# ELECTROPHORETIC ELUTION OF NUCLEIC ACIDS FROM GELS ADAPTED FOR SUBSEQUENT BIOLOGICAL TESTS

## Application for analysis of mRNAs from maize endosperm

Udo WIENAND, Zsuzsanna SCHWARZ and Günter FEIX
*Institut für Biologie III, Schänzlestr. 1, D-7800 Freiburg, FRG*

Received 13 November 1978
Revised version received 11 December 1978

## 1. Introduction

Gel electrophoresis has become a powerful tool for the analytical investigation of nucleic acid mixtures and for their preparative fractionation. However, the recovery of biologically active nucleic acids after electrophoresis has posed serious problems. The method of crushing the gel followed by elution often results in varying yields and partial loss of biological activity [1,2]. The method of electrophoretic elution of nucleic acids overcomes these problems. But, as applied so far, this method requires elaborate equipment and skilled handling and can lead to dilute solutions [3–5]. In all cases, the processing of several samples at the same time is rather time consuming. These disadvantages are overcome by the electrophoretic elution method described here. The procedure allows, without additional equipment, simultaneous elution of up to 20 samples/elution gel and has shown to yield, directly and with good recovery, biologically active mRNA, plasmid DNA and double-stranded DNA fragments. The performance of the method is demonstrated with mRNAs from maize endosperm and with DNA restriction fragments from a hybrid Col-plasmid.

## 2. Materials and methods

### 2.1. *Chemicals*

Salts were reagent grade from Merck/FRG. Acrylamide and methylenebisacrylamide were purchased from Serva/FRG. Seakam agarose was obtained from Marina Colloids Inc/USA. Urea (from Merck) was used without further purification.

### 2.2. *Enzymes*

Restriction endonucleases were prepared and assayed according to [6]. Polynucleotide kinase was from Biogenics Research Corp./USA and used as in [17].

The wheat germ system of protein biosynthesis was prepared and used with slight modifications as in [7].

### 2.3. *Nucleic acids*

Maize polysomes as well as maize mRNAs enriched for zein mRNA were isolated from the endosperm of 20 days post-pollination maize kernels as in [8]. Supercoiled DNA from the plasmid pZmc 134 [9] was isolated according to [10]. Defined fragments of double-stranded DNA were obtained by complete digestion with restriction enzymes.

### 2.4. *Gel electrophoresis*

Preparative polyacrylamide gel electrophoresis of RNA was performed in 3% polyacrylamide slab gels (2 mm thick) under denaturing conditions (7 M urea) for 3–5 h at 30–50 mA in the buffer system [11]. Immediately before electrophoresis the RNA was heated for 5 min at 100°C in sample buffer (20 mM MES buffer [12] (pH 6.0), 50 mM NaCl, 3 M urea, 0.5 mM EDTA) and quickly cooled in ice.

Preparative electrophoresis of DNA was carried out

in 1% agarose slab gels (4–8 mm thick) for 4–6 h at 100–200 mA in the buffer system [13] or according to [14].

After the electrophoresis of RNA or DNA, the gels or part of them were stained with ethidium bromide (4 μg/ml) followed by visualization of the nucleic acid pattern under ultraviolet light. Gel areas containing nucleic acids were cut out with a razor blade for subsequent elution. In the case of RNA gels, the gels were soaked for 2 h in electrophoresis buffer prior to cutting in order to remove most of the urea.

2.5. *Elution of nucleic acids*

The elution involves the preparation of an elution gel (A) followed by the electrophoretic elution of nucleic acids from a piece of a preparative gel (B):

(A) A horizontal 1% agarose gel lying on a glass plate (=elution gel) is connected with paper bridges to two vessels containing electrophoresis buffer and electrodes. For convenience this elution gel, which is made with the same buffer as the corresponding preceding preparative gel, is 2 mm thicker than the preparative gel. A rectangular-shaped hole of approx. twice the width of the gel piece to be eluted is cut out of the middle of the elution gel. Then a piece of dialysis tubing is slit open on one side, knotted at both ends and placed in the hole to form a lining. Care should be taken that the dialysis bag has contact with all sides of the hole. For simultaneous elution of several gel pieces, an elution gel with up to 10 or 20 rectangular shaped holes can be prepared, as demonstrated in fig.1, which depicts the elution set up. It should be noted that the same elution gel can be used successively for several eluting procedures.

(B) For the elution a piece of preparative gel containing nucleic acids is put into the hole of an elution gel coated with a dialysis bag (see (A)) positioning the gel piece in that part of the hole which is closest to the cathode (–) (see fig.1). The hole is then filled up to the upper edge of the inserted gel piece with electrophoresis buffer and electrophoresis is carried out for at least 5 h or overnight at 200 V (6–12 mA) in the cold room. To reduce evaporation of buffer a glass plate cover should be placed about 1 cm above the gel. After electrophoresis the buffer within the dialysis tube lining is thoroughly mixed by moving a Pasteur pipette along the wall of the tubing, gently drawing up the buffer and expelling it again, especially at the part of the hole nearest to the anode (+). The buffer is then removed from the hole and transferred to a plastic tube for subsequent ethanol precipitation. RNA was precipitated in the presence of 0.24 M ammonium acetate [15]. In the case of RNA eluted from polyacryl-

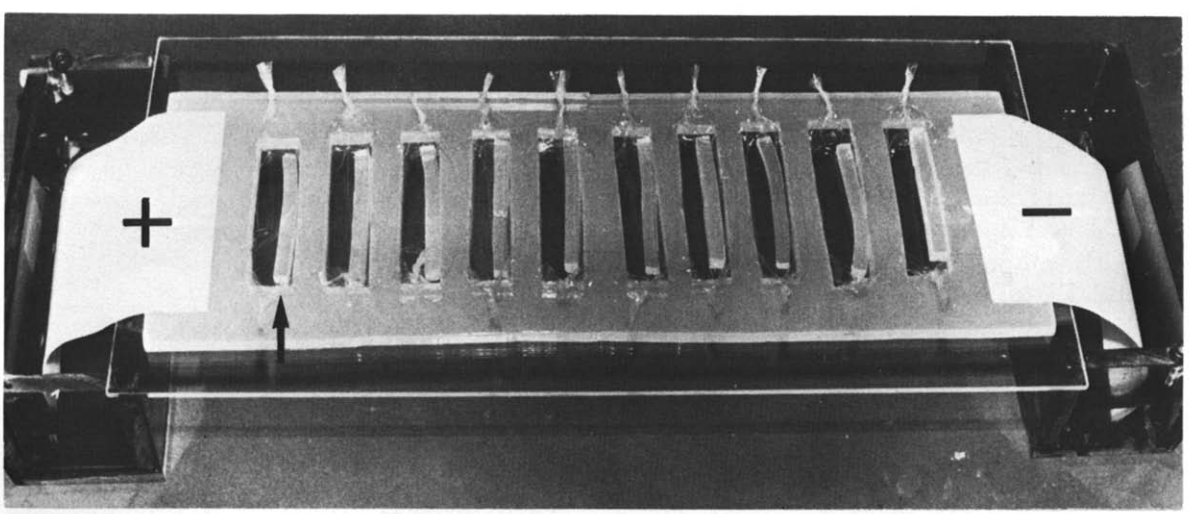


Fig.1. Elution gel containing 10 elution holes. The glass plate cover was taken off before taking this photo. The arrow indicates the position of an inserted gel piece.

amide gel pieces an ethanol-insoluble side product co-sediments with the RNA. This side product, however, does not interfere with the biological tests applied. DNA was precipitated in the presence of 0.3 M Na-acetate and 10 mM $Mg^{2+}$.

## 3. Results and discussion

This paper describes a fast and easy method for the electrophoretic elution of RNA and DNA from gels. The crucial point of the procedure is the use of a horizontal agarose gel [16] with rectangular-shaped holes into which gel pieces containing nucleic acids can be placed for electrophoretic elution. A great advantage of this arrangement is the possibility of processing up to 20 elutions at the same time (see fig.1). The elution gel is versatile in its application; it can accommodate polyacrylamide gel pieces as well as agarose gel pieces originating from slab gels or cylindrical gels. Because of the horizontal arrangement of the elution gel, low percentage gels used for separation of high molecular weight nucleic acids can be easily eluted. A coating of dialysis tubing in each hole serves to

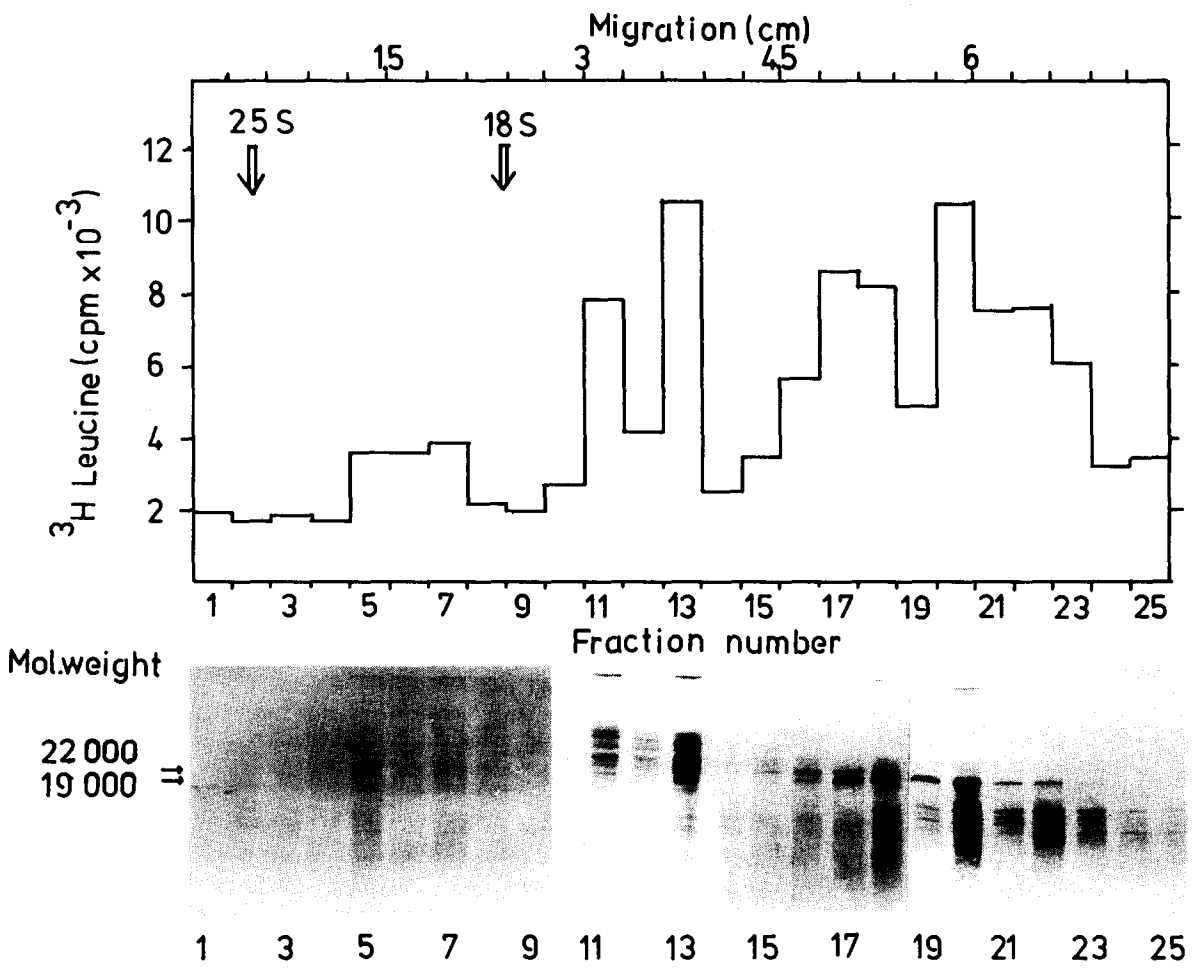


Fig.2. Analysis of eluted RNA fractions for mRNA activity. The mRNA activity of the fractionated poly(rA) containing RNA of maize endosperm after elution of the RNAs (see section 2) was tested by [$^3$H]leucine incorporation into trichloroacetic acid-insoluble material (upper part of figure) and by SDS–polyacrylamide electrophoresis (15% gel) of the synthesized proteins (lower part of the figure). Details of the protein biosynthesis and of the SDS–polyacrylamide electrophoresis have been described [8].

maintain the eluted nucleic acids within the hole and prevents entry of macromolecules and gel pieces from the agarose elution gel. The recovery of nucleic acids after the electrophoretic elution and ethanol precipitation is satisfying (50–70% for rRNA and mRNA, 70–90% for DNA fragments). The elution procedure does not lead to substantial degradation of the RNAs and DNAs as tested by analytical re-electrophoresis of the eluted nucleic acids. The eluted nucleic acids can be used directly for biological tests without further purification. Thus, eluted mRNA can be analysed for activity by in vitro protein biosynthesis. As the elution set up described allows the simultaneous elution of up to 20 samples, the method can be used to obtain without much effort a distribution profile according to size of active mRNAs from mRNA mixture after its electrophoretic separation. Figure 2 shows such a profile of the poly(rA) containing mRNAs of developing maize endosperm. The upper part of the figure shows [$^3$H]leucine incorporation values obtained from in vitro translations of the eluted RNAs in a wheat germ system. The total mRNA activity of all RNA fractions adds up to 30% of the activity of the unfractionated RNA. The arrows indicate the positions of maize ribosomal RNAs. The lower part of the figure depicts the sizes of the synthesized proteins as analyzed by SDS–polyacrylamide electrophoresis. As expected, the size of the synthesized proteins becomes smaller with a reduction in size of the mRNAs. In the case of the mRNAs coding for zein proteins (represented by the two main bands of 22 000 and 19 000 $M_r$ in fractions 15–19) the successful assay of the eluted mRNA fractions allowed the demonstration of two separable mRNAs for individual zein proteins [8].

Eluted DNAs can be used directly without further treatment as substrates for restriction enzymes and for polynucleotide kinase. It is demonstrated in fig.3A that individual DNA fragments can be isolated in a pure and undegraded form from a mixture of DNA restriction enzyme fragments (lane 1 and 2). The isolated DNA fragments can directly be used for further fragmentations with restriction enzymes as shown for the enzyme HpaII (lane 1 of fig.3B).

All 15 restriction enzymes tested so far yield the expected DNA fragment patterns. Furthermore, the eluted DNA fragments can be used directly as substrates for the polynucleotide kinase catalyzed phosphorylation of 5′-ends with [$\gamma$-$^{32}$P]ATP (efficiency = 50 000–250 000 cpm/pmol fragment) [17], as demonstrated by lane 2 of fig.3B. The labeled DNA fragments could be submitted without any complication to DNA sequencing [17].

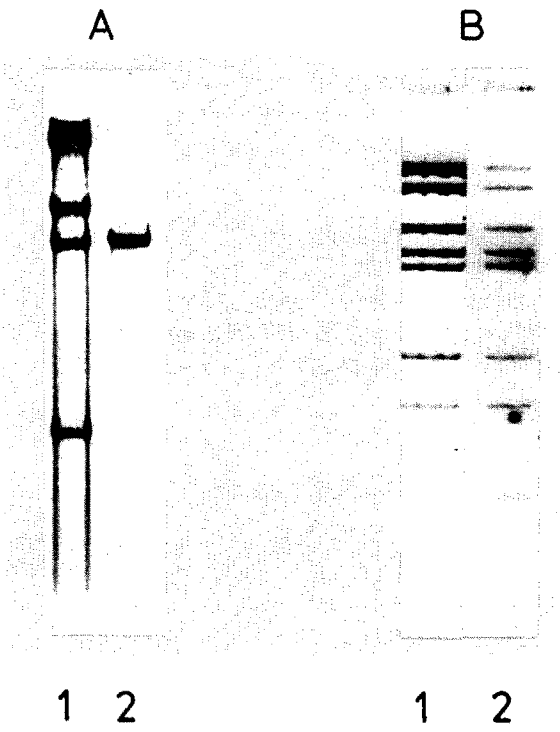


Fig.3. Gel electrophoresis of eluted DNA fragments before and after enzymatic reactions. (A) Plasmid pZmc 134 was digested with the restriction enzyme *Bam*HI and submitted to electrophoresis in 0.8% agarose as in section 2 (part A, lane 1). The third fragment from the top was eluted as detailed in methods and used for further experiments: one part of the fragment was reelectrophoresed in 0.8% agarose gel (part A, lane 2). (B) A second part of the eluted DNA fragment was digested with restriction enzyme *Hpa*II followed by electrophoresis in 7.5% polyacrylamide according to [18] (part B, lane 1); a third part was digested with *Hpa*II and submitted to a polynucleotide kinase reaction (see methods) and then electrophoresed in polyacrylamide under conditions of the preceding run (part B, lane 2). Lanes A1, A2 and B1 depict photographs of ethidium bromide staining, lane B2 shows an autoradiogramm.

In summary, all nucleic acids species eluted so far by the method presented here could be successfully used for biological tests indicating the potential usefulness of the method.

## Acknowledgements

The excellent technical assistance of Mrs E. Brutzer is gratefully acknowledged. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 46).

## References

[1] Dolja, V. V., Negruk, V. I., Novikov, V. K. and Atabekov, J. G. (1977) Anal. Biochem. 80, 502–506.
[2] Strongin, A. Ya., Kozlov, Tu. J., Debabov, V. G., Arsatians, R. A. and Zlochevsky, M. L. (1977) Anal. Biochem. 79, 1–10.
[3] Schuerch, A. R., Mitchell, W. R. and Joklik, W. K. (1975) Anal. Biochem. 65, 331–345.
[4] Sudgen, B., DeTroy, B., Roberts, R. J. and Sambrook, J. (1975) Anal. Biochem. 68, 36–46.
[5] Studier, F. W. (1973) J. Mol. Biol. 79, 237–248.
[6] Bickle, T. A., Pirrotta, V. and Imber, R. (1977) Nucl. Acid. Res. 4, 2561–2572.
[7] Davies, J. W., Aalbers, A. M. J., Stuik, E. J. and VanKammen, A. (1977) FEBS Lett. 77, 265–269.
[8] Wienand, U. and Feix, G. (1979) Eur. J. Biochem. in press.
[9] Bedbrook, J. R., Kolodner, R. and Bogorad, L. (1977) Cell 11, 739–749.
[10] Streek, R. E. and Hobom, G. (1975) Eur. J. Biochem. 57, 595–606.
[11] Peacock, A. C. and Dingman, C. W. (1967) Biochemistry 6, 1818–1827.
[12] Good, N. E., Winget, G. D., Winter, W., Conolly, T. N., Izawa, S. and Singh, R. M. M. (1966) Biochemistry 5, 467–477.
[13] Sharp, P., Sudgen, B. and Sambrock, J. (1973) Biochemistry 12, 3055–3063.
[14] Hayward, G. S. (1974) Proc. Natl. Acad. Sci. USA 71, 2108–2112.
[15] Osterburg, H. H., Allen, J. K. and Finch, C. B. (1975) Biochem. J. 147, 367–368.
[16] McDonnel, M. W., Simon, M. N. and Studier, F. W. (1977) J. Mol. Biol. 110, 119–146.
[17] Maxam, A. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560–564.
[18] Jeppesen, P. G. N. (1974) Anal. Biochem. 58, 195–207.