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Isolation of proteins and nucleic acids by electrophoresis on disposable gel columns

A simple and cheap one-step method to isolate proteins or nucleic acids by electrophoresis in disposable gel columns is reported. A disposable syringe was modified to host a gel column and an elution chamber. Starting from a crude extract of *E. coli*, the laboratory-made device allowed the isolation of the maltose binding protein (MBP) fused to a recombinant allergenic molecule with a molecular mass of 58 kDa, from a mixture of several proteins. Also, plasmid DNA could be isolated from a mixture containing chromosomal DNA and RNA, avoiding the use of organic solvents. Electrophoresis was performed at 150 V, 35°C, pH 8.0 and 8.3 for protein and DNA, respectively. The protein or the DNA obtained showed a yield of 80% and a purity grade of 90%, as estimated by densitometry.

Several methods are used for the purification of proteins or nucleic acids, including, among others, preparative and capillary electrophoresis, immunoaffinity chromatography, gel electrophoresis, and electroelution [1–7]. In selecting the most suitable method for purification, such parameters as complexity of procedure, yield, obtained purity, pH and temperature compatible with biological activity, time as well as the cost of the apparatus must be considered. Gel electrophoresis is probably the most frequently used method. In this report we describe a simple and cheap method that allows the isolation of a target protein or of a specific nucleic acid, by using a disposable polyacrylamide or agarose gel column, with a high rate of purity and recovery, in a single step, at constant pH and temperature.

The conic edge of a disposable plastic syringe was cut out and a dialysis membrane was glued (Attak, Loctite) to the opposite edge, at the bottom region of the column (Fig. 1). Two holes were then made at 0.3 cm from the bottom by using a heated syringe needle. Two tips (Eppendorf, 10 µL standard tips) were cut and glued in correspondence to the two holes. To form a layer 60% glycerol was then layered on and a magnetic bar (3 mm diameter × 1 cm length) was immersed into the glycerol layer. Ten % acrylamide (Sigma, St. Louis, MO, USA) was layered approximately to 4 cm in height, with a stacking gel of 0.4 cm (Fig. 1). After polymerization of the acrylamide gel, the column was connected to a peristaltic pump and placed in a beaker, containing the running buffer (25 mM Tris, 200 mM glycine, 0.1% SDS, pH 8.0; Fig. 2). The glycerol layer was removed with the acid of a peristaltic pump and a prerunning step was performed at 100 V for 30 min. The protein mixture was then loaded, approximately 5 µg of protein per mm² of gel, and electrophoresis was run at 150 V. At a flow rate of 0.7 mL/min, fractions were collected every 5 min after elution of the marker dye bromophenol blue (BPB). Since the agarose gel used for the purification of nucleic acid molecules did not adhere to the syringe walls, the O-ring of the syringe piston had to be inserted into the

syringe (Fig. 1). Further steps were similar to those described for polyacrylamide. The height of agarose was approximately 6 cm and the agarose concentration 0.8%. The mixture containing the target DNA to be purified was loaded (2 µg DNA/mm² gel) on top of the gel layer and electrophoresis was carried out at 100 V. At a flow rate of 0.5 mL/min, fractions were collected every 5 min after elution of BPB.

A recombinant clone prepared in our laboratory, coding for a fusion protein of 58 kDa, was grown to 0.5–0.6 OD₆₀₀ and then induced for 2 h with 0.3 mM isopropylthio-β-D-galactoside (IPTG). The harvested cells were suspended in loading buffer (10 × is 0.25 M Tris, 10% SDS, 0.1% BPB, pH 6.8), boiled for 2 min and then forced through a syringe needle Nr. 20 in order to break the DNA molecules and to reduce the viscosity of the solution. The cellular lysate was loaded onto the column, usually with a total load of 4 mg protein in a 50 mL syringe column. The plasmid DNA was prepared by the alkaline method [8], precipitated by isopropanol, dissolved in 10 mM Tris, 1 mM EDTA, pH 8.0, and loaded in the agarose column, usually with a total load of 400 µg of DNA in a 20 mL syringe column.

The standard conditions described above can be modified depending on the properties of the molecule to be isolated. The standard conditions were adopted to obtain in one step from a crude extract of *E. coli* the maltose binding protein fused to a recombinant protein of 58 kDa. The protein bands of the fractions were analyzed with a Bio-Rad Scanner, and approximately 90% purity and 80% yield were found (Fig. 3). The two major proteins (42 and 52 kDa) could be well resolved. The proteins in the collected fractions were dialyzed, concentrated and analyzed by ELISA and Western blotting [9]. The results obtained showed that biological activity was still present. Similarly, DNA was purified under standard conditions described above. Scanning of bands gave a purity of 90% and or yield of 80% (Fig. 4). The purified DNA was precipitated and used for bacteria transformation. Sequencing and restriction enzyme digestion showed a high degree of purity. An advantage of the reported method is the possibility to obtain a large amount of plasmids. Using a 20 mL syringe up to 400 µg of DNA could be purified, making this system a useful alternative to methods reported in the literature. Finally,

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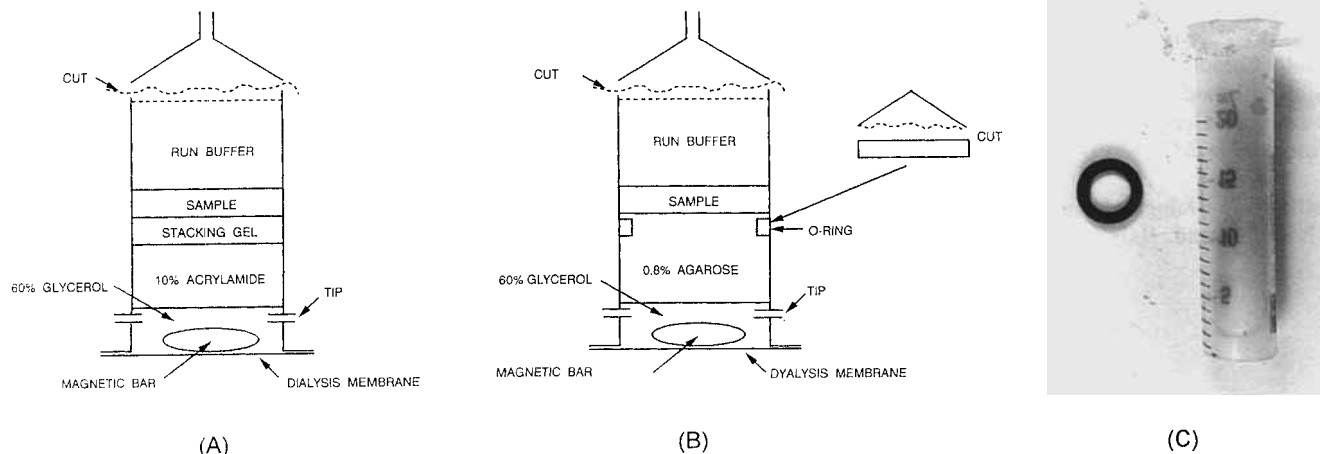


Figure 1. Scheme of the (A) polyacrylamide and (B) agarose column; (C) photo of the column. The gasket of the syringe piston was cut in order to obtain a rubber ring to be inserted into the syringe. Boiling agarose was cooled to 55°C and layered on the glycerol to completely cover the rubber ring. For other details see text.

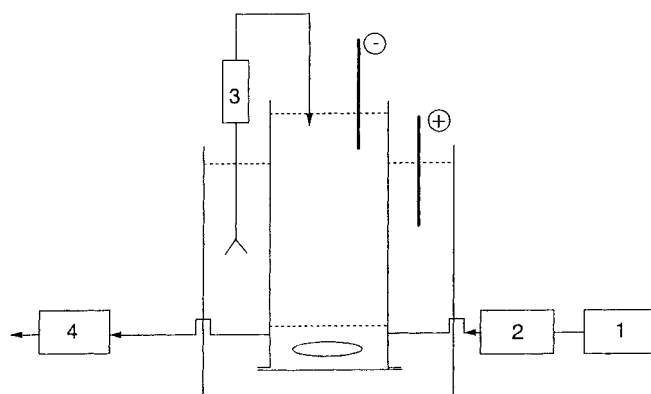


Figure 2. Scheme of the apparatus: 1, buffer tank; 2, peristaltic pump; 3, recycling buffer pump; 4, fraction collector.

the described method might also be used to purify DNA fragments and to fraction genomic DNA and mRNA. The apparatus can be easily assembled in every laboratory at a low cost and the procedure may be optimized for the isolation of specific proteins or nucleic acids, without use of organic solvents.

In conclusion, the described apparatus shows the following advantages: (i) Isolation of single components in one step from a complex mixture without additional purification, with high resolution and good yield, due to efficient mixing with a magnetic bar and low resistance in the elution chamber on passage of the current; (ii) constant temperature assured by immersion of the column in the buffer; (iii) possibility to change the temperature depending on the target molecule; (iv) constant

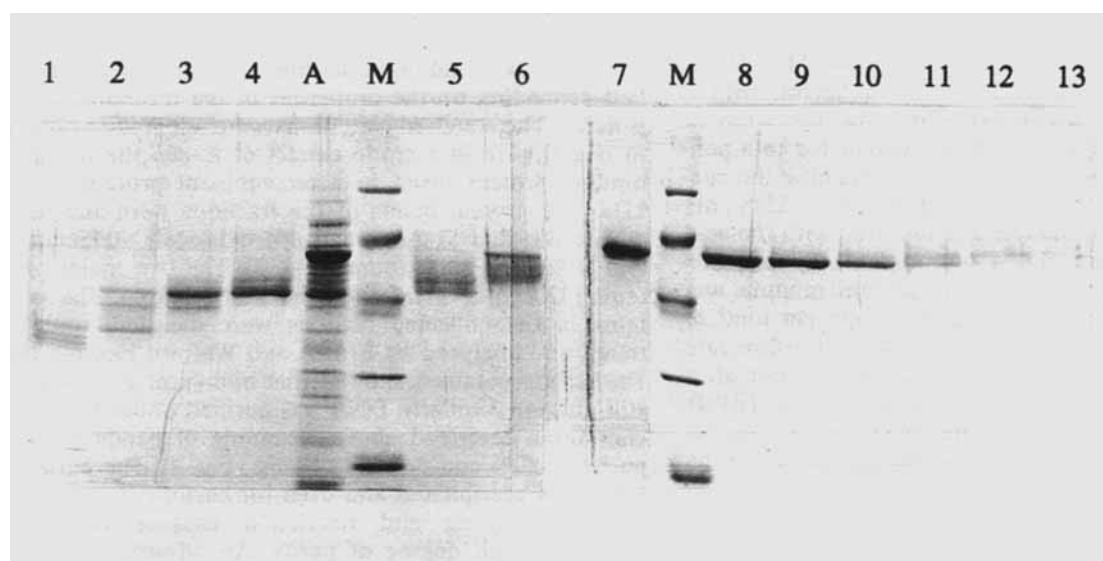


Figure 3. Fractions (1) to (13) eluted from the column, at a flow rate of 0.7 mL/min. The fractions were collected every 5 min and analyzed by SDS-PAGE in a 10% gel followed by staining with Coomassie Brilliant Blue. M, protein markers (21, 31, 42, 66 and 97 kDa); A, cellular lysate prepared as described in the text.

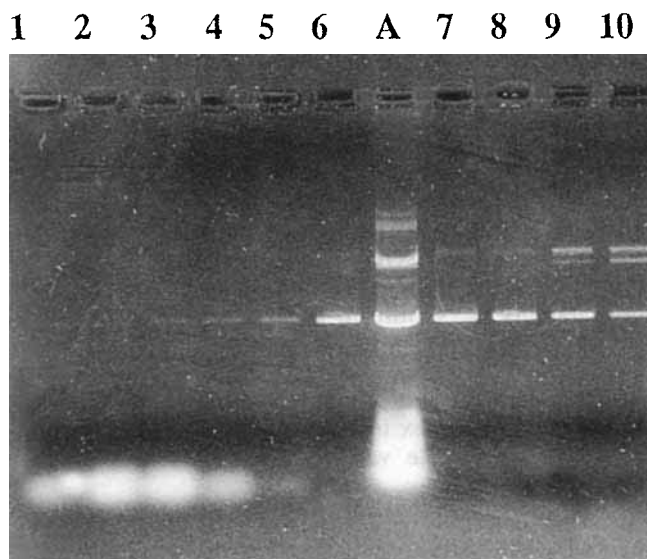


Figure 4. Fractions (1) to (10) eluted from the column, separated by horizontal agarose (0.8%) gel electrophoresis and stained with ethidium bromide. A, cellular lysate, prepared as described in the text.

pH due to the easy recycling of the buffer; and (v) low cost of a disposable system avoiding any contamination or cleaning after purification runs.

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