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A sensitive technique for detection of RNA with single-stranded probes

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A simple and sensitive method for definitive determination of coding strands and mapping of RNA-coding regions has been developed. This method involves two sequential hybridizations to RNA gel blots, first with unlabeled single-stranded M13 DNA containing a subcloned DNA fragment of interest, second with radioactively labeled M13 RF DNA. The technique possesses three significant advantages over standard hybridization methods. First, sensitivity is greatly increased, particularly with very small probes which cannot be labeled to sufficient specific activities using standard methods. An RNA species constituting only 0.03% of the 2 ug of poly(A)$^+$ RNA loaded on a gel can be detected by a 160 nt probe in a 4 hr exposure; the same RNA is not detected with the double-stranded form of the same subclone nick-translated with ^{32}P to a specific activity of 3 X 10^8 cpm/ug. The increased sensitivity is presumably due to the absence of competing strands in the first probe and the amplified size of the target for the second probe due to M13 sequences attached to the hybridized insert. Second, the purity of the single-stranded probe allows definitive coding strand assignments using just one DNA fragment subcloned in both orientations into M13mp18/19; no gel isolations of labeled DNAs, which are often cross-contaminated, are required. Third, the ease of this technique is appreciable. Many probes representing numerous regions along a large DNA fragment can be tested simultaneously, but only one, the M13 RF, needs to be radioactive; this reduces both cost and exposure to radioactivity.

RNA blots are prehybridized for 12 hours at 42°C in ~20 mL of prehybridization solution (5X SSC, 5X Denhardt's solution, 50 mM sodium phosphate buffer, pH 7.0, 250 ug/mL denatured carrier DNA, and 50% formamide). At least six single-lane blots can be prehybridized in the same bag. The filters are then hybridized individually for 30 hours at 42°C in ~10 mL of fresh prehybridization solution, to which dextran sulfate (10% final concentration) and the probe in ~100 uL 10 mM Tris, pH 8.0 have been added. Probes are prepared from 2 mL phage cultures in the same manner as are templates for dideoxy sequencing, and the ~1-5 ug of single-stranded DNA thus prepared are used in the hybridization. The probe solution is then removed, and the filters are washed together in 0.2X SSC, 0.1% SDS at 65°C for 1 hour. This washing is repeated four times without allowing the filters to dry out.

The prehybridization/hybridization/washing cycle is then repeated, but this time all the filters are hybridized together with M13 RF DNA which has been nick-translated with ^{32}P to a specific activity of ~3 X 10^8 cpm/ug, and the hybridization is carried out for only 20 hours. After the second round of washes, the filters are autoradiographed at room temperature.

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