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Short Communication

PRELIMINARY OUTCOMES REGARDING THE OPTIMIZATION OF SEPARATION OF PLASMIDS BY AGAROSE GEL ELECTROPHORESIS

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Abstract

The aim of this study was to find some of the necessarily conditions to improve the separation of plasmids (DNA molecules) by agarose gel electrophoresis. The preliminary results here presented shown that the separation of electrophoretic bands depend on the potential applied to the electrodes and on the composition and concentration of the running buffers. The separation was optimized for the following plasmids: pYES2, pCTCON2, Taq_pET22b+ and ChiA_pUC57. These plasmids have been in two forms, digested by restriction enzymes and undigested, in supercoiled form. By using 0.5X TBE running buffer and 180 V potential applied to the electrodes, the running time was decreased from 60 until 20 minutes.

Keywords: agarose gel electrophoresis, DNA plasmid, DNA electrophoresis, TAE, TBE.

1. Introduction

Agarose gel electrophoresis is the method that is most used for the separation of DNA fragments ranging from 100 bp to 25 kbp [1]. Agarose gel electrophoresis is a simple and

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inexpensive technique [2]. In order to separate DNA fragments by agarose gel electrophoresis, the samples containing DNA are loaded into pre-cast wells in the agarose gel and an electric current is applied. As DNA molecule contain phosphate groups, that have negative charges, the DNA fragments will run toward positive electrode. Due to the fact that DNA fragments have a uniform ratio of their molecular masses to total charges, these molecules will be separated according with their size, in a pattern such that the distance traveled is inversely proportional to the log of their masses[3].

When electrophoresis is running, there is a balance between beauty and efficiency; if the gel is run at high voltage, the samples move faster, but the bands are smeared and the gel can melt if the voltage is high enough. If the gel is running at low voltage, it takes long time, but the bands are clearer and nicer [4].

In DNA electrophoresis it is commonly used a constant voltage. During the running of the electrophoresis, the current (mA) increases and it warms the running buffer. To avoid this it is indicated to keep the voltage to a low value (10 v/cm of the gel). To a small size gel (7-10 cm length), the voltage used is ranging from 70 to 100V and the migration of the bands will run for 30-50 minutes. The voltage increasing over this ranging, using TAE or TBE buffers at normal concentration (1X), will increase migration speed of the DNAs, but the buffer and the gel will heat asymmetrically [5]. Increasing the time of electrophoresis do not necessarily produce a beneficial effect on the length or intensity of the comet tail of supercoiled form of DNA subjected to separation. These tails seems to consist of loops of DNA that are attached to the nuclear matrix, as the DNA fragments that are not supercoiled produce clear bands, without tails[6].

To visualize the results after electrophoresis, it is necessary to keep the gel in a buffer that contains a fluorophore or to include the DNA-binding fluorophore in gel before polymerization. Ethidium bromide is an usual DNA binding fluorophore. The disadvantage is that EtBr is its toxicity [4].

In this study, we present some preliminary results of the optimization of electrophoretic separation of plasmids in agarose gels. The optimal composition and concentration of the running buffer were selected and the influence of the potential applied to the electrodes on plasmid separation was investigated.

2. Materials and Methods

2.1. Materials and Reagents

The chemicals were of analytical grade or better and were purchased from Sigma via Redox Lab Supplies Com S.R.L: Tris (Tris(hydroxymethyl)aminomethane, #10708976001),

acetic acid (#320099), boric acid (#B7901), EDTA (Ethylenediaminetetraacetic acid # E9884), agarose (#A9539), ethidium bromide (#E7637).

To cast and run the gels, a horizontal electrophoretic system from BioRad (Bucharest, Romania) and a power supply (Consort EV202, BioRad, Bucharest, Romania) were used. The gels were photographed using a documentation gel system (ECX-F15.M, Suebia, Germany). The images were analyzed with GenAnalyzer software (http://www.gelanalyzer.com/).

2.2. Methods

Running Buffers. In this study two type of running buffer were used: TAE (Tris/Acetate/EDTA) and TBE (Tris/Borate/EDTA). Stock solutions of 50 times more concentrated than the working solution (50X) of TAE running buffer was prepared in dH_2O, mixing 48.4 g Tris, 11.44 mL acetic acid (glacial), 20 mL 0.5M EDTA to 200 mL final volume. Stock 10X TBE running buffer was prepared dissolving 108g Tris, 55g boric acid, 7.5g EDTA disodium salt in dH_2O to 1000 mL final volume.

Agarose gel preparation. Agarose gels with 0.8% agarose in running buffer were prepared by purring the hot solution (60^oC) in the casting mold (7 x 6 x 0.5 cm) of the electrophoretic system (Bio-Rad).

Sample preparation. As samples, there were used four plasmids, isolated using MINIPREP protocol[7], with molecular mass ranging from 5000 bp to 8000 bp: pYES2 (5.9 kbp), pCTCON2 (7,2 kbp), Taq_pET22b+(8 kpb), ChiA_pUC57 (5.1 kbp). For comparison reasons, in the electrophoretic experiments, the plasmids were used in native, supercoiled state and after digestion with EcoRI restriction enzyme. For this, at 30µL plasmid solution (2.696 µg/µL), 10 µL sterile H_2O, 3 µL restriction enzyme EcoRI (10u/µL) and 6µL 10X EcoRI buffer were incubated at 37^oC for 16h.

Agarose gel electrophoresis. Gel electrophoresis experiments were performed using 0.8% agarose gels and 7 X 6 cm mini-sub cell GT 8 gel rigs (Bio-Rad). Electrophoresis was run until the dye (bromophenol blue) arrived at less than 0.5 cm from the positive edge of gel. The gels were stained with EtBr (0.1 mg/mL) for 15 minutes and rinsed with dH_2O for 3 times. After staining, the gel is viewed and photographed in UV light.

3. Results and Discussions

A typical agarose gel electrophoresis of nucleic acids (plasmids) is presented in Figure 1. The first lane is the molecular weight scale (in kpb). The samples containing the plasmids digested by EcoRI migrate faster than the same plasmids which are not digested.

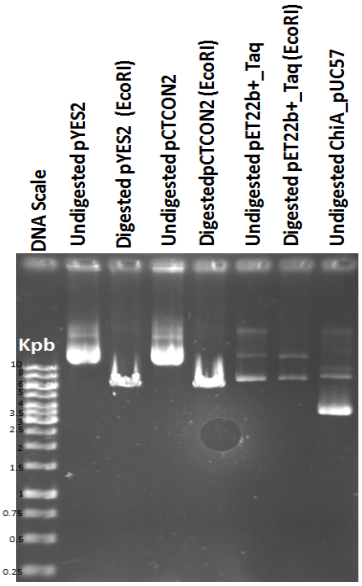

Figure 1. DNA agarose gel electrophoresis of supercoiled and digested plasmids. DNA scale is in the first column and plasmids in the others lanes. The voltage applied to the electrodes was 80V, running buffer TBE 1X and migration time was 60 minutes.

As one on the aims of this study was to find the conditions that lead to a reduction of the total running time of the electrophoresis, the first series of experiments were directed to reveal the influence of the potential (voltage) applied to the gel on the velocity of migration of nucleic acids.

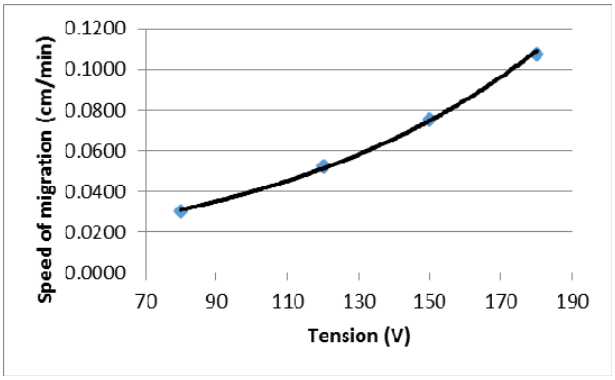

Figure 2 The variation of the speed of migration of undigested plasmid pYES2 according to the potential applied to the electrodes, using 0.5X TBE running buffer

From **Figure 2**, one can see that the higher the voltage, the higher is the migration speed of nucleic acids. The best correlation with these results is an exponential equation ($y=a\cdot e^{b\cdot x}$) where y is the speed of migration of nucleic acids (in mm/min) and x is the voltage applied to the agarose gel. In **Table 1** there are presented the values of coefficients **a** and **b** for the equations obtained for the plasmids used as samples. For the samples pYES2 and pCTCON2, in native state, that are supercoiled, the values of parameters **a** (quite similar with the slope in the case of linear regression line) are smaller than in the case of the same plasmids, but

analyzed after digestion with EcoRI. In these cases, the enzyme cuts the double helix and the nucleic acid chain become uncoiled.

From **Figure 2** one may see that when the voltage is increased from 80 to 160V, the speed of migration rise from 0.3 to 0.8 mm/min. At high voltage, the temperature of the gel can increase above 40ºC and the agarose gel can melt. Without a proper device to thermostat the system, the voltage cannot be increased above 180 V and, consequently, the velocity of the nucleic acids cannot be higher than 1 mm/min.

Table 1. Parameters of the exponential equations that express the dependence of the speed of migration of plasmids (mm/min) on the voltage applied to the agarose gel

Plasmid	a	b	R^2
pYES2 (undigested)	0.113	0.012	0.9986
pYES2 (digested)	0.147	0.011	0.9998
pCTCON2 (undigested)	0.114	0.012	0.9975
pCTCON2 (digested)	0.148	0.011	0.9997
Taq_pET22b+ (undigested)	0.145	0.011	0.9991
Taq_pET22b+ (digested)	0.145	0.011	0.9993

The experiments done with running buffers TBE and TAE have revealed that with TAE, the temperature of the gel is higher than with TBE, at the same buffer concentration. For this reason, the rest of experiments were performed in TBE. Another factor that was taken into consideration was the concentration of the running buffer. As it is depicted in Figure 3, the best results, here expressed as higher migration speed of the nucleic acid used as sample, were obtained when the concentration of the TBE running buffer was 0.5X.

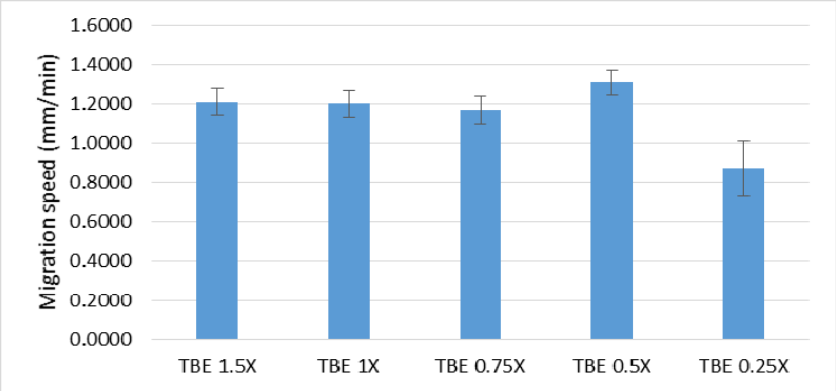

Figure 3 Migration speed of plasmid pCTCON2, digested with EcoRI, in different TBE concentrations

Considering only these two modifications, i.e. the use of 0.5 X TBE running buffer instead of 1X TBE or 1X TAE and a potential applied to the electrodes of 180 V, we have reduced the running time from 60 to 20 minutes, and, in the same time, we have decreased the price of the analysis with 25%. In the experiments yet to come, we will optimize other parameters of the agarose gel electrophoresis, aiming to increase the selectivity and further decrease the price of the analysis.

4. Conclusions

For the separation of some plasmids with chain length ranging between 5000 bp and 8000 bp the best results were obtained when 0.5X TBE running buffer was used. Using this buffer and a potential of 180V applied to the electrodes, the running time of separation was reduced until 20 minute. Beside a 3 times reduction of the time of electrophoretic separation, we have to consider a reduction with 25% of the price of analysis, when half of the concentration of normal buffer was used.

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