Benchmarks

method of fixing samples before antibody staining (6), and it would be interesting to assess the suitability of such samples for FISH.

Quantitation of successful FISH in tissue sections is not straightforward because of sample-to-sample variation, nuclear truncation in sectioning and variations across the slide in both tissue density and section width. Accurate comparison of FISH sensitivity following microwave vs. protease treatment would require a much wider study and is not reported here. The speed and simplicity of microwaving combined with both FISH results that can be consistently scored and the low rate of sample failure make it our method of choice for prostatic tissue and should make it useful in interphase FISH on tissue sections in general.

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Quantitative Recovery of Nucleic Acids from Excised Gel Pieces by Isotachopheresis

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The purification of nucleic acid molecules using electrophoresis is a widely applied technique in molecular biology, in which nucleic acid is recovered from excised slices of polyacrylamide and agarose gels. Although many different experimental approaches to this technique are applied, quantitative recovery is difficult. Problems of preparative nucleic acid purification are (i) loss or damage of material being recovered, (ii) contamination by the gel material and (iii) time inefficiency (e.g., mechanical elution using shaking).

We report here a simple and gentle electro-elution method for the quantitative recovery of either RNA or DNA from either polyacrylamide or agarose gels. Unlike conventional electro-elution procedures, the nucleic acids are eluted into a gel filtration matrix that is contained in a small chromatography

column. This is a simplification of the isotachopheresis procedure originally described by Öfverstedt et al. (1). The methodology takes advantage of the fact that ions of different electrophoretic mobilities will migrate with the same velocity in an electric field (isotachopheresis) that is separated by two solute zones. Since in the past we have only cited the original work by Öfverstedt et al., we have been asked by other researchers to provide a detailed protocol of the simplified isotachopheresis method that we have used routinely for more than a decade.

Principle of the method. The column material (Sephadex® G-50 fine;

Figure 1. Schematic outline of the column used for the isotachopheresis. A chromatography column is packed with Sephadex G-50 fine (matrix). The column is closed with a three-way valve, which itself is closed by means of a dialysis membrane attached with a piece of silicon tubing. The column is placed in a beaker filled with running buffer, in which an electrode is placed. The gel piece containing nucleic acids of interest is placed horizontally on top of the Sephadex gel bed and covered with the trailing electrolyte. The upper end of the column is closed with a lid that contains an electrode.

Amersham Pharmacia Biotech, Uppsala, Sweden) is equilibrated with 40 mM Tris-HCl (running buffer), in which Cl^- serves as the leading electrolyte. The gel piece containing nucleic acids of interest is placed on top of the column and covered with 100 mM ϵ -amino-caproic acid, an amphoteric ion that has a slightly negative net charge in aqueous solution and migrates in the electric field slowly (terminating electrolyte). When voltage is applied, the nucleic acids elute from the gel piece. Because of the different migration paces of the leading and the terminating electrolytes, the nucleic acid, which has an intermediate velocity, is concentrated at the boundary between the two ionic environments. The position of the migrating boundary can be visualized during the electrophoresis by supplementing the terminating electrolyte with traces of phenol red (which co-migrates with the nucleic acid) be-

fore beginning the procedure. When the boundary has reached the lower part of the column, the electric field is switched off, and the nucleic acid is collected in a 1.5-mL reaction tube. At this stage, one can take advantage of the gel filtration effect of the Sephadex to separate the nucleic acid from the low-molecular-weight phenol red. However, because of its solubility in alcohol, phenol red can also be removed by a subsequent ethanol or isopropanol precipitation.

We have used this method in the past on a regular basis for both RNA and DNA to recover between 90%–99% of highly purified material. Unlike conventional electro-elution, the nucleic acid does not make contact with the dialysis membrane. Because the Sephadex resin is separated physically from the eluate, it is impossible to contaminate the purified nucleic acid. Also, denaturing agents like urea are

removed, because they do not migrate under the conditions used. For that reason, the isotachopheresis method is especially useful for the gentle recovery of sensitive, *in vitro*-synthesized RNA transcripts. However, we also use this form of electro-elution routinely with all other kinds of nucleic acids, including synthetic DNA and RNA oligonucleotides, circular and linear RNA, double-stranded RNA, DNA restriction fragments, polymerase chain reaction (PCR) products, genomic DNA and vector DNA. We have eluted RNAs of up to 3 kb and DNAs of 10 kb, but this should not be regarded as upper size limitation of the procedure. The size of the nucleic acid that can be eluted is limited by the matrix of the excised gel—especially by the percentage of polyacrylamide or agarose. Any nucleic acid that elutes from the gel slice within 2 h in an electric field of 20 V/cm will be recovered efficiently.

Benchmarks

Preparation of the Sephadex. All solutions used are autoclaved, especially when RNA is being eluted. Twenty grams of Sephadex G-50 fine are equilibrated with 4× TE (1× TE = 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) by stirring with a magnetic bar. This column material is allowed to sediment and is then adjusted with 4× TE to reach equal volumes of sedimented and liquid phases. After stirring again, the material is placed in 100-mL bottles and autoclaved.

A stock solution of approximately 100 mL of 100 mM ϵ -amino-caproic acid is prepared in an autoclaved glass bottle using autoclaved water. A few crystals of phenol red are added, so that the solution is red. Approximately 2 mL of the solution are needed per run. The stock solution is stored frozen at -20°C.

Preparation of the Luer-lok® three-way valve. A 2-cm-long piece is cut from a 2-cm-wide conventional dialysis membrane and the tube is cut open, to create a piece of membrane that is 2 × 4 cm. The membrane is washed with 40 mM Tris-HCl (pH 8.3), placed around the lower part of the three-way valve and attached to it by a tight silicon tubing such that the outlet is sealed by the dialysis membrane. The valve is filled with running buffer from bottom to top using a Pasteur pipet, taking care that no air bubble remains in the valve. **Note:** It has proven helpful to have a second three-way valve ready during the isotachopheresis run, since air bubbles might form at the interface between the three-way valve and the end of the column, thereby blocking the flow of the current applied. In this case, the three-way valve has to be replaced quickly to prevent diffusion of the nucleic acids on the column.

Preparation of the column. The column is prepared as illustrated in Figure 1. An empty 0.8- × 4-cm Poly-Prep® Chromatography Column (Bio-Rad, Hercules, CA, USA) is attached to a stand support and washed as follows: (i) 1× with 0.1 M NaOH, (ii) 1× with 0.1 M HCl, (iii) 2× with autoclaved water and (iv) 2× with 40 mM Tris-HCl (pH 8.3) (these washing steps are necessary only when RNA is to be eluted). The Sephadex G-50 fine solution is used to fill the column and allowed to sediment up to about 2 cm from the end of the column. The column material is washed with 2 vol of 40 mM Tris-HCl (pH 8.3). The eluate of the column during washing is collected in a beaker, and the pH is checked using pH paper. After the second wash, a membrane-closed and buffer-filled valve is then attached to the column. Using the outlet of the valve, any residual running buffer from the top of the column will be eluted so that the surface of the gel matrix is just immersed. The column is placed into a beaker that contains running buffer such that the dialysis membrane-closed outlet of the valve is hanging into the buffer. A platinum electrode is placed into the buffer. The gel piece is placed horizontally on top of the Sephadex gel bed. Using a Pasteur pipet, the gel is carefully covered with about 2 mL 100 mM ϵ -amino-

caproic acid, supplemented with traces of phenol red. The upper part of the column is then closed with a lid containing an electrode.

Isotachopheresis run. An electric field of 20 V/cm (limiting) is applied. This will result typically in an initial current of about 3 mA, which decreases during the run. Shortly after voltage is applied, the phenol red will accumulate at a sharp zone that slowly migrates through the column. A typical run lasts about 2 h. At the end of the run, when the phenol red is approximately 3 mm away from the lower end of the column, the electric field is switched off, the three-way valve is removed and the nucleic acid is recovered by collecting approximately 400 μ L of the column eluate. For complete recovery of the nucleic acid a portion of the phenol red fraction can be collected. The drops that are eluted before the phenol red contain most of the nucleic acid. If desired, the nucleic acid can be purified further by a phenol/chloroform extraction and concentrated by a subsequent NaOAc/ethanol precipitation.

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