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Comparison of DNA migrations in two clamped homogeneous electric field chambers of different sizes Relation between sample thickness and electrophoresis time

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Abstract

We present here a method to compare the mathematical descriptions of DNA migration per pulse as a function of pulse time. It is based on obtaining robust estimates and variances of DNA reorientation time, migration velocities during and after DNA reorientation; and on the statistical comparisons of these estimates. We demonstrated an equal description for the migration per pulse of each DNA molecule separated under identical conditions in clamped homogeneous electric field (CHEF) and miniCHEF chambers. However, miniCHEF resolved the patterns in shorter times, because it uses thinner samples. The relationship between sample thickness and CHEF run time is also presented. © 1998 Elsevier Science B.V.

Keywords: Clamped homogeneous electric field; Migration velocities; DNA reorientation time; Sample thickness; Pulse parameters; Chromosomes; DNA

1. Introduction

The duration of pulsed field gel electrophoresis (PFGE) runs should be the time required to separate DNA molecules in well-defined bands. It depends on the DNA size range to be separated and on the selected experimental conditions [1]. Recently, we designed and constructed miniCHEF (CHEF= clamped homogeneous electric field) and miniTAFE (TAFE=transverse alternating field electrophoresis) apparatus to separate high-molecular-mass DNA at higher electric fields in shorter run times [2]. Mini equipments were constructed by reducing the distance between the opposite electrode pairs but maintaining the original relations between gel lengths and the electrode array dispositions constant [2]. These small chambers require thinner samples and less buffer volume [2,3]. A recent study demonstrated that the miniCHEF chamber can be used to distinguish circular and linear DNA molecules in 4 h [4], indicating that it could support common CHEF applications.

Reduction of the electrophoresis time is especially useful to perform experiments which require several PFGE runs; in particular, to resolve the molecular karyotypes of complex genomes, or to study factors

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affecting DNA migration. Microscopy studies of DNA migration in gel electrophoresis [5,6] suggest that the behaviour of DNA molecules does not depend on the dimensions of the electrode platform. However, it remains to be determined if DNA molecules migrate and are resolved similarly in PFGE chambers of different sizes under the same electrophoretic conditions.

Maintaining constant the driving and frictional forces in a CHEF [7] chamber, the distances travelled by a molecule in the gel depend on the pulse times and the number of pulses, whereas the migrations per pulse [8] should be related only to the pulse times. Therefore, to contrast the migrations of a DNA molecule in CHEF and miniCHEF, it is sufficient to compare the migrations per pulses at different pulse times. The migration per pulse of a DNA molecule as a function of pulse time is mathematically described by three parameters: the molecule reorientation time (t_r) and the migration velocities during and after DNA reorientation (v_r and v_m, respectively) [9,10]. Then, if a molecule has the same t_r, v_r and v_m in CHEF and miniCHEF, it will migrate in large gels and minigels the same distances at each combination of pulse time and number of pulse.

The resolution of the molecules depends on the migrated distances and band widths [11]; and at constant electric field, temperature and DNA concentration [1], the band width is related to the insert slice size [12]. Thus, in CHEF and miniCHEF different patterns should be resolved for identical driving forces, pulse times and number of pulses; and different run times should be required to similarly resolve the patterns.

Here, we studied if the *Saccharomyces cerevisiae* chromosomes migrated equal distances during a pulse in CHEF and miniCHEF gels under identical pulse time, driving forces and frictional forces. To perform these comparisons, we obtained robust estimates and variances of the t_r, v_r and v_m of each chromosome separated in both chambers, and compared them. We also studied the resolution patterns of these molecules in both chambers at identical driving force, frictional force and pulse time. Finally, we verified if samples of different thicknesses required distinct run times to give a particular resolution pattern. To carry out this, we coelectrophoresed yeast chromosomes embedded in plugs of different thicknesses. Sample thickness has not been considered as a factor influencing PFGE run times. In the data obtained here, we show that the sample thickness affects the electrophoresis time.

2. Hypothesis and statistical procedures

At any given pulse time, the resolution between DNA molecules of sizes Z_j and Z_{j+1} is $R_{j,j+1}$, where $R_{j,j+1}=|D_j-D_{j+1}|/b_w$ [11], D is the migrated distance, b_w the band width, j indexes any molecule, and $j+1$ the next molecule in the pattern. Taking $D=dN_p$, where d is the migration per pulse [8,7] and N_p the number of pulses, $R_{j,j+1}$ can be expressed as:

$$R_{j,j+1} = N_p|d_j - d_{j+1}|/b_w \quad (1)$$

2.1. Hypothesis on DNA migration and resolution in CHEF and miniCHEF

In a CHEF chamber the migrations per pulse of a molecule should depend exclusively on the driving force, the frictional force and the pulse time. Thus, under a selected combination of run parameters in CHEF and miniCHEF chambers the migrations per pulse of the molecules (d_j) at any pulse time must be independent on the chamber dimensions. If we demonstrate the above proposition, then, according to Eq. (1), the variation introduced in the number of pulses (N_p) to obtain similar resolution patterns in these two chambers is attributable to the differences in the band widths, because the width of the bands depends on the plug thicknesses and CHEF and miniCHEF use plugs of different thicknesses [2,3].

2.2. Identification of the main variables to test the hypothesis

To statistically prove the hypothesis exposed above, we will use the following identification for the variables: YC will be the yeast chromosomes separated by CHEF and miniCHEF electrophoresis. The smallest chromosome will be identified as YC_1, the next molecule in the pattern as YC_2, and so on. The slow migrating chromosome in the patterns will be named YC_p. The variable j will be used to refer

the values of the subscripts; thus j ranges from 1 to p, and p is the number of molecules resolved in each run. In each CHEF experiment the DNA molecules will be electrophoresed at a given pulse time (t_p) for a particular number of pulses (N_p). Thus, in the experiment using the smallest pulse time we have the combination (t_{p1}, N_{p1}), in the next experiment we will have (t_{p2}, N_{p2}), until the last run, when we will have (t_{pm}, N_{pm}), where m is the number of CHEF and miniCHEF runs. We will use the variable i to index t_p and N_p and to refer the values of these subscripts. For each chromosome (YC_j) we will have an array of migrated distances in the gels (D_{ji}) at different pulse times (t_{pi}), and the corresponding migrations per pulse (d_{ji}). The reorientation time (t_r) of a molecule (YC_j), the migration velocity during the molecule reorientation (v_r) and after it (v_m) will be called t_{rj}, v_{rj}, v_{mj}. To identify the data or results obtained using miniCHEF or CHEF chambers, we will use the variable name M or L, respectively, that is $d(M)_{ji}$ and $d(L)_{ji}$, $t_r(L)_j$, $t_r(M)_j$, etc. By identical electrophoretic conditions in CHEF and miniCHEF chambers we mean the use of the same electric field, temperature, agarose concentration and buffer ionic strength.

2.3. A method for the simultaneous comparisons of the migrations per pulse at various pulse times

For the molecules YC_j and YC_{j+1} separated in CHEF and miniCHEF chambers under identical conditions, at pulse time t_{p1}, for the number of pulses N_{p1}, their corresponding migrations per pulse d_{j1} and $d_{(j+1)1}$ would be

$$\left[d(L)_{j1} = d(M)_{j1}\right] \text{ and}$$
$$\left[d(L)_{(j+1)1} = d(M)_{(j+1)1}\right] \text{ at } t_{p1} \quad (2)$$

Eq. (2) means that at t_{p1} $[d(L)_{j1} - d(L)_{(j+1)1}] = [d(M)_{j1} - d(M)_{(j+1)1}]$. Therefore, for a number of DNA molecules p (j varies from 1 to p) separated in CHEF and miniCHEF under different m pulse durations (t_{p1}, t_{p2},.., t_{pm}; i varies from 1 to m), for the number of pulses (N_{p1}, N_{p2},.., N_{pm}) we have to prove that

$$\left[d(L)_{ji} - d(M)_{ji}\right] = 0$$

for all molecules (YC_j) at all pulse times (t_{pi}) (3)

The migration per pulse (d) is a function of t_r, v_r and v_m. In the complete interval of pulse time, d is described [9,10] by

$$d = \nu_r t_p \text{ for } t_p \le t_r \quad (4)$$

$$d = \nu_m (t_p - t_r) \text{ for } t_p > t_r \quad (5)$$

Thus, the hypothesis proposed in Eq. (3) will be proved using the experimental data of migrations per pulse (d_{ji}) obtained at different pulse times (t_{pi}), [$d(L)_{ji}$, t_{pi}] and [$d(M)_{ji}$, t_{pi}], estimating t_{rj}, v_{rj} and v_{mj} for each YC_j, and statistically demonstrating that $[v_r(L)_j - v_r(M)_j] = 0$, $[v_m(L)_j - v_m(M)_j] = 0$ and $[t_r(L)_j - t_r(M)_j] = 0$. If we estimate the variances (S^2) of the parameters, $S^2 v_{rj}$, $S^2 v_{mj}$ and $S^2 t_{rj}$ (for data coming from both chambers), we can calculate the t-statistic [13] and perform the comparisons. Calling the parameters P_r and their variances $S^2 P_r$ we have for t

$$t = \left|\left[P_r(L)_j - P_r(M)_j\right]\right| / \left\{\left[S^2 P_r(L)_j / m(L)\right] + \left[S^2 P_r(M)_j / m(M)\right]\right\}^{0.5} \quad (6)$$

2.4. Determination of DNA reorientation times and migration velocities

Under constant electrophoretic conditions, but varying the pulse time, the migration per pulse of the YC_j molecule is described in the complete interval of pulse time by two distinct linear functions of pulse duration [9,10]. In CHEF and miniCHEF experiments, we obtained for each YC_j molecule sufficient ordinate pairs [(d_{ji}, t_{pi}), $i = 1...m$] to fit these two functions (for simplicity, the symbols for the identification of the chambers are not used here). Using a regression method, we related them during reorientation as:

$$d_{ji} = (a_0)_j + (a_1)_j t_{pi}$$

for given j, and i ranging between 1 and lim (7)

and after reorientation as:

$$d_{ji} = (b_0)_j + (b_1)_j t_{pi}$$

for given j, and i ranging between lim + 1 and m'' (8)

where $(a_0)_j$ and $(a_1)_j$ and $(b_0)_j$ and $(b_1)_j$ are the

regression coefficients of Eqs. (7) and (8), respectively (the migration per pulse of each molecule has two slopes, see examples in Fig. 1). Eq. (7) is valid from t_{pj1} to $t_{pj(\lim)}$, and Eq. (8), from $t_{pj(\lim+1)}$ to $t_{pj(m'')}$. The $t_{pj(\lim)}$ is the pulse time that marks the end of reorientation [10], and $t_{pj(m'')}$ is the last pulse time after the reorientation linearly relating the array (d_{ji} and t_{pi}) (always $\lim < m'' \leq m$). By analogy of Eqs. (7) and (4), $v_{rj} = (a_1)_j$ [10]. Rearranging Eq. (5) we have

$$d = -v_m t_r + v_m t_p$$

By analogy between Eq. (8) and the above equation $v_{mj} = (b_1)_j$ and $t_{rj} = -(b_0)_j/(b_1)_j$ [9]. The variances of the regression coefficients ($S^2(a_1)_j$ and $S^2(b_1)_j$) are the variances of the velocities v_{rj} and v_{mj}, respectively, while t_r variance ($S^2 t_{rj}$) is approached here by

$$S^2 t_{rj} = [(b_0)_j/(b_1)_j]^2 \left[(S^2(b_0)_j/(b_0)_j^2 + S^2(b_1)_j/(b_1)_j^2 \right]$$

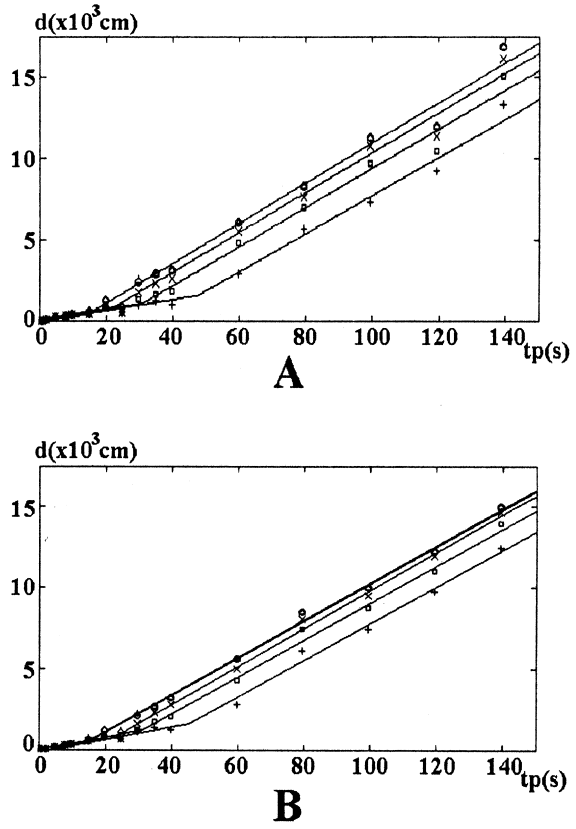

Fig. 1. Plot of the migration per pulse (d_{ji}) as a function of pulse time (t_{pi}) in miniCHEF (A) and CHEF (B) for yeast chromosome V (+), IX (□), III (×), VI (○) and I (△). All experiments were done in 1.5% agarose, 0.5×TBE buffer, 20°C, at 5.8 V/cm and for an electrophoresis run time of 7 h.

2.5. Robust regression and variance estimations

Data outliers are commonly found in experimentation and they have deleterious effect on the estimates of the coefficients of the regression equations. To avoid this effect on t_r, v_r, and v_m estimates and on the final statistical comparisons, we modified the original calculation procedure [9,10] using a robust regression method instead of the classical least squares. We obtained the *M*-estimates of the regression coefficients (generically called here c_0, c_1, the intercept and slope, respectively) and their variances ($S^2 c_0$ and $S^2 c_1$). These *M*-estimates were obtained as previously described [14] and are the arguments that minimized the summation of the absolute deviations E_i. To facilitate comprehension we will not use the chamber identification here, and for a molecule YC_j that has the array of migrations per pulse and pulse times (d_{ji}, t_{pi}) we will rewrite this array without the subscript j as (d_i, t_{pi}). Then c_0 and c_1 are

$$(c_0, c_1) = \arg\min \Sigma |E_i|$$

where $E_i = d_i - (c_0 + c_1 t_{pi})$; i varies between 1 and n; and n is the number of ordinate pairs to fit each regression equation. Here, $n = \lim$ when $t_p \leq t_r$ and $n = m'' - (\lim + 1)$ when $t_p > t_r$. Each coefficient of variance $S^2 c_k$ (where $k = 0, 1$) was calculated considering that

$$S^2 c_k = t_{pkk}^{-1}/4[f(0)]^2$$

where t_{pkk}^{-1} are the diagonal elements of the inverse of the design matrix [15–17]. Assuming for error distribution the function $g_\lambda(E)$

$$g_\lambda(E) = e^{-(E/\lambda)^2}/\left[\lambda/(2\pi)^{0.5}\right]$$

we have for $f(0)$

$$f(0) = \left[\Sigma g_\lambda(E_i)\right]/n, \text{ for } i \text{ between 1 and } n$$

Here, λ is estimated as the argument that maximized the function.

$$\lambda = \arg\left\{\max\left[\log f_{\lambda}^{(-i)}(E_i)\right]\right\},$$

for i between 1 and n; and $\lambda > 0$

where $f_{\lambda}^{(-i)}(E_i)$

$$f_{\lambda}^{(-i)}(E_i) = [\, g_{\lambda}(E_i - E_w)\,]/(n-1)$$

for all i; w between 1 and n, $w \neq i$

Calculations of c_k [15], λ, $S^2 c_k$ were done by programming iterative algorithms which used least square estimates as initial values.

3. Experimental

3.1. CHEF and miniCHEF experiments for data acquisition

Saccharomyces cerevisiae (strain 196-2, α, his-, a kind gift of M. Luzzati, France) chromosomes were simultaneously separated in our CHEF and miniCHEF laboratory-made chambers [7,18,2] by alternating the electric fields with a common switching unit. The switching unit is commanded by the hardware and software that automatically sets and checks the pulse time and the number of pulses [19]. Agarose plugs (0.85×0.85×0.2 cm, length×width×thickness), containing yeast DNA ($6.25 \cdot 10^9$ cells/ml), were prepared as described [20–22]. Half sectioned plugs, maintaining the original slice thickness (0.2 cm), were used in CHEF experiments, whereas, sectioned plugs having half of the original thickness (0.1 cm) were used in miniCHEF experiments. One dimension of the plug matches the slot deepness, the other is related to the width of the comb teeth, whereas the smallest dimension, that corresponds to the teeth's thickness, is what we have called the sample thickness. The smallest dimension can be visualized in the gel photographs. As a control we also cast samples in a special mold (0.25×0.4×0.1 cm) that generates 0.1 cm thick samples [3].

To compare DNA migrations, in some experiments the samples were electrophoresed in CHEF and miniCHEF chambers under the same pulse times in 1.5% agarose gels, 0.5×TBE (1×TBE: 89 m*M* Tris, 89 m*M* boric acid, 2 m*M* EDTA, pH 8.3), at 20°C, 5.8 V/cm, for 7 h [the used pulse times (t_{pi}) are in Fig. 2]. Additional CHEF and miniCHEF experiments were done to quantify the run times required to achieve a given resolution pattern of yeast chromosomes. We ran large gels and minigels until similar well-resolved patterns were obtained.

Other experiments were done to study the effect of sample thickness on the electrophoresis times in miniCHEF chambers. To cast the samples of 0.200, 0.160, 0.135, 0.100 and 0.070 cm thick, PTFE bars of these thicknesses were placed between two microscope slides. We sealed them with saran wrap, pressed each mold with clamps and finally cast the samples. All samples had $6.25 \cdot 10^9$ cells/ml. To validate the different N_p values (according to the relation N_p/b_w or N_p/t_h; t_h: sample thickness) that these samples required to give similar resolutions between the molecules, we coelectrophoresed the

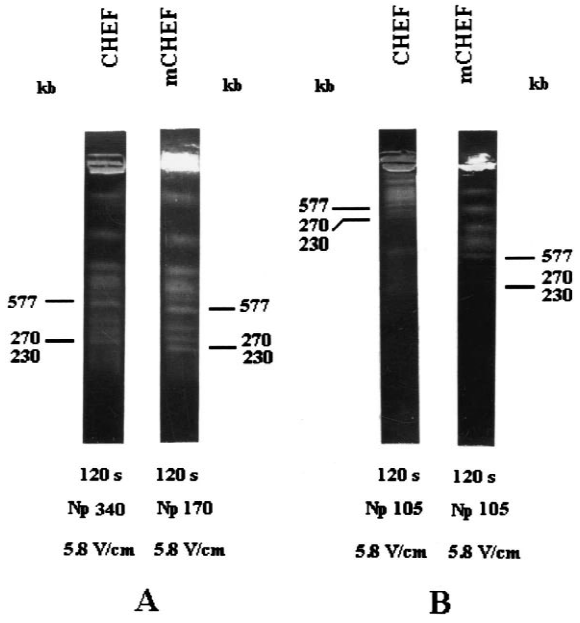

Fig. 2. Electrophoretic patterns of *S. cerevisiae* chromosomes at 120 s of pulse time, 20°C and 5.8 V/cm. They were obtained in 1.5% agarose gel, 0.5×TBE in CHEF and miniCHEF chambers for different run times. (A) 11 h and 20 min for miniCHEF and 22 h and 40 min for CHEF; (B) 7 h for both methods. Photography magnification factor (ratio between the gel length in the photographs and its real length): (A) miniCHEF 1.438, (B) miniCHEF 1.589. Photography reduction factor (ratio between the real gel length and its length in the photographs): (A) CHEF 1.364, (B) CHEF 1.411.

samples with different thicknesses in the miniCHEF chamber at 50 s of pulse time as follows: in lane number one of the gel we loaded at zero time the 0.200 cm plug, which was electrophoresed for 172 pulses; then we stopped the run and loaded the 0.160 cm plug in the second lane. Both plugs were coelectrophoresed for 108 pulses; then we stopped the run again, and loaded the 0.135 cm plug in the third lane. We continued the coelectrophoresis for 151 pulses and stopped it to load the 0.100 cm plug in the fourth lane. Then, we applied 129 pulses, stopped the run again and loaded the 0.070 cm plug in the fifth lane. The coelectrophoresis ended after a total of 861 pulses. In this way the samples of different thicknesses were coelectrophoresed for distinct N_p in a single gel. To assure constant ionic strength and pH during the coelectrophoresis, every hour we replaced 50 ml (12.5%) of the buffer in the chamber with fresh buffer. The gels were stained with ethidium bromide (0.5 μg/ml) and photographed.

3.2. Initial data processing and software

The photographs were scanned by a Quick Scan Densitometer (Desaga, Heidelberg, Germany) and the peaks were manually digitised using a graphic tablet (Apple II, graphic tablet). The band width (b_w) and the band position (D) were determined by fitting the heights (y) of the peaks to a Gaussian function of the positions in the gels (x). By the log–log transformation (natural logarithms) of the variables, we have the function

$$\ln y = \left(\ln\left\{1/\left[\sigma(2\pi)^{0.5}\right]\right\} - \mu^2/\sigma^2\right) + \left(2\mu/\sigma^2\right)x - \left[1/\sigma^2\right]x^2$$

that can be described by a second degree polynomy of log y and powers of x. Then, we calculated $b_w = 2(-1/a_2)^{0.5}$, and $D = -a_1/(2a_2)$; where a_1 and a_2 are the slopes of the polinomy.

Migrations per pulses of the yeast chromosomes I, VI, III, IX and V of 230, 270, 315, 440 and 577 kilobase pairs (kb), respectively [23] were calculated at each pulse time (t_{pi}) as the migrated distance in the gel (D_{ji}) divided by the corresponding number of pulses (N_{pi}) ($d_{ji} = D_{ji}/N_{pi}$). From CHEF and miniCHEF experiments performed at different pulse times (a total of m runs), and for each YC_j (each chromosome), we obtained $[d(L)_{ji}, t_{pi}]$ and $[d(M)_{ji}, t_{pi}]$ and used them to estimate $t_r(L)_j$, $v_r(L)_j$, $v_m(L)_j$, $t_r(M)_j$, $v_r(M)_j$, $v_m(M)_j$ and the respective variances (the index j varies from 1 to 5). The resolutions ($R_{j,j+k}$; where k is an integer between $j+1$ and p) were calculated according to Eq. (1).

Public domain and laboratory-written software were used to perform calculations. The latter was written in Turbo-Pascal, version 7.0, running on Windows or MS-DOS operating system for an AcerMate 466d IBM compatible microcomputer.

4. Results and discussion

4.1. Similar migrations per pulse of DNA molecules under the same electrophoretic conditions in CHEF and miniCHEF

Graphically, the functions relating DNA migration per pulse of yeast chromosomes with the pulse times displayed similar shapes in the complete interval of pulse time for CHEF and miniCHEF data, as is shown in Fig. 1A (MiniCHEF) and Fig. 1B (CHEF). The pairs $[d(L)_{ji}, t_{pi}]$ and $[d(M)_{ji}, t_{pi}]$ obtained for each chromosome (YC_j) in CHEF and miniCHEF experiments were fitted to Eqs. (7) and (8). For all molecules, the robust regression coefficients $(a_1)_j$, $(b_0)_j$, and $(b_1)_j$ of the straight lines, calculated during and after the molecule reorientation, differed from zero, whereas $(a_0)_j$ was equal to zero (significant level of 0.05). From the results of the regression analyses, we determined t_{rj}, v_{rj} and v_{mj} and the variances for each molecule in both chambers (Table 1) (the data of Table 1 permit the calculations of the regression coefficients according to their relations to t_{rj}, v_{mj} and v_{rj}). The statistical comparisons between the parameters obtained in CHEF and miniCHEF for each chromosome revealed that $[t_r(L)_j - t_r(M)_j] = 0$, $[v_r(L)_j - v_r(M)_j] = 0$ and $[v_m(L)_j - v_m(M)_j] = 0$ for most of the studied molecules (significant level of 0.05. Table 1). The t_r and v_m obtained for the 230 kb yeast chromosome in both chambers significantly differed. However, aside from this difference, that was due to the presence of a leverage point in the 230 kb data (M-estimates of the regression coefficients are affected by leverage points), the results

Table 1
Comparisons between the reorientation times and migration velocities of *S. cerevisiae* chromosomes in CHEF and miniCHEF

Parameters (Pr)	DNA sizes (kb)									
	230 ($j=1$)		270 ($j=2$)		315 ($j=3$)		440 ($j=4$)		577 ($j=5$)	
	mCHEF	CHEF	mCHEF	CHEF	mCHEF	CHEF	mCHEF	CHEF	mCHEF	CHEF
v_r	4.09	4.24	4.09	3.61	4.35	3.61	4.27	4.09	3.32	3.74
($\cdot 10^5$ cm/s)	$m=4$	$m=4$	$m=4$	$m=8$	$m=5$	$m=8$	$m=7$	$m=7$	$m=10$	$m=10$
S.D.	$1.12\cdot10^{-7}$	$4.04\cdot10^{-6}$	$1.12\cdot10^{-7}$	$1.40\cdot10^{-5}$	$7.87\cdot10^{-6}$	$1.67\cdot10^{-6}$	$6.90\cdot10^{-6}$	$3.11\cdot10^{-6}$	$4.59\cdot10^{-6}$	$4.57\cdot10^{-6}$
P(t) for H_0	0.73		0.74		0.38		0.82		0.53	
v_m	11.99	10.62	12.18	10.90	11.54	10.83	11.56	10.56	10.32	10.31
($\cdot 10^5$ cm/s)	$m=12$	$m=10$	$m=12$	$m=6$	$m=10$	$m=6$	$m=8$	$m=8$	$m=6$	$m=6$
S.D.	$5.59\cdot10^{-6}$	$1.85\cdot10^{-6}$	$6.49\cdot10^{-6}$	$9.85\cdot10^{-6}$	$8.28\cdot10^{-6}$	$1.07\cdot10^{-5}$	$9.76\cdot10^{-6}$	$5.87\cdot10^{-6}$	$1.89\cdot10^{-5}$	$5.31\cdot10^{-6}$
P(t) for H_0	0.03		0.29		0.61		0.40		0.99	
t_r	9.81	8.29	10.57	10.59	14.35	14.63	20.05	18.82	30.17	28.16
(s)	$m=12$	$m=10$	$m=12$	$m=6$	$m=10$	$m=6$	$m=8$	$m=8$	$m=6$	$m=6$
S.D.	0.58	0.18	0.69	1.18	1.15	1.63	1.82	1.13	5.79	1.53
P(t) for H_0	0.02		0.99		0.89		0.58		0.74	

t_r, v_r and v_m were determined under the conditions described in Fig. 2. m=Number of experimental points; S.D.=$\sqrt{S^2}$; and P(t) for H_0 is the probability of $[P_r(M)_j - P_r(L)_j]=0$.

confirmed, with 0.95 certain probability, that with an identical pulse time, driving force and frictional force, each molecule moves at an equal velocity in CHEF and miniCHEF; and it spends the same time for reorienting itself. Therefore, under similar conditions, the migration per pulse of each yeast chromosome in CHEF and miniCHEF chambers has a common mathematical description as a function of pulse time; and thus, the chromosome migrated the same distances during the application of a pulse. Eq. (3) was proved for most of the studied molecules (YC_j).

The results justified that migration data obtained in large and mini chambers can be analyzed together to determine how run factors affect DNA migration [24]. We also provided a method to avoid the influence of data outliers in t_r, v_r and v_m estimations. The calculation of parameter variances also permitted the development of a procedure to strictly compare DNA migration per pulse in a wide interval of pulse times.

4.2. DNA molecules are resolved in a shorter time in miniCHEF than in CHEF under the same driving force and pulse time

To obtain almost identical patterns of *S. cerevisiae* chromosomes in CHEF and miniCHEF under equal pulse time (120 s) and electrophoretic conditions (5.8 V/cm, 20°C, buffer TBE×0.5, 1.5% agarose) we used different run times (Fig. 2A). Fig. 2A shows the patterns obtained in miniCHEF and CHEF at 170 and at 340 pulses, magnified (by a factor of 1.438) or reduced (by a factor of 1.364), respectively, to overlap them. Resolutions between the YC_j and YC_{j+1} (or YC_{j+k}) molecules were similar in both chambers, giving an average resolution ratio [R(M)/R(L)] close to one (Table 2, upper section). Despite we obtained similar resolutions in these gels (Fig. 2A), each chromosome migrated different distances in both chambers (see in the upper part of Table 2 the distances migrated by chromosome V in CHEF and miniCHEF). Using the above experimental conditions but identical run times (105 pulses), the patterns obtained in CHEF were poorly resolved, whereas the ones obtained in miniCHEF were better defined (Fig. 2B). The resolution ratios [R(M)/R(L)] between the YC_j and $YC_{(j+1)}$ (or YC_{j+k}) molecules were greater than 1.5, but each particular chromosome migrated the same distance in both chambers. In the CHEF and miniCHEF gels of Fig. 2B, the 270 kb molecule migrated 1.25 and 1.25 cm; the 315 kb molecule 1.22 and 1.18 cm; and the 440 kb molecule 1.12 and 1.10 cm, respectively. These results evi-

Table 2
Resolutions ($R_{j,j+k}$) between the YC_j and YC_{j+k} yeast chromosomes in two different experiments

N_p	t_h	D_{577kb}	$R_{j,j+k}$ of simultaneous CHEF (L) and miniCHEF (M) runs at 120 s of pulse time									
			CZ-577	CZ-440	CZ-315	CZ-270	577-440	577-315	577-270	440-315	440-270	315-270
(L) 340	0.2	3.14	12.26	14.25	15.39	16.61	2.09	3.14	4.36	1.05	2.27	1.22
(M) 170	0.1	1.68	12.65	14.87	16.09	17.30	2.22	3.44	4.65	1.22	2.43	1.21
Ratio R(M)/R(L)			1.03	1.04	1.05	1.04	1.06	1.10	1.07	1.16	1.07	0.99
			$R_{j,j+k}$ of MiniCHEF run at 50 s of pulse time									
861	0.200	1.53	**3.23**	9.38	–	–	**6.15**	–	–	–	–	–
689	0.160	1.18	2.58	9.27	11.93	14.14	6.68	9.34	11.56	2.66	4.87	2.22
581	0.135	1.06	2.58	9.80	12.60	14.70	7.22	10.02	12.12	2.80	4.90	2.10
430	0.100	0.85	2.66	9.75	12.58	14.89	7.09	9.92	12.23	2.84	**5.14**	2.30
301	0.070	0.59	2.28	**9.03**	11.73	13.76	6.75	9.45	11.48	2.70	4.73	2.03
	Mean	$\bar{R}_{j,j+k}$	2.67	9.45	12.21	14.37	6.78	9.68	11.85	2.75	4.91	2.16
	Variance	S^2R	0.121	0.107	0.199	0.270	0.174	0.114	0.146	0.007	0.029	0.015
	Mean S.E.	MSE	0.155	0.146	0.223	0.259	0.187	0.169	0.191	0.042	0.085	0.603
	Relative standard deviation	R.S.D. (%)	5.8	1.5	1.8	1.8	2.8	1.7	1.6	1.5	1.7	2.8

The upper section shows the results of the simultaneous CHEF and miniCHEF runs. The lower section shows the resolutions achieved in miniCHEF by coelectrophoresing samples of different thicknesses (t_h) for different numbers of pulses (N_p).
D_{577kb} is the distance migrated in the gels by chromosome V of yeast; YC_1, YC_2, YC_3, YC_4 and YC_5 are the compression zone (CZs) of the 577, 440, 315 and 270 yeast chromosomes, respectively. R(M)/R(L) is the resolution ratio of the molecules separated in miniCHEF and CHEF. Electrode distances in CHEF and miniCHEF apparatus are 28.4 and 11.2 cm, respectively. Significant differences between $R_{j,j+k}$ and the mean are printed in bold face.

denced that identical run durations, pulse times and electrophoretic conditions do not give similar patterns in these chambers. We proved that $d(L)_{ji} = d(M)_{ji}$ at all (t_{pi}) assayed. As $D_{ji} = d_{ji}N_{pi}$, the distances migrated by a yeast chromosome in miniCHEF and CHEF chambers for 170 and 340 pulses (Fig. 2A), respectively, are necessary different, whereas for 105 pulses (Fig. 2B) the chromosome has to migrate the same distances in both chambers.

We have also demonstrated that using different N_p similar resolutions were obtained in both chambers (Fig. 2A). The electric field (5.8 V/cm), temperature (20°C), ionic strength and agarose concentration in CHEF and miniCHEF were similar, therefore, the above results are independent of these factors. The samples loaded in the gels had identical DNA concentration, but they differed in their thicknesses (0.2 and 0.1 cm for CHEF and miniCHEF, respectively); thus, replacing in Eq. (1) our previous results (see that $[d(L)_{ji} - d(L)_{(j+1)i}] = [d(M)_{ji} - d(M)_{(j+1)i}]$), it is reasonable that to obtain similar resolutions ($R_{j,j+1}$ or $R_{j,j+k}$) in CHEF and miniCHEF; N_p must vary proportionally to the band widths of the patterns and thus, to the thicknesses of the samples. It is known that changes in the insert slice size modify band width [12] and resolution [11], but sample thickness had not been considered a variable that affects PFGE run time. The data obtained from our experiments suggested this effect.

4.3. Relationship between the number of pulses and sample thicknesses to obtain a given resolution in the CHEF system

Based on Eq. (1), we approached the relationship between the thicknesses of the plugs and the number of pulses. To achieve constant $R_{j,j+k}$ by coelectrophoresing the DNA embedded in samples of different thicknesses, the ratio N_p/b_w has to be maintained constant. For a particular DNA concentration the band widths are proportional to the sample thicknesses; thus, we can use N_p/t_h instead of N_p/b_w. This is simple because N_p/t_h is known at the beginning of the experiment. Using this ratio and taking the electrophoresis time (t_e) as $t_e = 2t_pN_p$, we

can predict how sample thickness would influence PFGE run time.

To validate these predictions, we separated yeast chromosomes in the miniCHEF chamber under the conditions described in Fig. 3. DNA samples of identical concentrations, but immobilized in plugs of 0.200, 0.160, 0.135, 0.100 and 0.070 cm thick were coeletrophoresed (see Section 3.1) at 50 s of pulse time for numbers of pulses (N_p) that maintained constant the ratio N_p/t_h (see the N_p in Fig. 3B). In this experiment we selected the ratio $N_p/t_h = 4303$, which in conventional CHEF experiments, performed at 50 s of pulse time, resolves the 230, 270, 315, 440 and 577 kb chromosomes well.

When yeast DNA was embedded in 0.200 cm plugs the molecules migrated farther from the slot, and the 230, 270 and 315 kb yeast chromosomes left the minigel (Fig. 3A, lane 1) after 861 pulses (Fig. 3B, lane 1). When DNA was embedded in 0.160 cm plug thick, the 230 and 270 kb chromosomes migrated near the bottom of the gel and almost left it (Fig. 3A, lane 2) after 689 pulses (Fig. 3B lane 2). Consequently, yeast DNA embedded in plugs of 0.200 or 0.160 cm thicknesses would give the characteristic electrophoretic pattern of 50 s of pulse time by separating the molecules in the 10 cm length conventional CHEF gel. The molecules embedded in the agarose plugs of 0.135, 0.100 and 0.070 cm received 581, 430 and 301 pulses, respectively (Fig. 3B, lanes 3–5); and they migrated nearer to the slots, but nearly up to half of the minigel length (Fig. 3A, lanes 3–5). Thus, these samples gave satisfactory patterns in a 3.9 cm gel length (Fig. 3A, lanes 3–5). These results suggested that the thickness of the sample, the run time and the gel length are closely related factors, as they are in conventional electrophoresis and chromatography.

For any selected j and k, the resolutions $R_{j,j+k}$ were almost constant among the assayed sample thicknesses (see columns of Table 2, lower section). To verify if an individual $R_{j,j+k}$ (see columns of Table 2, lower section) falls into the mean confidence intervals, we excluded the particular $R_{j,j+k}$ from the statistic calculations (the mean and the

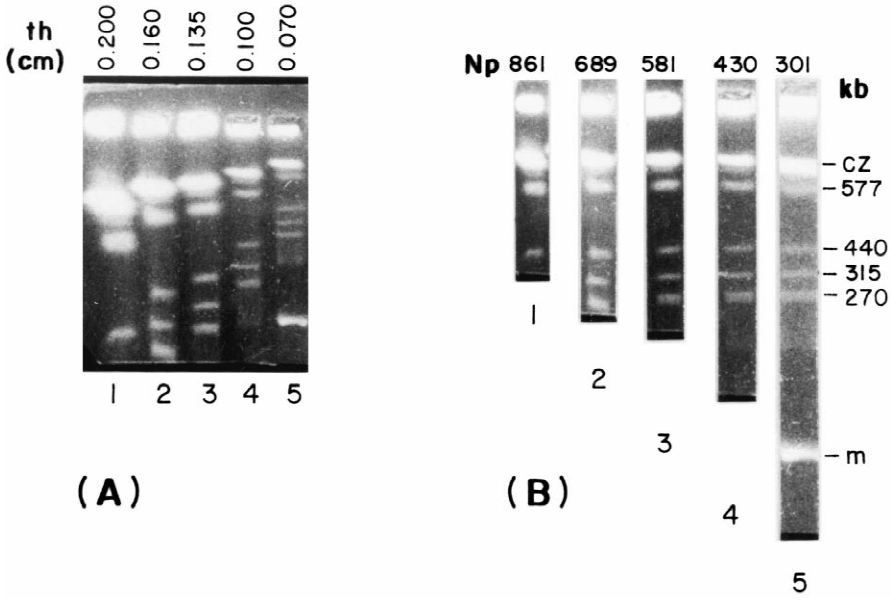

Fig. 3. (A) Electrophoretic patterns of *S. cerevisiae* DNA embedded in agarose plugs of different thicknesses (t_h). The samples were coelectrophoresed in miniCHEF at 50 s of pulse time for the number of pulses (N_p) indicated in the Figure under the conditions shown in Fig. 2. Photography reduction factor in part A: 1.069 (ratio between real gel length and its length in the photograph). (B) Enlargements of each lane of the gel to visualize similar patterns. For lane 1: 0.71, for lane 2: 0.88, for lane 3: 0.97, for lane 4:1.21 and for lane 5: 1.72. They were determined by calculating GB/GA, where GA and GB are the distances between the top of the slot and the end of the gel in parts A and B, respectively. Mitochondrial DNA is indicated by ''m''.

mean error), and tested for 0.95 certain probability (Student-t probability) if it ($R_{j,j+k}$) would fall into $\bar{R}_{j,j+k} \pm$CL (CL: confidence limits). We repeated this procedure with all YC_j and YC_{j+k} molecules. Nearly 91% of all $R_{j,j+k}$ fell into the corresponding confidence intervals of the means $\bar{R}_{j,j+k}$ (see bold values in Table 2, lower section), demonstrating that maintaining constant the ratio N_p/t_h, we can predict correctly the numbers of pulses that give a constant $R_{j,j+k}$. Small mean standard errors and relative standard deviations lower than 6% for $R_{j,j+k}$ were obtained (Table 2).

By maintaining constant the ratio N_p/t_h the patterns were similarly resolved for samples of different thicknesses (Table 2). However, due to the dissimilar N_p applied to each lane during the coelectrophoresis, the positions of the migration boundaries (D_j) of each particular yeast chromosome (YC_j), and thus ($D_j - D_{j+k}$), were different among the gel lanes (Fig. 3A). As the sample becomes thinner, each particular ($D_j - D_{j+k}$) proportionally decreased, and the pattern resolved in a particular lane resembled a magnified (or reduced) picture of the others (Fig. 3A). To appreciate identical patterns in these lanes, and artificially the same D_j and ($D_j - D_{j+k}$), each lane of the gel has to be magnified or reduced until the patterns overlapped (Fig. 3B, lanes 1–5). This approach provided patterns that look similar; as if they were replicas of a single experiment. The ratio N_p/t_h also worked well with CHEF and miniCHEF data (Table 2).

For samples of a particular thickness, electrophoresed in a particular gel at a given pulse time, the resolution (R) between two molecules increases as we augmented the number of pulses (or the electrophoresis time) [1]. Here, we proved that we can obtain a desired resolution between the molecules in a shorter time by electrophoresing thinner samples. Thus, the thickness of a sample is an important factor in PFGE runs.

Our data also proved similar migration per pulse in CHEF and miniCHEF under the conditions selected in this work, and suggested that the size of the CHEF chambers did not influence the migration per pulse of DNA molecules. Finally, from the constructive outlook, we concluded that the design of the accessories, used to cast agarose plugs, is relevant when dealing with the construction of efficient PFGE sets.

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