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Note

Recovery of RNA from polyacrylamide slab gels

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Methods for separation of individual species of RNA or separation of RNA into size classes frequently involve column chromatography or density gradient sedimentation. Both methods have inadequate resolution of closely spaced species with contamination of one class or species of RNA with another. Polyacrylamide gel electrophoresis does permit extremely high resolution but current methods for recovery of RNA from slab gels are cumbersome, time consuming and produce low yields. They involve cutting out a gel band and re-electrophoresing the RNA cast in a cylindrical gel, into a dialysis bag[1]. Alternatively, specially constructed equipment that has buffer flowing through the gel of dialysis membrane at the outlet of the gel has been used[2]. Recently a technique involving use of a high salt gradient has been described[3] for use in recovering protein from polyacrylamide gels. In our experience this last technique does not prevent low-molecular-weight RNA from passing through the high salt layer while high-molecular-weight RNA precipitates at the high salt interface. The method described below does permit high yield recovery of low-molecular-weight RNA from slab gels and is both simple and rapid.

The basic method involves electrophoresing RNA into a polyacrylamide slab gel and then removing the RNA from the gel by electrophoresis through a solution containing a dialysis membrane barrier. First I shall describe the membrane barrier and then illustrate how a single species of RNA or a whole class of high- or low-molecular-weight RNA can be recovered from a slab gel.

METHODS

Gel apparatus

A standard slab gel apparatus (Bio-Rad Labs.), was used with modification of the polyvinyl chloride (PVC) spacers between the glass plates. Spacers were cut from 3 mm thick PVC with two sets of protrusions at the top and bottom of the gel, as in Fig. 1.

Membrane barrier

A dialysis membrane (12 × 1 cm) is cut out longitudinally along the length of one side of a flat section of dialysis tubing with a 3500 MW cutoff (Fisher Scientific). The dialysis membrane that has been cut out is then folded along its length to form

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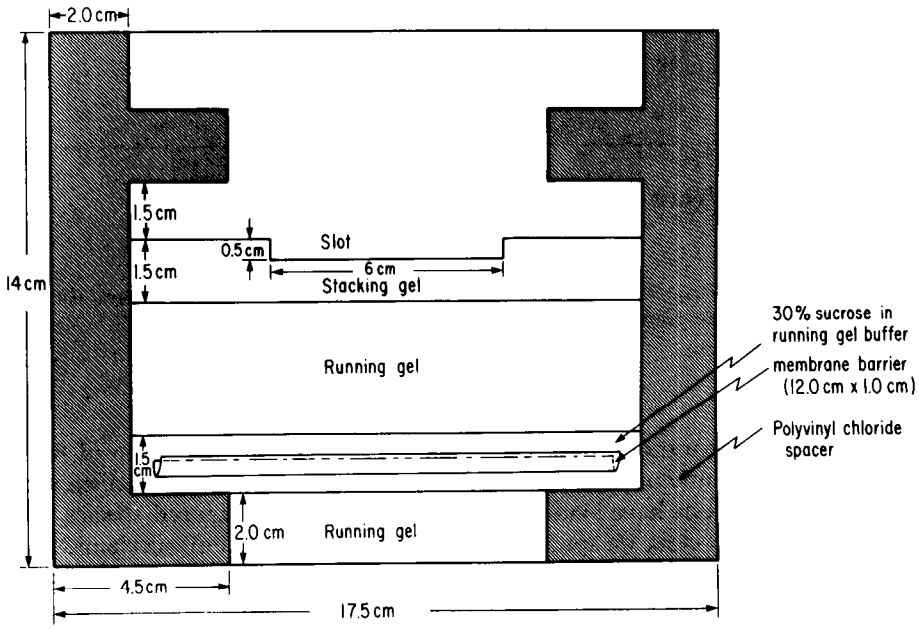

Fig. 1. Standard slab gel apparatus with modified polyvinyl chloride spacers between the glass plates.

a U-shaped through. The folded membrane, slightly shorther than the width of the slab gel, is then inserted between the glass plates of the electrophoresis apparatus using a section of narrow diameter plastic tubing to correctly position the barrier parallel to the top edge of the acrylamide gel. Since the two sides of the barrier repel one another, the sides of the membrane barrier press closely against the glass walls of the gel apparatus.

Recovery of low-molecular-weight RNA

Polyacrylamide gels, sample buffer, gel buffers, electrophoresis buffer and conditions of electrophoresis followed those of Philippsen and Zachau[2].

To recover low-molecular-weight RNA [*e.g. E. coli* tRNA (Sigma)] from a sample without high-molecular-weight RNA contamination, an aliquot of the sample is first electrophoresed on an appropriate analytical gel (1.5 mm thick). After staining of the gel with 0.2% methylene blue and silver if necessary[4], a measurement is taken of the distance between the bottom of the gel and the desired point at which the higher-molecular-weight RNA is to be excluded. All species of RNA which have not migrated past that point will be excluded from the RNA to be recovered from a preparative gel. A preparative gel (3 mm thick) is then made to a level 1.5 cm below the point determined from the analytical gel.

The membrane barrier is then added with its crease inferior and just above the gel. A solution of 30% sucrose in running gel buffer is added so that it rises above the sides of the membrane barrier to the level of the point determined from the previous gel. A running gel is then formed on top of the sucrose solution followed by a stacking gel. Before pouring the stacking gel an 6-cm PVC spacer can be inserted

0.5 cm below the surface of the forming spacer gel. After polymerization of the stacking gel the slot forming spacer is removed. Up to 300 μl of sample in sample buffer is then put in the slot. Electrophoresis buffer is added and the sample is then electrophoresed until the marker dye has reached the bottom of the gel. The polarity of electrophoresis is reversed and the current resumed for 5 min to remove RNA adhering to the membrane. The electrophoresis apparatus is then inverted.

A long spinal needle with a trocar is then inserted through the original lower gel section (now uppermost) into the sucrose containing buffer. The trocar is removed and a second needle with its trocar is inserted into the sucrose buffer. The sucrose buffer is carefully aspirated into a syringe attached to the second needle while air enters the region through the first needle. The aspirate is put into a dialysis bag. The space containing the membrane barrier is flushed back and forth with water inserted through the second syringe and the washing added to the dialysis bag for dialysis against water. The RNA containing dialyzate is then lyophilized.

Recovery of high-molecular weight RNA

By careful use of a long needle and a spatula the lowest section of separation gel, below the section containing sucrose buffer is carefully removed. Flushing with reservoir buffer helps to remove all loose pieces of gel and the membrane barrier. With the apparatus remaining inverted, running gel solution is then poured into this section of the gel apparatus. When it is polymerized the apparatus is returned to its proper upright position. A new membrane barrier is next inserted above the stacking gel with its crease upwards. A solution of 30% sucrose in running gel buffer is then added above the stacking gel apparatus just covering the membrane barrier. This is followed by reservoir buffer.

The electrophoresis is then resumed with the original polarity reversed so that the RNA migrates upwards. Electrophoresis is continued for a time period at least one hour longer than the original electrophoresis so as to assure that all the high-molecular-weight RNA has come out of the gel. As a final step, the polarity of electrophoresis is reversed for 5 min to remove any RNA tightly adhering to the membrane.

The top reservoir buffer is then carefully removed and discarded. The 30% sucrose buffer is aspirated and added to a dialysis bag along with a wash of the same space. The membrane barrier can also be added to the dialysis bag. After dialysis the recovered RNA is lyophilized.

Recovery of a single RNA band

A stained or unstained band is sliced from a slab with a razor blade. The band is inserted horizontally in the center between the glass plates when the slab gel apparatus is assembled. Running gel buffer is then poured into the apparatus until it just covers the inserted band. After polymerization a membrane barrier is put just over the inserted band with its crease upwards. 30% sucrose in running gel buffer is then added until it covers the barrier. This is followed by further running gel. After polymerization electrophoresis is performed until the RNA has migrated out of the gel and into the membrane barrier. The polarity of electrophoresis is then reversed for 5 min. The 30% sucrose buffer is removed and the area flushed as above. The RNA is then dialyzed and lyophilized.

RESULTS AND DISCUSSION

An amount of 50 μg of *E. coli* tRNA was applied on a 5% stacking gel on top of an 8% separation gel. After electrophoresis into the membrane barrier and dialysis, 20% of the recovered RNA was electrophoresed in parallel with samples of tRNA (1–10 μg per sample). A comparison of the stained bands showed a recovery of about 80% of the tRNA applied to the preparative gel. Similar yields were found from elution of ^{3}H-labeled tRNA. Staining of bands with methylene blue prior to electrophoretic elution did not quantitatively change the recovery yield of single bands.

The use of a membrane barrier prevents the RNA from migrating further than the surface of the barrier. The current is carried through the gel by small ions which pass through the semipermeable membrane setting up parallel lines of force in a homogeneous electric field. The construction of the spacers narrows the field so that it is not as wide as the membrane barrier. Therefore, the current goes directly through the membrane rather than attempting to curve around the edges or ends of the membrane. In addition, use of a sample slot of smaller width than the entire gel further limits the side diffusion of the sample as it migrtes through the gel. The RNA thus gets trapped inside the vertex of the membrane barrier. The current is reversed briefly after electrophoresis to release any RNA adhering to the membrane surface.

We have found the use of a membrane barrier simple, reproducible, and an inexpensive alternative to cumbersome elution apparatus. The technique has allowed us to separate very small RNA species (less than 4S) from larger-molecular-weight species with no detectable contamination of either group of RNA with the other as determined by an ultrasensitive high resolution silver stain method[4].

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