High-Resolution Preparative-Scale Purification of RNA Using the Prep Cell[1]

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Milligram-scale purification of RNA with high resolution is required for spectroscopic and X-ray crystallographic characterizations, as well as for clinical trials. The presence of many conformations of the same RNA sequence makes it particularly difficult to purify RNA using column chromatography. Therefore, denaturing gel electrophoresis is commonly used for RNA purification (1, 2). However, most gel electrophoresis methods suffer low capacity, requiring 8–15 gels for a typical spectroscopic or X-ray structural experiment (3). We previously reported a method for automated large-scale purification of an RNA ribozyme from other transcription components using the Bio-Rad Prep Cell (4). Here we test a new model of the Prep Cell (Fig. 1) which is three times longer than the original Model 491 apparatus and also investigate the sample recovery of the Model 491 apparatus. We found that the new apparatus offers significant improvement in resolution and loading capacity, and that the sample recovery of ~90% for Model 491 apparatus is better than that typically obtained from either electroelution or crush-and-soak methods. This method can be easily adapted to large-scale purification of other nucleic acids.

Materials and Methods

A 100-ml-scale transcription was performed as previously described to obtain milligram quantities of the 34-mer 5′-GGCGACCGUGAUGAGGCCGAAAGGCCGAAACAUU-3′ (4). The crude transcript was ethanol-precipitated and reconstituted in 5 ml of 1.5× TBE[4] (135 mM Tris–borate, 3 mM EDTA). The sample was then concentrated using Centricon-10 units (Amicon, Beverly, MA) to a final volume of 1–2 ml. During this concentration procedure, the centricon was washed several times with 1.5× TBE to partially remove unincorporated NTPs and thus increase the solubility of the transcript.

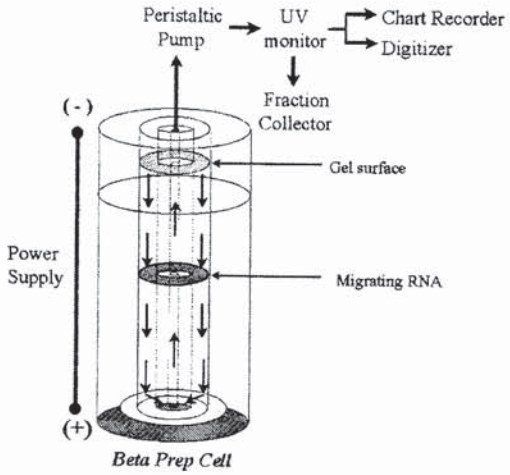

FIG. 1. Schematic diagram of the modified Prep Cell. This model is identical to the Model 491 except the lower chamber has been elongated to accommodate a longer gel tube. The RNA is loaded onto the cylindrical PAGE gel where it migrates down the gel toward the positive electrode (see the arrows), just as in traditional electrophoresis. The separated RNA species are then pulled through the capillary tubing located in the center of the cooling core by a peristaltic pump into a UV monitor and finally into a fraction collector. A cellulose membrane with a molecular weight cutoff below that of the RNA is placed at the bottom of the Prep Cell gel to prevent the purified RNA from escaping the system while still allowing passage of conducting ions. A digitizer was also added to allow collection of a computerized chromatogram in addition to the hardcopy chromatogram generated by the strip chart recorder.

Purification of the RNA transcripts was performed using the Model 491 Prep Cell and prototype Prep Cell along with equipment donated by Bio-Rad Laboratories, including the Model 1327 Econo-Recorder, EM-1 Econo UV Monitor, Model 2128 Fraction Collector, EP-1 Econo Pump, and the Powerpac 1000 power supply. In addition to the chart recorder, the data were simultaneously digitized using a ComputerBoard DAS-08 digitizer. A 20% acrylamide/8 M urea gel (120 ml for a 13-cm gel, 260 ml for a 30-cm gel) was prepared in the 37-mm i.d. large Prep Cell gel tube and allowed to polymerize for 3 h. During the polymerization process, the gel was cooled using the recirculation pump connected to the cooling core and a 1-liter beaker of ice-water. To prevent crystallization of the urea during the cooling process, room-temperature water was passed through the pump until polymerization began and the gel started to get warm, at which point ice was added to the beaker. For each run, 130 μl of crude transcript was combined with an equal volume of formamide, heat denatured, and loaded onto the Prep Cell gel. We found that increasing the power to 15 W allowed for a shorter running time without loss in resolution. Therefore, the following separation conditions were used: 20% denaturing polyacrylamide, 15 W con-

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[4] Abbreviation used: TBE, Tris–borate–EDTA.

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stant power, 1.5× TBE running buffer, 1 ml/min elution rate, and 8 ml/fraction.

Results and Discussion

Percentage sample recovery. To determine how much RNA can be recovered from the Prep Cell gel, duplicate runs were performed using the Model 491 Prep Cell. For each run, 2–3 mg of purified 34-mer hammerhead ribozyme (quantified by UV absorption using ϵ_{260nm} = 285,483 M^{-1} cm^{-1}) was loaded onto a freshly prepared 13-cm, 20% PAGE Prep Cell gel. After completion of the run, the fractions containing RNA were combined, concentrated, desalted, and quantified by UV absorption. Of the 3.1 mg loaded in the first run and the 2 mg loaded in the second, 2.8 and 1.8 mg were recovered, respectively. The corresponding average recovery is 90%. This recovery is better than that typically obtained from both conventional electroelution (70–80%) (5) and crush-and-soak methods (60–80%) (6). The improved recovery is attributed to less postelectrophoresis manipulation.

Effect of gel height. The maximal gel height for the commercially available Model 491 Prep Cell is 13 cm, compared to 40 cm for most preparative PAGE gels. To investigate whether a longer gel would offer better resolution, we used a beta Prep Cell model with a maximal height of 40 cm, supplied by Bio-Rad Laboratories (Hercules, CA). Figure 2A shows a comparison of chromatograms from purification runs using 13- and 30-cm Prep Cell gels, each loaded with an RNA transcript containing 0.8 mg of 34- and 35-mer hammerhead ribozyme (as determined by quantifying these bands on an analytical PAGE gel prior to Prep-Cell purification). The operating conditions were constant for each run. As expected, better resolution is achieved with the 30-cm gel. Individual peaks corresponding to $n-1$, n, $n+1$, and $n+2$ transcripts are visible. To obtain comparable resolution on the 13-cm gel, less than half as much crude transcript can be loaded (4).

The Prep Cell can be used to purify even larger quantities of RNA. An RNA transcript containing ~7 mg of 34- and 35-mer hammerhead ribozymes was loaded onto a 30-cm Prep Cell gel. Although resolved peaks corresponding to the 34- and 35-mer are not visible in the chromatogram, individual 8-ml fractions contain pure 34-mer RNA (Fig. 2B). Depending on the purity requirement of the particular application, those fractions containing less pure RNA can be pooled and repurified using the same Prep Cell gel to recover RNA of higher purity.

In summary, the Prep Cell method provides high-resolution preparative purification of RNA with high percentage sample recovery. In addition, the longer apparatus has the advantage of allowing resolution of n from $n+1$ transcripts even when several milligrams

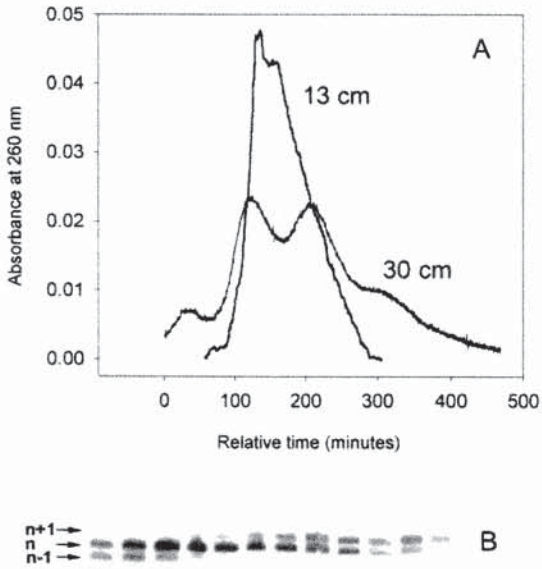

FIG. 2. (A) Overlaid chromatograms from purifications using 13- and 30-cm Prep Cell gels. Because the elution time differs for the two gel lengths, the origin of the time axis shown corresponds to a point just before peak elution and does not correspond to the start of each run. The origin corresponds to 15.5 h (930 min) and 47.5 h (2850 min) for the 13- and 30-cm runs, respectively. (B) Analytical PAGE gel of Prep Cell peak fractions from a 30-cm Prep Cell run in which an RNA transcript containing 7 mg of 34- and 35-mer hammerhead ribozyme was loaded. Aliquots of every other fraction from the RNA peak were loaded onto a 19 cm × 29 cm × 0.7 mm gel and stained with ethidium bromide.

is loaded. This method requires a significantly lower amount of acrylamide gel solution (~260 ml for the 30 cm Prep Cell) than a typical preparative PAGE gel (~720 ml for a 40 × 60 × 0.3 cm gel), needs no attended operation once the sample is loaded, and requires minimal postelectrophoresis manipulation. Unlike the crush-and-soak (6), electroelution (5), or ultracentrifugation methods, the gel is not destroyed and can be used up to three times without significant loss of resolution. These features make the Prep Cell method a viable alternative to traditional purification methods, especially for laboratories that need large quantities of RNA on a routine basis. This method has also been adapted to high-resolution purification of milligram quantities of other nucleic acids, such as phosphorothioate DNA and RNA in our laboratory.

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An Improved Method for the Purification of Large DNA Fragments from Agarose Gels Using Wizard *Plus* SV Columns

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The isolation of DNA fragments from agarose gels is an integral step of many molecular biological protocols. Of the numerous techniques developed to recover DNA fragments from agarose gels (1), the direct elution of DNA from the agarose matrix by centrifugation through a filter is the simplest. Different filters have been used including cotton-filled pipet tips (2), glass wool (3), blotting paper (4), paper slurry (5), commercial barrier pipet tips (6), and Wizard minicolumns (7). While the Wizard minicolumn is a convenient and reproducible means to directly elute DNA from agarose gels, it suffers from two limitations as a filter. First, the standard Wizard columns are of low capacity and its small opening can make the insertion of the agarose gel slice awkward. Second, the DNA yield is often poor, particularly with large DNA fragments. This problem is, however, not confined to the use of Wizard colums because low yields of large DNA fragments have been observed with other filter systems (2–6).

We describe the use of Wizard *Plus* SV miniprep DNA purification columns (Promega, Madison, WI) for the rapid isolation of DNA fragments from agarose gels. The yield of large DNA fragments is improved by preequilibrating the gel slices in a neutral salt buffer and freezing before centrifugation (8).

Two 30-ng samples of λ–*Hin*dIII-digested DNA marker were electrophoresed in parallel on a 0.7% agarose gel in 1× Tris–acetate–EDTA buffer (1). The gel was stained with ethidium bromide and the 23-, 9-, and 6.5-kb bands were excised from both lanes under UV transillumination. Individual DNA fragments were eluted using either of the following two protocols:

1. The original Wizard DNA gel elution protocol of Wolff and Hull (7). Briefly, gel slices were placed in individual standard Wizard columns held in 1.5-ml Eppendorf tubes and the DNA was eluted by centrifugation at 14,000*g* for 12 min. DNA was precipitated by the addition of 0.1 vol of 3 M sodium acetate and 1 vol of isopropanol, followed by centrifugation at 14,000*g* for 10 min.

The Wizard Plus SV column with salt equilibration and freezing protocol. Gel slices were placed within individual 2-ml Eppendorf tubes containing 1 ml of salt buffer (300 mM sodium acetate, 50 mM Tris–HCl, 1 mM EDTA, pH 8.3). The gel slices were allowed to equilibrate for 30 min at room temperature before the gel slice was transferred, with minimal buffer, to individual Wizard *Plus* SV miniprep DNA purification columns held in 1.5-ml Eppendorf tubes. After freezing the gel slices for 10 min at −70°C, the excised DNA fragment was eluted by centrifugation at 14,000*g* for 12 min. DNA was precipitated by the addition of 1 vol of isopropanol followed by centrifugation at 14,000*g* for 10 min.

DNA samples were resuspended in 10 μl of TE (10 mM Tris–HCl, pH 7.4; 1 mM EDTA, pH 8.0) and the DNA recovery quantified (Table 1) using the Fluro-S MultiImager (Bio-Rad, Hercules, CA) after electrophoresis on a 0.7% agarose gel with 30 ng of λ–*Hin*dIII DNA marker (Fig. 1).

TABLE 1

Efficiency of DNA Recovery from Agarose Using either the Original Wizard Direct Elution Method (7) or the Presented Improved Wizard *Plus* SV Salt/Freeze Method

Fragment size (kb)	Original method (%)	Improved method (%)
23	33	75
9	45	85
6.5	40	80

The Wizard *Plus* SV column, in combination with a salt preequilibration and freeze step, provides a reliable method for the direct elution of large DNA fragments from agarose gels. Their large capacity and opening make them particularly convenient when excising large gel volumes. The addition of a salt buffer preequilibration and freezing step provides for a significant increased recovery of large DNA fragments (Table 1). We have isolated DNA fragments using this technique from 0.4 to 3% agarose. Finally, DNA purified using this method has proven suitable for a range of molecular biological procedures, including plasmid preparation, DNA ligations, DNA sequencing, PCR amplification, and restriction enzyme digestions (9).

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