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Setting-up a Field Inversion Gel Electrophoresis System

D. FOLOGEA, TATIANA VASSU, ILEANA STOICA, ORTANSA CSUTAK, ELENA SASARMAN, DIANA SMARANDACHE, SIMONA SOARE, RALUCA GHINDEA, CRISTINA MORARU, ROBERTINA IONESCU, M. ATAMAN
MICROGEN-Center for Research in Genetics, Microbiology and Biotechnology, University of Bucharest, Faculty of Biology, Intr. Portocalelor 1-3, sector 5, 060101-Bucharest, Romania

Abstract

Electrophoresis is one of the best known methods for separating DNA molecules. Using an external static field, DNA electrophoresis can be performed up to 20-50 kbp, over this limit all DNA molecules showing the same mobility. In order to rise this limit more than 1 Mbp, a lot of experimental variants of Pulsed Field Gel Electrophoresis has been developed. In this paper a simple way to obtain a computer controlled Field Inversion Gel Electrophoresis is presented. Our device was tested with very good results on a commercial DNA marker.

Keywords: Field Inversion Gel Electrophoresis, Electrocariotyping

Introduction

Agarose gel electrophoresis represents one of the most powerful techniques used for DNA molecule separation [1]. Most applications use a single pair of electrodes to generate the electric field, constrained to be uniform and oriented in a single direction. The electrophoresis is obtained by placing DNA samples in a solid matrix and the molecules will migrate toward the anode by a process termed reptation. The separation depends on the sieving properties of the gel matrix. Unfortunately, in conventional electrophoretic separation of DNA molecules on agarose gel, the largest molecule that can be separated are about 20-50 kilobase pairs (kbp).Larger molecules than this limit will show the same mobility in static electric field and will co migrate as a unique lane in the gel.

In order to avoid this drawback, some special electrophoretic techniques have been carried out. These techniques are based on the observation that changing the electric field orientation will force the DNA relaxing and reorientation in a size dependent manner [2]. There are a lot of implementations of this technique, like FIGE (Field Inversion Gel Electrophoresis) [2, 3], CHEF (Contour Clamped Homogenous Electric Field) [4,5], OFAGE (Orthogonal Field Alternating Gel Electrophoresis), and TFAGE (Transverse Alternating Field Electrophoresis) [6].

Field Inversion Gel Electrophoresis seems to be the simplest way to achieve large DNA molecules separation, at least up to 1 Mpb. Basically, the electric field is switched to the opposite direction at certain moments. Every time the direction of the electric field is changed, the DNA will be forced to orientate along the new direction of the electric field. The reorientation process is size dependent, as well as the relaxation to the unperturbed state when the electric field is switched off [3, 6-9].

The aim of this paper is to present how to set up a simple and performant FIGE system, computer controlled, starting from a conventional electrophoresis system. Also, some results about large DNA separation using this device are presented.

Materials and Methods

Conventional Electrophoresis Equipment

A standard electrophoresis system (BioRAD, USA), containing a gel box, electrophoresis power supply, electrophoresis tank and cables have been used for our experiments. Actually, any horizontal submerse gel electrophoresis gel system is appropriate.

Computer Controlled Voltage Inversion

In order to obtain the electric field inversion, two relays are the most convenient solutions. The delay in electric field applications introduced by these relays is no more than 30 msec, an acceptable value. The relays will be wired as shown in Figure 1. Relays control will be achieved from the parallel port (LPT1) of a computer, through a small interface with role in separate the LPT port from relays. An optically isolated interface will be a better solution.

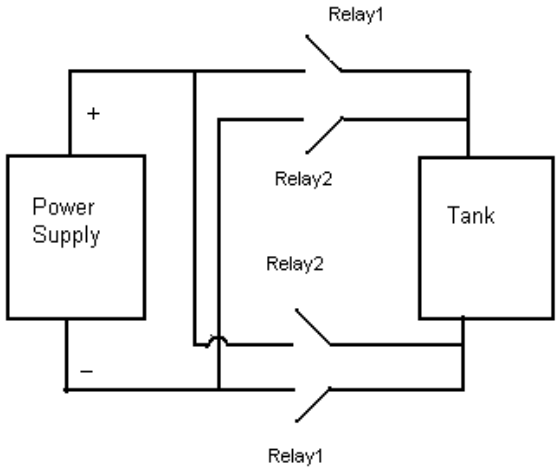

Figure 1. A simplified wiring diagram for relays

Anyway, the most important task is to command the relays from PC. In this respect, is necessary to write on LPT ports (DATA Bits) the values that we need, starting from LSB (Less Significant Bit). Only three different states have to be achieved: forward direction migration, reverse direction migration and pause (no migration). These states constitutes one cycle and the running time will consists from a lot of cycles, with different switching intervals (switch time ramping). These states and the action for them are presented in (**Table 1**) and will be obtained writing 1, 2 or 0 values to the LPT port.

Table 1. The necessary states of DATA Bits, relays, effects and number written on LPT

Bit 0 (LSB)	Bit 1	Relay1	Relay2	Effect	Number written on port
1	0	Close	Open	Forward	1
0	1	Open	Close	Reverse	2
0	0	Open	Close	Pause	0

Testing the FIGE System

In order to determine the size of large DNA molecules, we used a standard strain *S. cerevisiae* YPH80 (SIGMA), with known chromosomes number and size (**Table 2**). The electrophoresis gel has been prepared using special agarose for pulsed field running gel (SIGMA), 1% concentration, prepared in TBE 0.5X buffer (Tris 0.089M, boric acid 0.089M, EDTA 0.002M, pH=8). The total running time of 36 hours at 56 V (10 cm gel length) had the following steps: initial forward time (Fwi-10 sec), final forward time (Fwf- 160 sec), reverse time –Rev = 1/3 Fw, pause = 1/10 (Fw or Rev), linear switch time ramping.

Table 2. Chromosome number and length for *S. cerevisiae* YPH 80 (SIGMA)

Band no.	Chromosome	Size (kbp)
1.	XII	2200
2.	IV	1640
3.	VII	1120
3.	XV	1100
4.	XVI	945
5.	XIII	915
6.	II	815
7.	XIV	785
8.	X	745
9.	XI	680
10.	V	610
11.	VIII	555
12.	IV	450
13.	III	375
14.	VI	295
15.	I	225

Results and Discussions

In order to test the device functionality, a small computer program has been conceived, using BASIC® language. Usually, the LPT I/O address is 378H [10]. This can be checked on computers start, running BIOS Setup. Defining the port number as p = **3***16*16+**7***16+**8** (or the corresponding numbers –bolded- for a different I/O address), the instruction line for writing a number to LPT is:

OUT (P,n)

where n will be 1 (forward), 2 (reverse) or 0 (no electric field, pause).

The major advantage of such a device is that any switch time ramping will be available by software. Usually, linear, logarithmic or randomic ramps are used. In our case any combination of these are possible, including any pause interval. These characteristics are not common for FIGE commercial devices.

The other six DATA bits (not used) can allow to control, for example, a power supply with 64 voltage levels, in order to run Asymmetric Voltage FIGE or Zero Integrated Field Electrophoresis (ZIFE). From computer, any switch time interval can be established from 0.5 sec to 24 hours, covering any FIGE application. The total running time has no limit, but a FIGE experiment is lasting no more than few days.

The main results obtained for the commercial marker are presented in **Figure 2**. As shown, 10 chromosomes are separated using our running program.

Figure 2. Large DNA size molecules separated by FIGE using the home made device (starting from the top)

Conclusions

A full computer controlled FIGE system home made has a lot of advantages regarding switch time ramping or other parameters. Without using a thermostat or a buffer recirculation pump, the DNA can be separated up to 2 Mpb. We hope improving the software package and the experimental conditions to rise the separation limit up to 6 Mpb, in order to extend the elctrocariotyping capabilities of the device.

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