

Rapid transfer of small RNAs from a polyacrylamide gel onto a nylon membrane using a gel dryer

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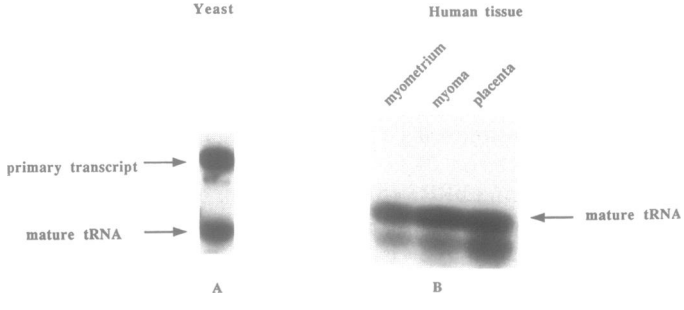
Small RNAs such as tRNAs are best separated on polyacrylamide gels. For hybridization analysis the RNAs are generally transferred onto a nylon membrane using an electroblotting system. As an alternate method we have repeatedly used a vacuum gel dryer to transfer small RNAs from polyacrylamide gels onto Hybond N^+ membrane. Autoradiograms of blots obtained with this method are shown below. In A, total RNA from a yeast strain which accumulates precursors of a mutant tyrosine-inserting ochre suppressor SUP4-o tRNA (1) was fractionated on a denaturing gel, transferred onto the membrane and hybridized with a probe complementary to yeast tyrosine tRNA; the mature 78 nucleotides tRNA and the precursor tRNA (about 115 nucleotides long) are readily transferred to the membrane and give a strong hybridization signal. In B, total tRNA extracted from human tissues was electrophoresed on a semi-denaturing gel and hybridized with a probe complementary to human tyrosine tRNA. In this case only mature tRNA can be detected after hybridization indicating that very little precursor tRNA accumulates in these cells. We have checked that 40% to 50% of mature tRNA is transferred from the gel onto the membrane. Longer RNA molecules are not as well transferred (for a 223 nucleotides long RNA for example, 20% of the molecules bind to the membrane). This technique is therefore best suited for small RNAs like tRNAs, tRNA precursors, snRNAs or 5S RNAs.

Experimental procedure: Denaturing gels were 8% polyacrylamide-7M urea made up in TBE buffer pH 8.3 and were run at room temperature using 0.5×TBE as the running buffer. The semi-denaturing gels were 10% polyacrylamide,4 M urea made up in TBE buffer pH 8.3 and were run in the cold room using TBE as the running buffer. The thickness of the gels can be varied between 0.5 mm and 1 mm. After electrophoresis the gel and the membrane were equilibrated in 25 mM phosphate buffer pH 6.5 for 10 min. At that stage gels can be stained with ethidium bromide in order to visualize the RNAs. The gels were layed on plastic wrap and most of the liquid was allowed to drip off the gel, a Hybond N^+ membrane was layed on top of the gel and a piece of 3 MM paper on top of the nylon membrane. The blot sandwich was placed in the vacuum gel dryer with the 3 MM paper facing the vacuum plate of the dryer. The gel was dried under vacuum at 80°C for 2 h. After drying the gel which sticks to the membrane can be easily removed by soaking a few minutes in 25 mM phosphate buffer. Because the transfer is done at 80°C there is no need to fix the RNA on the membrane by baking or UV irradiation. The transferred RNA bands can be visualized under UV light. The blot was then prehybridized in 6×SSPE, 0.2% bovine serum albumine (BSA), 0.2% Ficoll, 0.2% polyvinylpyrollidone (PVP), 0.1% SDS, 50 μg/ml denatured salmon sperm DNA and hybridized with the appropriate labelled probe in 6×SSPE, 1.0% SDS.

REFERENCE

1. Wilhelm,M.L., Keith,G., Fix,C. and Wilheim,F.X. (1992) *Nucleic Acids Res.* **20**, 791–796.



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