18 h at 42 °C. The RNA blot is washed according to Amersham's manual. Briefly, twice in 5 × SSPE for 15 min at 42 °C, once in 1 × SSPE/0.1 % SDS for 30 min at 42 °C, once in 0.1 × SSPE/0.1 % SDS for 15 min at room temperature.

This protocol is suitable, *e.g.* for detection and localization of specific sequences, and/or for identification of virus strains and isolates (Fig. 1).

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