Field Inversion Gel Electrophoresis of Large DNA Molecules Extracted from a *Saccharomyces cerevisiae* Strain

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Abstract

Electrokaryotyping is one of the most recent and important methods for characterisation, and identification of yeast strains. Until now a great number of electrokaryotyping systems have been described, among which FIGE allow the separation of DNA molecules from 50 kbp up to 2 Mbp. Since such equipments have relatively high prices, we designed a new FIGE system based on apparatus already existing in our laboratory. This paper also describes an improved technique for obtaining intact chromosomal DNA molecules from a Saccharomyces cerevisiae S288c strain (ATCC 26108). We succeeded to obtain, by variation of electric field parameters, nine of the thirteen S. cerevisiae chromosomes normally isolated by FIGE, which allow us to consider that our system is highly competitive and can be used for obtaining data with taxonomical value.

Keywords: electrokaryotyping, FIGE, *Saccharomyces cerevisiae*, optimization, taxonomy

Introduction

An easier and more accurate characterization of chromosomes from lower eukaryotes (protozoa and fungi) was possible due to the emergence and development of specific electrophoretic techniques that allow the separation of intact chromosomal DNA molecules. Since classic citogenetic and genetic analysis are difficult to use, until recently, the only possibility for the differentiation and determination of chromosome number in fungi (e.g. yeasts), was the analysis of linkage groups. On the other hand, in yeasts, for example, a major impediment is the small size of chromosome and the persistence of nuclear membrane during mitosis and meiosis and their small size.

The idea of a possible electrophoretic separation of intact DNA molecules as an alternative for karyotyping by classical methods was possible only in 1982, when David

Schwartz constructed a new kind of electrophoretic system and showed that the electric mobilities of DNA molecules of some hundred base pairs strongly depend on their dimensions in the presence of two alternative electric fields. Design of pulsed field electrophoresis (PFGE) technique and the later modifications of this method allowed new ways of studying yeast chromosomes [3; 4], these data completing the more large classical information on yeast taxonomy [1; 9]. Also, Southern blotting on electrokaryotypes has greatly increased the molecular resolution in establishing the linkage groups in yeasts [13].

The first electrokaryotyping experiments on yeasts used *Saccharomyces cerevisiae* as experimental model [5; 8], and the 16 chromosomes of *S. cerevisiae* were separated in 13 electrophoretic lanes, due to the co-migration of some of the chromosomes with close dimensions (XIII and XVI, VII and XV, V and VIII) [12].

Nowadays, many types of systems for electrokaryotyping are available [2; 6; 7; 10], each presenting particular characteristics as their utilization is related to the dimensions of the chromosomes that are expected to be separated. The FIGE (Field Inversion Gel Electrophoresis) technique is highly recommended for the separation of chromosomal DNA molecules up to 2 Mbp [3]. As the electrokaryotyping systems have relatively high prices, we thought to design a new, highly competitive system based on apparatus that already exists in our laboratory. At the same time, we succeeded to improving the technique for obtaining intact chromosomal DNA molecules.

Materials and Methods

Yeast strains

The *S. cerevisiae* S288c strain (ATCC 26108) from the Microbial Collection of the Laboratory of Microbial Genetics and Biotechnology from the Faculty of Biology, University of Bucharest, was maintained by cultivation on YPGA medium (g/L yeast extract 10, peptone 10, glucose 20, agar-agar 20). In order to determine the size of DNA molecules isolated from *S. cerevisiae* S288c, we used a standard strain of *S. cerevisiae* YPH 80 (SIGMA) (**Table 1**).

Table 1. 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	IX	450
13	III	375
14	VI	295
15	I	225

Preparation of high molecular weight DNA

In order to obtain intact chromosomal DNA molecules we used a new technique based on those described by CARLE & al. [5] and SPENCER [11]. 5 mL yeast culture incubated for 18 – 20 h at 28ºC, in YPG medium, were centrifuged for 5 min at 6000 rpm, and the cells were washed twice with 1.5 mL solution EDTA 50 mM, pH 7.5 and then resuspended in 0.9 mL of the same solution. The suspension was mixed with 0.9 mL agarose low melting point 1% (prepared in sol. EDTA 50 mM, pH 7.5, at 45ºC). For the lysis of the cellular wall a solution of lyticase (10 mg/mL) was used. The mixture was purred in special agarose plugs and incubated for 15 min at 4ºC. The agarose plugs were then incubated for 48 h, at 37ºC with 10 mL solution EDTA 0.5 M and Tris-HCl 0.05 M, pH 7.5 and 750 μl β-mercaptoethanol. The plugs were incubated for 48 h, at 50ºC with sol. EDTA 0.5 M, pH 8, sarkosyl and proteinase K (20 mg/mL). After washing them twice with solution EDTA 0.5 M, pH 8, the plugs were incubated at 4ºC in the same solution.

Electrophoresis conditions

The specific electrophoresis conditions were established according to the expected sizes of the DNA molecules to be separated. For electrokaryotyping we used a system consisting in: **(1)** a 386 PC for programming the forward and reverse pulse time, the total running time and the number of cycles; **(2)** an apparatus for nucleic acids agarose electrophoresis (BIORAD); **(3)** an electric field invertor computer programmed, developed in our laboratory. The field inversor device is based on a small computer interface, having large capabilities in establishing the switch time ramp. A recirculation or thermostate system is not required.

We used special Agarose for Pulsed Field Electrophoresis Running Gel (SIGMA) concentration 1.5%, prepared in buffer TBE 0.5X (Tris 0.089 M; boric acid 0.089 M; EDTA 0.002 M, pH 8).

The electric conditions for electrophoresis were optimized throughout two experiments, using as starting point the conditions described by SPENCER & al. [11], for the separation of DNA molecules with sizes up to 2 Mbp.

(A) The first experiment had a total running time of 12 h at 65 V, with the following steps: initial forward running time (*Fwdi*) – 6 sec.; final forward running time (*Fwdf*) – 60 sec.; reverse running time (*Rvs*) – 1/3 *Fwdi*; pause for forward running (*P fwd*) – 1/10 *Fwdi*; pause for reverse running (*P rvs*) – 1/20 *Rvs*, linear switch time ramping.

(B) The second experiment had a total running time of 18 h at 53 V, with the following steps: *Fwdi* – 3 sec.; *Fwdf* – 60 sec.; *Rvs* – 1/3 *Fwdi*; *P fwd* – 1/10 *Fwdi*; *P rvs* – 1/20 *Rvs*, linear switch time ramping.

Results and Discussions

Preparation of high molecular weight DNA

The method used for preparation of high molecular weight intact chromosomal DNA was modified in different steps compared with those mentioned in the literature. The lysis of the cell wall and the membrane permeabilisation of the cells embedded in agarose plugs were done by incubation and washing with: **(1)** enzymes - for lyticase a final concentration of 1 mg/mL was established, **(2)** solutions (EDTA 0.5M and Tris-HCl 0.05M – pH 7.5) and **(3)** detergents - sarkosyl 1%. The inhibition of the activity of DN-ases was done using EDTA sol. 0.5M. For protein removal we used proteinase K in final concentration of 2 mg/mL .

Our method has the advantages of using fewer solutions, a shorter washing time and the fact that the samples (e.g the DNA plugs) can be stored at 4°C for 3 – 4 weeks without diminishing the integrity of DNA molecules.

Optimization of electrophoretic parameters

Our experiments aimed a good separation of chromosomal DNA molecules of different dimensions and obtaining of electrophoretic lanes with high resolution. Therefore, we made some improvements regarding the switching time ramping.

We performed more experiments using various parameters. For two of these experiments we obtained data similar to those described for *S. cerevisiae* in other papers.

(A) The first experiment resulted in seven bands (**Figure 1**). The comparative study of these bands with those described by SPENCER & al. [11], revealed that six of the bands correspond to chromosomes with sizes up to 700 kbp: *I* - 245 kbp; *VI* - 280 kbp; *III* - 360 kbp; *IX* - 450 kbp; *V* and *VIII* (that comigrate due to close dimensions) - 555 kbp; *XI* - 680 kbp. The seventh band (named *C*) consists in chromosomal DNA molecules that have not yet separated in these electrophoretic conditions.

These results confirmed the method used for preparation of DNA molecules and provided new data regarding the variation of electric parameters.

(B) In the second experiment we modified the electric parameters for obtaining larger chromosomes. Thus: (i) the pulse time was increased for allowing the larger DNA molecules to reorientate in the electric field; (ii) the total migration time was increased; (iii) the voltage was decreased. The variation of these parameters allowed a better separation of molecules larger than 700 kbp and a better resolution of bands.

We obtained 8 bands (**Figure 2**), from which seven were identified as chromosomes: (1) the first seven chromosomes obtained also in the previous experiment (*I, VI, III, IX, V* and *VIII, XI*); (2) the chromosome number *X* – 7450 kbp. The 8th band (named *C*) corresponds to chromosomal DNA molecules that did not separate under these electric conditions.

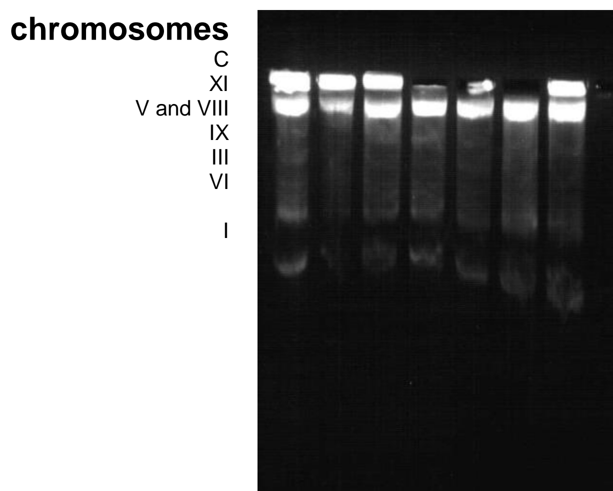

Figure 1. Electrophoretic pattern of *S. cerevisiae* S288c chromosomes.

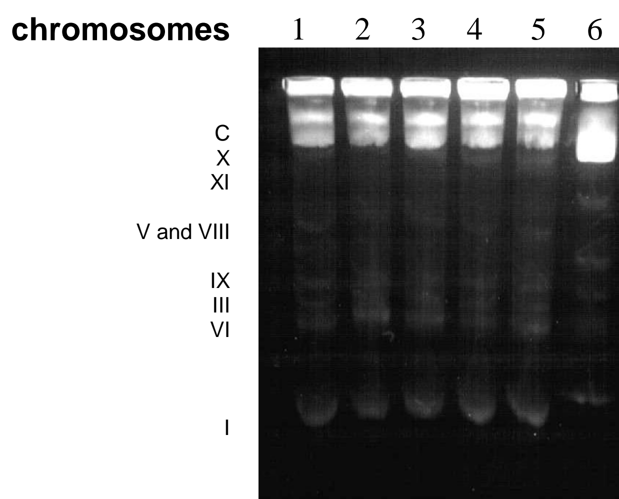

Figure 2. Electrophoretic pattern of *S. cerevisiae* chromosomes (lane: 1-5 - *S. cerevisiae* S288c, 6 - *S. cerevisiae* YPH80).

Conclusions

Our results can have major impact, as this electrokaryotyping system is as well competitive as well as accurate in providing taxonomical data. We underline that the system that we designed allows electrophoretic separation of nine out of the sixteen chromosomes from *S. cerevisiae* strains. We are expecting even better results using a recirculation and thermostat system.

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