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Elena Nassonova
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Pulsed Field Gel Electrophoresis: Theory, Instruments and Application

E. S. Nassonova

Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia
e-mail: nosema@mail.ru

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Abstract—Pulsed field gel electrophoresis (PFGE) is a technique for the fractionation of high-molecular-weight DNA ranging from 10 kb to 10 Mb by electrophoresis in agarose gel with an electric field that alternates (pulsates) in two directions. This technology plays a key role in modern genomics, as it allows manipulations with DNA of whole chromosomes or their large fragments. In this review, we discuss (1) the theory behind PFGE; (2) different instruments based on the principle of pulsed field, as well as their advantages and limitations; (3) factors affecting the DNA mobility in PFGE gel; and (4) practical applications of the technique.

Key words: PFGE, DNA fractionation, chromosomal DNA.

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Abbreviations: CHEF, contour-clamped homogeneous electric field; FIGE, field inversion gel electrophoresis; OFAGE, orthogonal field-alternating gel electrophoresis; PFGE, pulsed field gel electrophoresis or pulsed field gradient electrophoresis (as previously used); RGE, rotating gel electrophoresis; TAFE, transverse alternating field electrophoresis.

Pulsed field gel electrophoresis (PFGE) is a technique enabling separation of DNA molecules in agarose gel by using two alternating electric fields, with vectors directed to each other at obtuse angle (Smith et al., 1990). PFGE fractionates large DNA molecules in the size range from 10 kb to 10 Mb (Orbach et al., 1988; Kuspa et al., 1989; Cooney, 1990). Within this range are chromosomal DNA of prokaryotes and lower eukaryotes; therefore, it is possible to manipulate the DNA of whole chromosomes or their large fragments (for higher organisms). It is for this reason that the technique is a key instrument in modern genomics.

The purpose of the review is (1) to consider theoretical basis; (2) to describe available PFGE instruments, as well as their advantages and limitations; (3) to discuss factors affecting molecule separation; and (4) to survey the current applications of PFGE.

HISTORY AND THEORY OF PFGE

Schwartz and coworkers (Schwartz et al., 1982; Schwartz and Cantor, 1984) were the first to succeed in fractionating high-molecular-weight DNA by electrophoresis. Previously, the electrophoresis of large DNA molecules was infeasible for two main reasons. First, with routine methods of DNA isolation and purification, it was impossible to obtain intact DNA of large size. Deproteinized DNA of a whole chromosome has a contour length of a few millimeters and diameter of 20 nm. These molecules with axis ratio of about 10^6 are very sensitive to the shift force; therefore, they are cleaved if the routine methods of isolation are applied. Second, under conventional electrophoresis, DNA molecules of more than 50 kb migrate in gel with similar velocities and, therefore, are not separated by size.

To separate DNA of whole chromosomes of the budding yeast *Saccharomyces cerevisiae* with sizes of 200–2200 kb, Schwartz and coworkers suggested two fundamentally new approaches that allowed them to solve the problem of purifying and fractionating large DNA molecules. The authors (1) developed a new technique that resulted in the isolation of intact chromosomal DNA from cells embedded in low-melting agarose and (2) designed a device for electrophoresis with periodical alterations of the direction of the electric field, which made it possible to fractionate large DNA molecules in agarose gel.

The authors assumed that molecule separation in pulsed field occurred because different-sized molecules required different time for reorientation. They considered large molecules to be reoriented for longer when electric field was switched and, therefore, remained behind smaller molecules. It resulted in size fractionation of the molecules. Further experiments showed that this elegant hypothesis was not exactly correct. The mechanism of fractionation lies in diverse forms of DNA motion in gel, which determine different rate of migration of different-sized molecules. The observations on DNA stained with fluorochromes showed that

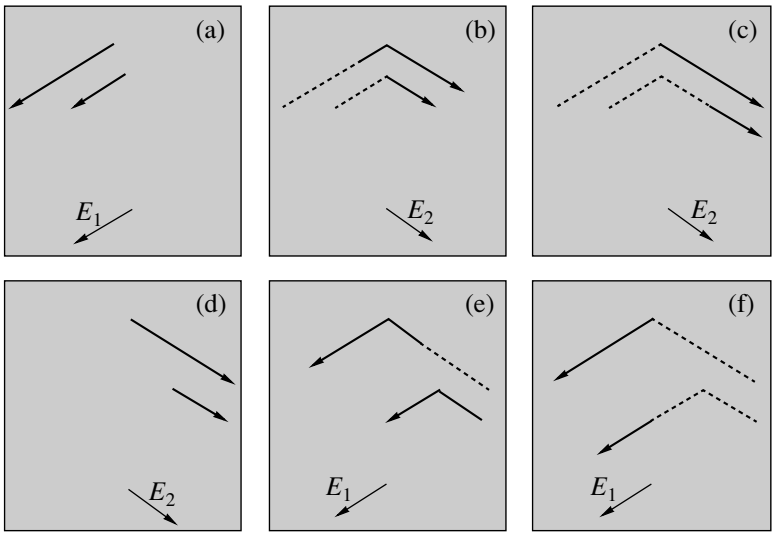

Fig. 1. Schematic drawing showing the mechanism of DNA fractionation during the pulsed field gel electrophoresis (modified after Gurrieri et al., 1996). When the electric field is switched, DNA molecules first start backward motion in the gel led by their former trailing tails. Each molecule moves back by a distance proportional to its size (a), (b). Then, the molecules commence onward motion in a new field (c). As a result, longer molecules remain behind the shorter ones. With every new field switch, the retardation of longer molecules becomes more profound (d), (e), (f). E_1, E_2 are vectors of electric field strength; long and short arrows show long and short DNA molecules, respectively. Arrow heads indicate the direction of migration of the molecules and, therefore, the positions of their leading ends. Dashed lines show tracks of molecule migration in gel.

molecules moving in gel by an electric field are elongated in the direction of the field strength vector (Bustamante et al., 1990; Gurrieri et al., 1996). When the electric field is switched, DNA molecules start to migrate in a new direction by one of their ends; elongated DNA conformation is retained. If the angle between the strength vectors of alternative fields, i.e., the so-called "reorientation angle", is 90°, any end of molecule may become a leading one. If the angle is less than 90°, at the moment "anterior" end of the molecule becomes the leading one. If the angle is more than 90°, the "trailing" end of the molecule becomes the leading one. In PFGE devices, reorientation angles are greater than 90°; therefore, when field is switched, molecules begin to move in a new direction led by their trailing ends and, in the beginning, they should do backward motions (Fig. 1). Molecules retreat at distances proportional to their size; hence, large molecules remain behind the small ones. The efficiency of molecule separation is defined by the size, the number of switching of field direction, and duration of intervals of stable orientation of the field (Southern et al., 1987; Southern and Elder, 1995).

PULSED FIELD GEL ELECTROPHORESIS INSTRUMENTS

In the device designed by Schwartz and Cantor (1984), the field was switched in certain intervals at a 90° angle. The gel was placed into the chamber for horizontal electrophoresis. One field was homogeneous and generated by two rows of dot electrodes. The other field was nonhomogeneous and generated by a number of dot electrodes as cathode and one dot electrode as anode (Fig. 2). This system became known as "pulsed field gradient electrophoresis" (PFGE). The authors considered the voltage gradient to be a prerequisite for DNA molecules to be separated in pulsed field; therefore, they used electrode configurations ultimately producing a nonhomogeneous field. In this system, the angle between vectors of field strength varied in different gel regions (110°–150°); therefore, molecules equal in size migrated with different rates depending on their initial position in gel; this complicated the comparison of the electrophoretic mobilities of DNAs run on neighbor lanes making it impossible to accurately estimate size.

Later, a number of instruments for electrophoresis in pulsed field were suggested that differed in electrode configurations and, consequently, in the reorientation angle. The abbreviation "PFGE" suggested by Schwartz and Cantor (1984) is currently applied for all methods and instruments for DNA electrophoresis where the principle of pulsed field is used. However, further experiments showed that a non-homogeneous field is not an obligatory requirement for successful DNA separation. Because most current systems are based on homogeneous fields, the abbreviation "PFGE" got a new interpretation (pulsed field *gel* electrophoresis).

Almost at the same time as a description of the first device for electrophoresis in pulsed field was published

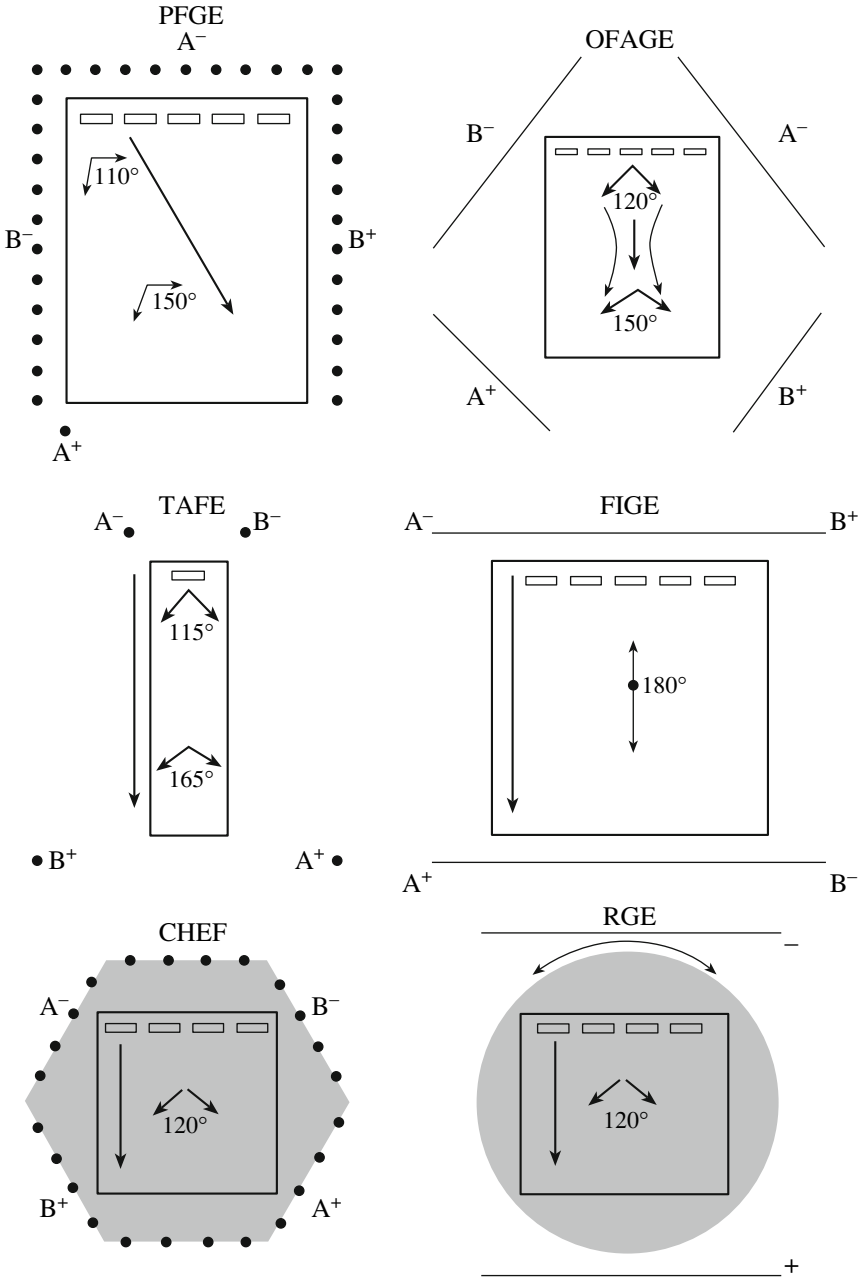

Fig. 2. Schematic drawings showing electrode geometries in commonly used instruments for pulsed field gel electrophoresis (modified after Mezhevaya et al., 1993; Lai et al., 1989). Systems with nonhomogenous electric fields: (PFGE) pulsed field gradient electrophoresis; (OFAGE) orthogonal field-alternating gel electrophoresis; (TAFE) transverse alternating-field electrophoresis. Systems with homogeneous electric fields: (FIGE) field inversion gel electrophoresis; (CHEF) contour-clamped homogeneous electric field; (RGE) rotating gel electrophoresis. Short thin arrows show the vectors of field strength of alternative electric fields; reorientation angle is indicated inside. Wide arrows point to the net direction of migration. Symbols A^+ and A^-, B^+ and B^- indicate positions of electrode pairs of alternative electric fields. Electrodes are indicated by dots in PFGE, TAFE, and CHEF schemes and by straight lines in OFAGE, FIGE, and RGE schemes.

(Schwartz, and Cantor, 1984), Carle and Olson (1984) reported another variant of the technique, which was named "orthogonal field-alternating gel electrophoresis" (OFAGE). In OFAGE device, the arrangement of electrodes was symmetric and their lengths were unequal. The longer electrode was used as a cathode and the shorter one as an anode. In this system, the reorientation angle varied from 120° to 150° in different gel regions. In OFAGE gels the central lanes were straight that allowed comparison of electrophoretic

mobilities of molecules migrating in neighbor lanes. However, the lanes close to the gel edges remained curved.

Gardiner and coworkers (Gardiner et al., 1986) suggested a system with gel placed vertically in the chamber and alternating electric fields oriented not along, but across the gel. The authors called the system "transverse alternating-field electrophoresis" (TAFE). The word "transverse" is referred to the orientation of the vector of field strength with respect to the gel plane. The field in this system is also nonhomogeneous, as the reorientation angle increased from the gel top to the bottom from 115° to 165°, which results in the reduced field strength and the limited rate of DNA migration at the bottom of the gel. The decrease in the rate of molecule migration leads to the self-sharpening (Cantor et al., 1988) or band-sharpening effect (Gardiner, 1992). The bands become narrower and more distinct but arranged close to each other. Band sharpening complicates TAFE standardization and obtaining of reproducible results but may be useful for particular purposes, e.g., for the accurate separation of molecules with similar sizes. Moreover, in spite of the nonhomogeneous electric field, this vertical variant of pulse field gel electrophoresis ensures strictly rectilinear molecule migration and, consequently, all lanes (including marginal ones) are straight and parallel. It allows comparison of electrophoretic mobilities of molecules migrated in different lanes.

Common disadvantages of the systems for electrophoresis in pulsed field described above include a nonhomogeneous electric field, with variation in both field strength and reorientation angle. It results in variable rate of migration of DNA molecules depending on their location in the gel. This leads to appearance of curved lanes (in PFGE and OFAGE) and band sharpening effect (in TAFE), thus complicating optimization of electrophoresis conditions. The following experiments showed that nonhomogeneous electric field and voltage gradient were not obligatory requirements for molecule separation in pulsed field. Further technological progress was based on the development of systems with homogeneous pulsed field.

Carle and coworkers (Carle et al., 1986) proposed to utilize a standard horizontal chamber with two parallel electrodes of equal lengths and to invert periodically the polarity of the applied electric field, i.e., the reorientation angle in the system was 180°. The net forward migration of DNA was retained because of a difference in the duration or in voltage of the forward and reverse fields.

The method was referred to as "field inversion gel electrophoresis" (FIGE). The advantages of the technique are the usability of the instrument (any standard chambers for conventional DNA electrophoresis are suitable) and independence of DNA mobility from its position in a gel. The shortage is nonlinear dependence of the migration rate of DNA from its size resulted in band inversion effect. Each value of pulse time, the interval at which the direction of the electric field is switched, corresponds to a particular range of the size of DNA molecules migrating at a very low rate. Larger and smaller molecules migrate with notable rates, which may produce artifact fractionation. Electrophoresis with field inversion was not recognized as suitable for the accurate determination of DNA size.

Chu and coworkers (Chu et al., 1986) developed a device for pulsed field gel electrophoresis with a controlled homogeneous electric field (contour-clamped homogeneous electric field, CHEF). Under these conditions, the path and the rate of migration of DNA molecule do not depend on its position in gel; neither band inversion nor band sharpening are observed. In this system, multiple dot electrodes are arranged around a hexagonal contour and clamped to potentials appropriate for a homogeneous field (Fig. 3). With CHEF, it is possible to achieve straight lanes and stable DNA separation. CHEF is the mostly widely applied of the known variants of electrophoresis in pulsed field, as it ensures the most effective fractionation of DNA molecules in a wide range of sizes. For example, it is this system that allows the almost complete separation of chromosomal DNAs from the budding yeast *Saccharomyces cerevisiae*. Using other instruments only 11–12 bands were visualized in the yeast molecular karyotype as some heterologous chromosomes migrated in a single band (Schwartz, and Cantor, 1984; Carle, and Olson, 1985). In CHEF gel with fractionated chromosomal DNAs of *S. cerevisiae* Chu and coauthors observed 15 bands, i.e. the researchers succeeded in separation of 14 from 16 yeast chromosomes (Chu et al., 1986).

In addition to the devices for pulsed field gel electrophoresis mentioned above, there are electrophoretic instruments in which pulsed field is generated not by switching of field strength on electrode pairs but by the rotation of gel placed on a horizontal platform, which turned to a given angle in certain intervals (rotating gel in a constant field, RGCF, Anand, 1986; rotating gel electrophoresis, RGE, Southern et al., 1987) or the gel is stationary, while cathode and anode are the parts of rotating rotor (rotating-field gel electrophoresis, ROFE, Ziegler et al., 1987).

Other modifications of pulsed electrophoresis are described as well; however, most of currently utilized commercial instruments are based on the CHEF principle, e.g., CHEF-DR® III pulsed field electrophoresis system (Bio-Rad Laboratories, United States) and Gene Navigator™ system (GE Healthcare Bio-Sciences, Sweden).

FACTORS DETERMING EFFICIENCY OF MOLECULE SEPARATION

In addition to factors mentioned above that are critical for DNA isolation and fractionation in pulsed field, such as meticulous methods for native high-molecular-

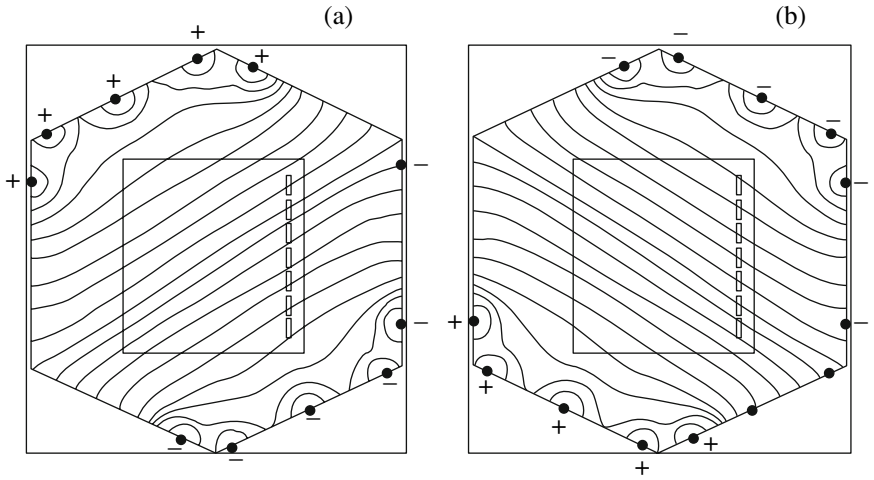

Fig. 3. Homogeneous pulsed electric field in a CHEF instrument. Schematic drawing is based on the data from the user manual to the Gene Navigator system (GE Healthcare Bio-Sciences, Sweden). Shown are isofield lines and electrodes generating the alternative electric fields; (a), (b) two alternative directions of the vectors of electric field strength.

weight DNA purification and specific electrode arrangement in the electrophoresis chamber, there are a number of parameters that also affect molecule mobility and separation, including pulse time and a mode of its switching, field strength, reorientation angle, run time, agarose concentration, buffer concentration, and temperature. All of these parameters are interdependent.

Pulse time (switch time, switch interval) is the time interval for switching the direction of the field. The first experiments on DNA fractionation in a pulsed electric field showed that the pulse time is the most critical parameter for molecule separation. The larger the size of fractionated molecules, the less frequently the switching of electric fields should be performed. Schwartz and Cantor suggested that the time required for the reorientation of DNA molecule in a new field depends on its size. They believed that the optimal pulse time for DNA molecules of a given size was the time in which molecules succeeded in reorienting and only began to move in a new direction. Larger molecules within the same pulse interval had not enough time to reorient themselves; therefore, they began to move in a new direction later, i.e., their movements were inhibited. The smaller molecules would migrate in the same direction for too long and, consequently, their separation would be ineffective (Schwartz and Cantor, 1984). According to the current concept of the mechanisms of DNA migration in a pulsed field, it is more correct to take into consideration the time needed for the reciprocal movement rather than the time required for reorientation (Gurrieri et al., 1996). However, the general rule revealed by researchers at the beginning of PFGE development is still the same; with a given pulse time only molecules of a particular size class occur under optimal conditions for separation and may be effectively fractionated. Thus, to fractionate DNA in a broad size range it is necessary to find the regime of sequential increase of pulse time (Fig. 4a). The change of pulse time during electrophoresis is called "ramping." This parameter may be increased throughout a run either constantly or discontinuously over a range of discrete values. Continuous increase of pulse time from minimum to maximum of specified values during a given time interval is called "interpolation". An alternative variant, the stepwise increase in pulse time, is called "stepping."

Molecule resolution in gel is characterized by band spacing and band sharpening. The area in the gel where effective DNA separation is achieved is called the "resolution zone", or "resolution window." The dependence between the rate of DNA migration and molecule size is linear in the resolution zone; therefore, it is possible to precisely estimate the size of DNA molecules in this zone (Figs. 4b and 4c).

Field strength. Resolution is the function both of the pulse time and electric field strength. As a whole, the higher the field strength, the higher the rate of molecule migration. However, the higher the molecule size, the more this dependence deviates from the linear function (Cantor et al., 1988). To separate molecules of less than 1 Mb, the field strength applied is 6–10 V/cm. The lower the field strength, the higher the resolution; however, the size range of separated molecules is narrower (Birren et al., 1988). An excessively high field strength (higher than 10 V/cm) results in weak molecule separation and the appearance of so-called "smears," i.e., zones of uniform DNA staining with visually unidentified single bands (Mathew et al., 1988b; Chu, 1990). For large molecules (more than 1 Mb), high field strength results in a "trapping effect," i.e., spontaneous permanent molecule immobilization in gel due to specific irreversible changes of their conformation. Such molecules are fractionated preferentially at a low field

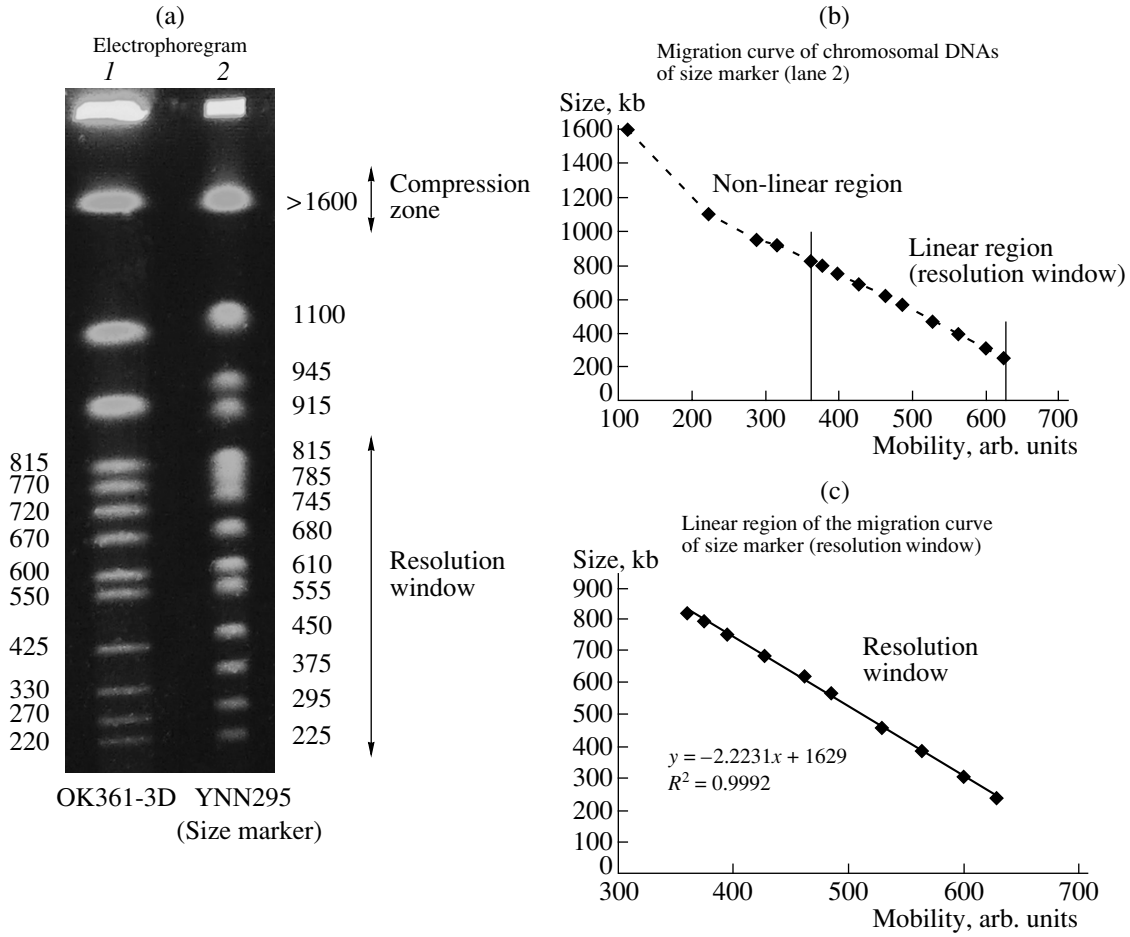

Fig. 4. Example of fractionation and size determination of chromosomal DNA by pulsed field gel electrophoresis with pulse time ramp. (a) Fractionation of chromosomal DNA of the yeast *Saccharomyces cerevisiae*. Lane *1*—OK361-3D strain; lane *2*—YNN295 strain (Sigma–Aldrich, United States) used for size markers. Electrophoresis was carried out in 1% agarose gel in 0.5 × TBE at +14°C in the apparatus for contour-clamped homogeneous electric field, CHEF-DR® III pulsed field electrophoresis system (Bio-Rad Laboratories, United States). The run time was 24 h at 6 V cm^{-1} with pulse time interpolated between 60 and 120 s. (b) Migration curve of chromosomal DNA of *S. cerevisiae*, strain YNN295 used as size markers (dash line); (c) a region of the curve, which corresponds to the resolution window where relationship between the mobility and the size is approximated by linear function. Using instruments of MS Excel, a trend line (solid line) was drawn, the function (y) was determined, and the R-squared value (R^2) was calculated for this region. Based on the obtained equation, the sizes of chromosomal DNA of the strain OK361-3D were calculated; (a) mean values calculated from the results of five independent runs are shown on the right. Sizes of chromosomal DNA of the marker strain YNN295 are indicated in (a) on the left. A compression zone is a region at the upper part of the gel with comigrating DNAs that remained unresolved under applied conditions.

strength (1–3 V/cm) (Gunderson and Chu, 1991; Gurrieri et al., 1999).

The pulse time and the field strength are interrelated factors. PFGE resolution is determined by the following "window function" (Chu, 1990): $W = E^{1.4} \times Tp$, where E is the electric field strength and Tp is the pulse time. The higher the value of the function, the larger the sizes of DNA molecules that can be separated effectively. It is obvious that the separation of DNA molecules of the same size range may be achieved with different pulse time if the field strength is changed so that the W function value remains constant.

Reorientation angle. This parameter in some PFGE devices is fixed; in others, it varies from 90 to 180°. It was demonstrated that the change of the reorientation angle did not notably affect the migration of molecules less than 1 Mb. The mobility of larger molecules increases with decrease of the angle; however, the band resolution in gel is getting reduced (Clark et al., 1988; Chu and Gunderson, 1991). It was found empirically that the best separation is achieved with an angle value of more than 110°. (Cantor et al., 1988). Most commercially available instruments for PFGE use a fixed angle of 120°.

Agarose concentration in gel. The agarose concentration in gel has less effect on the mobility of DNA molecules during electrophoresis in pulsed field than in constant one. It was found that resolution is somewhat better for 1.2% agarose gels than for 0.9% gels. Further increase in agarose concentration merely decreases the mobility and hence increases the run time without improving the resolution (Mathew et al., 1988a). In practice to separate molecules with size less than 3 Mb 1.0–1.5% agarose gels are commonly used.

Composition and temperature of electrophoresis buffer. Buffers with high ionic strength, Tris-borate (0.5 × TBE) or Tris-acetate (1 × TAE) are commonly used for PFGE. The electrophoretic mobility of DNA molecules in pulsed field depends vigorously on the buffer temperature; the higher the temperature, the higher the rate of molecule migration (Mathew et al., 1988a). PFGE is usually performed at +12–16°C (Chu, 1990). At higher buffer temperatures, the bands become wide and diffuse. It results in notably reduced resolution and appearance of smears. It is considered that increase in buffer temperature leads either to molecule conformation preventing their rapid reorientation in gel or to modification of agarose matrix properties (Mathew et al., 1988a). Thus, the maintenance of the permanent temperature of the electrophoresis buffer is a prerequisite for the good quality of DNA molecule separation.

APPLICATION OF THE TECHNIQUE

Pulsed field gel electrophoresis is widely used in prokaryote and eukaryote genomics. PFGE in combination with other molecular methods makes it possible to localize cloned sequences on chromosomes, to perform physical genome mapping, and to specify the fine structure of physical and genetic chromosome maps in various organisms. It is the application of electrophoresis in pulsed field to the fractionation of high-molecular-weight fragments of DNA digested with endonucleases resulted in the first map of the genome of *Escherichia coli* strain K12 in 1987 (Smith et al., 1987), as well as in the publication two years later of the first complete restriction map of the genome of an eukaryotic organism, the fission yeast *Schizosaccharomyces pombe* (Fan et al., 1989). The technique was successfully applied to eubacteria and archaea genome mapping (Smith and Condemine, 1990; Dingwall et al., 1990; Bautsch, 1992; Fujiwara et al., 1996), as well as for analysis of replication in these organisms, in particular for localization of the origin and terminus of replication, for estimation of the initiation time, the rate of fork movement and the order of gene replication in the cell cycle (Pyle and Finch, 1988; Ohki and Smith, 1989; Dingwall and Shapiro, 1989).

The patterns of restriction fragments of bacterial chromosomal DNA digested with endonucleases and separated with PFGE are used to identify microorganism strains in medicine, industry, and agriculture (see, e.g., Correia et al., 1994). The method is used also in molecular ecology, for example, for fingerprinting of viral assemblages in natural inhabitants (Steward, 2001).

Pulsed field gel electrophoresis was a power stimulus for the large-scale mapping of entire genomes and individual chromosomes in eukaryotes. The technique is applied to mapping of long genes, e.g., Duchenne muscular dystrophy gene (Burmeister and Lehrach, 1986); it has also been used to create chromosome-specific and artificial yeast chromosome libraries, as well as to screen them (Ecker, 1990; Ougen and Cohen 1995; Ragoussis, 1995; Larin, 1995).

One of the most important applications of PFGE is the molecular karyotyping of low eukaryotes, the small size of whose chromosomal DNA makes them amenable for separation in pulsed field. Molecular karyotyping allows the number of chromosomes and the genome size to be estimated, as well as the genome dynamics to be followed, in particular chromosome rearrangements and resultant chromosome polymorphism, which is common for many unicellular eukaryotes to be revealed. The analysis of the chromosome set and genome dynamics is informative for understanding of intra- and interspecies karyotypic variability (see, e.g., Henriksson et al., 2002), as well as of chromosome evolution in closely related species (Henriksson et al., 1995; Britto et al., 1998; Fischer et al., 2000, 2006; Dujon, 2006). A comparison of molecular karyotypes of fungi and protozoan isolates of different origin is widely used in clinical diagnostics and agricultural microbiology (Giannini et al., 1986; Taylor et al., 1991; Fraissinet–Tachet et al., 1996; Taylor et al., 1999 and others). PFGE is also essential in the first stages of the projects on fungi and protozoan genome sequencing (Gardner et al., 2002; Eichinger et al., 2005).

In conclusion, it should be stressed that PFGE greatly facilitates the structural and functional analysis of genome in various organisms. There are a number of variants of the technique, the most widely used is the contour-clamped homogeneous electric field (CHEF). The molecule mobility and separation in pulsed field depend on the number of interrelated parameters, the most significant of which are the pulse time and the regime of its increase. A sequential increase in the pulse time in the stepping or interpolation mode facilitates the fractionation of DNA molecules of different sizes. PFGE is a vital instrument in modern genomics, as it permits the separation of chromosomal DNA and their large fragments.

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