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Journal of Chromatography A, 855 (1999) 723–726

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Modified apparatus for voltage gradient gel electrophoresis

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Received 29 March 1999; received in revised form 3 June 1999; accepted 3 June 1999

Abstract

We built a modified version of voltage gradient gel electrophoresis system to correct distortions in nucleic acids electrophoretic migration patterns occurring at the edges of the gel when the original voltage gradient apparatus is used. The new device allows correct fractionation of nucleic acids also when electrophoresis is performed at high voltages.

Keywords: Voltage gradient gel electrophoresis; Instrumentation; DNA

1. Introduction

In 1993, a voltage gradient gel electrophoresis (VGGE) system was built to obtain a decreasing voltage gradient along the gel during electrophoretic fractionation of nucleic acids in an agarose matrix [1]. This procedure was aimed at obtaining a good separation of high-molecular-mass fragments in the gel (i.e. long runs) and, at the same time, to prevent low-molecular-mass fragments running out from the gel. It was also demonstrated that the voltage gradient effect produced an enhancement of the sharpness of each single nucleic acids band by reducing its thickness [2], thus giving an overall enhanced resolution of the different molecular components in the gel as compared to conventional electrophoresis.

Probably as a consequence of the VGGE device structure, a slight, sometimes troublesome, oblique distortion on nucleic acids migration pattern occurred at the edges of the gel.

In this paper we test a new version of VGGE system which overcomes this drawback, thus obtaining correct electrophoretic migration patterns, even when electrophoresis is carried out at high voltages.

2. Experimental

The new apparatus consists of two rectangular Plexiglas plates, 2 mm thick, whose dimensions depend upon the size of the electrophoretic tank, glued with the edges of a rectangular Plexiglas plate (2 mm thick) placed slantwise over the gel, with an angle of 18° with respect to the gel surface (Fig. 1a). Both lateral plates were cut in order to produce a recess (Fig. 1a) which would allow the gel tray, modified as in the original voltage gradient system [1] and shown in Fig. 1b, to have a tight fit with the VGGE apparatus (Fig. 1c).

Electrophoretic migrations were performed using

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00704-9

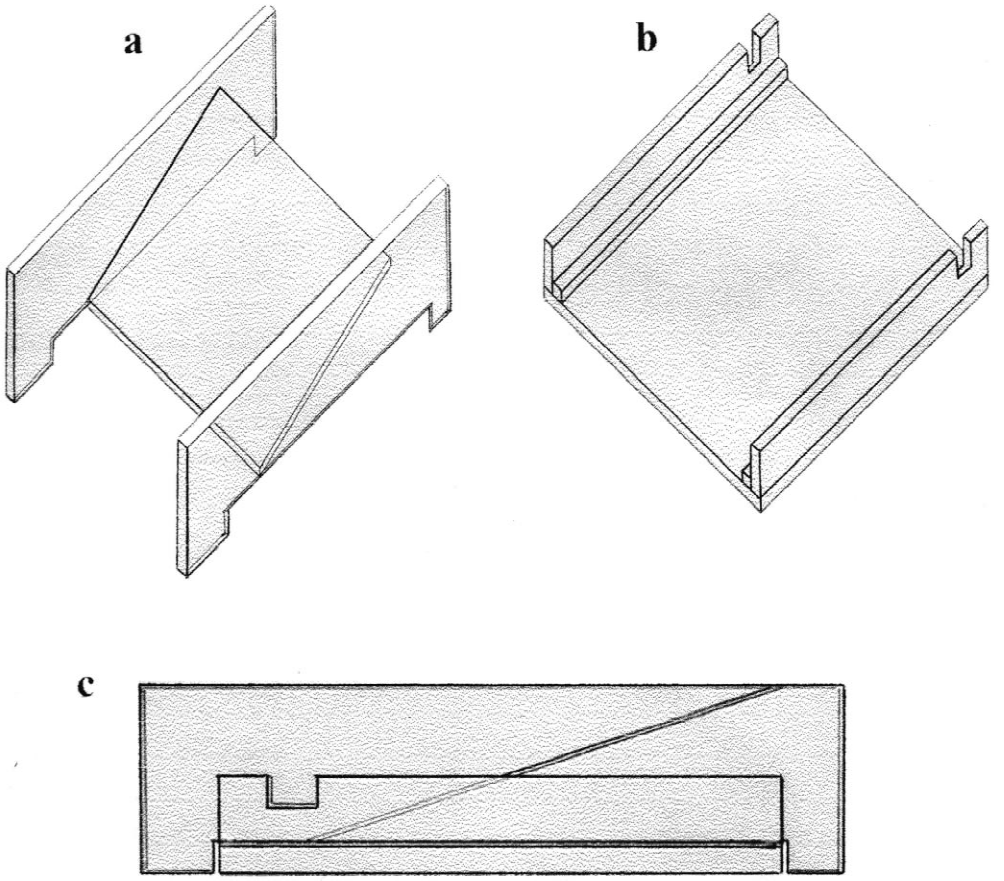

Fig. 1. Schematic drawings of the new version of VGGE apparatus (a) and of the modified gel tray (b). The two pieces (a and b) are mounted to form a single block, as shown in (c).

the same tank, in 1% agarose gel in 1× TAE buffer (40 m*M* Tris, pH 8.0; 2 m*M* EDTA, pH 8.0), at 3 V/cm [3] in the experiments shown in Fig. 2a and b, and at 6.5 V/cm in the experiments shown in Fig. 2 c and d.

In all the electrophoreses, 0.5 μg of Boehringer's (Manheim) 500 base pairs (bp) ladder and λ-EcoRI DNA markers per track were used.

3. Results and discussion

In addition to the main peculiarities of voltage gradient migration we discussed above, VGGE allows very fast nucleic acids fractionations with an enhanced resolution of bands when compared to conventional electrophoresis [1,2]. So we currently use VGGE fractionation of nucleic acids in our laboratory routine. But, as mentioned above, VGGE sometimes produces a slight deflexion of nucleic acids components in the more external gel tracks. This is shown in Fig. 2a, in which different DNA markers were fractionated by VGGE at 3 V/cm. As shown in Fig. 2c, distortion was dramatically enhanced when electrophoresis was carried out at high voltage.

We believe that this distortion is caused by a non rectilinear current flux because of the structure of VGGE apparatus itself, as shown in Fig. 3a. The variability in the frequence and in the degree of this phenomenon we have encountered in the long period we have used this system supports this hypotesis,

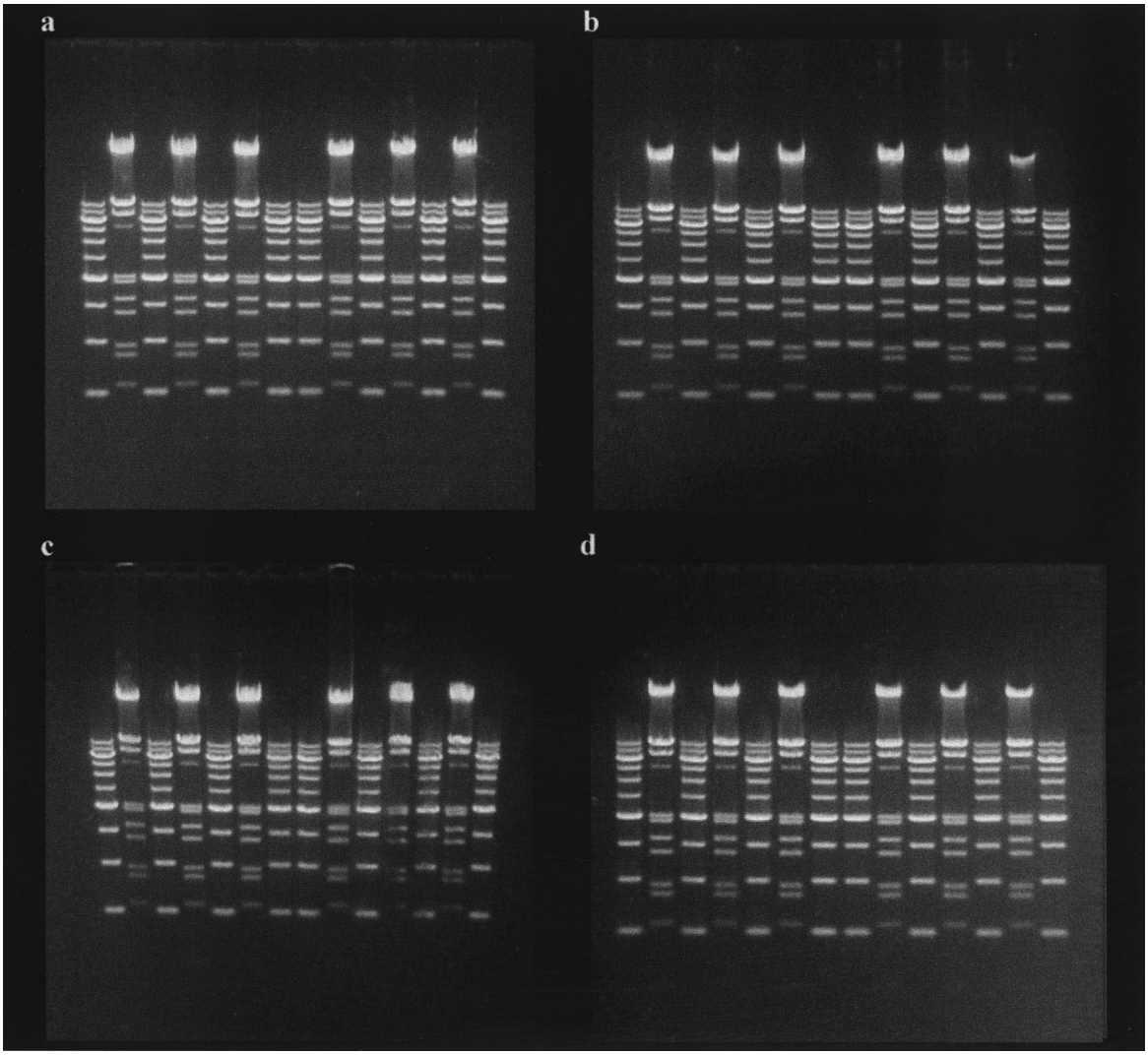

Fig. 2. Voltage gradient electrophoresis of different DNA markers (500 bp ladder, 1st, 3rd, 5th, 7th, 8th, 10th, 12th, 14th track and λ-EcoRI, 2nd, 4th, 6th, 9th, 11th, 13th track) at 3 V/cm (a and b) and at 6.5 V/cm (c and d), using original VGGE system (a and c) and the new VGGE version (b and d).

since different electrophoresis conditions (i.e. different agarose concentrations, different voltages, different buffers) are routinely used in our laboratory.

In the new VGGE version, the two lateral Plexiglas plates form a single geometrical block with the gel tray (Fig. 1c) and, as they protrude towards both the electrodes, they contribute to align the current flux before it creates voltage gradient (Fig. 3b).

The result is a correct electrophoretic fractionation of molecules along the whole gel, as shown in Fig. 2b. This also occurs when electrophoresis is carried out at a high voltage (Fig. 2d), the same which causes strong deflexions when the original VGGE system is used (Fig. 2c).

Acknowledgements

We are deeply indebted to Dr. Karoly Onorato for computer analyses and for preparing drawings and

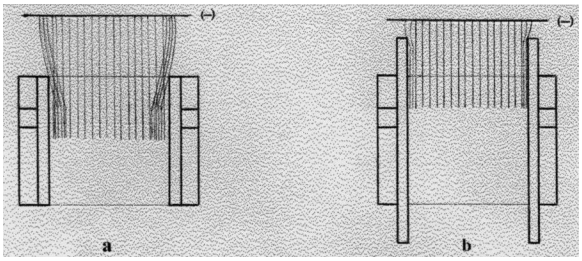

Fig. 3. Schematic drawings of the supposed current flux during electrophoresis using the original VGGE system (a) and using the modified VGGE version (b).

figures. This work was partially supported by funds of ‘‘Ministero dell’ Università e della Ricerca Scientifica e Tecnologica’’ (M.U.R.S.T.) 60% and 40%.

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