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Pulsed-Field Gel Electrophoresis (PFGE) Technique and its use in Molecular Biology

Esin (HACIOĞLU) BASIM
Süleyman Demirel University, Faculty of Agriculture, Department of Plant Protection,
32260 Çünür, Isparta - TURKEY

Hüseyin BASIM
Akdeniz University, Faculty of Agriculture, Department of Plant Protection,
07058, Antalya - TURKEY

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Abstract: In recent years, the use of pulsed-field gel electrophoresis (PFGE) in the molecular biology area has been subject to much research. PFGE is a powerful tool for characterizing various strains at the DNA level, obtaining relevant information on genome size and constructing the physical and genetic map of the chromosome of bacteria that are poorly understood at the genetic level as well as in separating chromosomes in microorganisms, and in the long-range mapping of mammalian genes. PFGE also has advantage of examining the elongated and oriented configuration of large DNA molecules in agarose gels at finite field strengths. In this review, the use of PFGE in molecular biology, the general characteristics of PFGE, different types of PFGE and factors affecting PFGE are introduced.

Key Words: Pulsed-Field Gel Electrophoresis (PFGE), CHEF, Molecular Biology, Biotechnology, Restriction Enzymes.

Pulsed-Field Jel Elektroforez (PFGE) Tekniği ve Moleküler Biyoloji Alanında Kullanımı

Özet: Son yıllarda, moleküler biyoloji alanında pulsed-field jel elektroforez (PFGE)'in kullanımı birçok araştırmaya konu olmuştur. PFGE, DNA düzeyinde çeşitli strainlerin karakterize edilmesinde, genom büyüklükleri hakkında bilgi elde etmede, genetik düzeyde anlaşılamamış bakteri kromozomlarının fiziki ve genetik haritalarının oluşturulmasında,mikroorganizmaların kromozomlarının ayrılmasında, ve memeli genlerinin büyük çaptaki haritalanmasında kullanılan etkili bir metottur. PFGE; agaroz jel içerisindeki büyük DNA moleküllerinin uygun bir alanda değişik şekillerde hareket etmesini sağlayan bir avantaja da sahiptir. Bu derlemede, PFGE'in moleküler biyoloji alanında kullanımı, PFGE'in genel özellikleri, çeşitli tipleri ve PFGE'i etkileyen faktörler sunulmuştur.

Anahtar Sözcükler: Pulsed-Field Jel Elektroforez (PFGE), CHEF, Moleküler Biyoloji, Biyoteknoloji, Restriksiyon Enzimler

Introduction

Much of the rapid progress that is being made in molecular biology today depends upon the ability to separate, size and visualize DNA molecules. The most common technique for this purpose is that of standard agarose gel electrophoresis. Gel electrophoresis (1) is one of the most commonly used separation techniques in the modern biology laboratory. Its ubiquity arises from both the simplicity and versatility of the technique. Electrophoresis has found widespread use in biological assays, and in the purification and separation of proteins and nucleic acids. The physical mapping of genes and DNA sequencing both depend on separation by gel electrophoresis (1). Conventional gel electrophoresis of DNA molecules is carried out by placing DNA in a solid matrix (i.e. agarose or polyacrylamide) and inducing the molecules to migrate through the gel under a static electric field. DNA fragments from 100 to 200 base pairs (bp) up to 50 kilobase pairs (kb) are routinely separated by conventional gel electrophoresis techniques. Above 50 kb, because of the size of the molecules, the sieving action of the gel is lost, and fragments run as a broad, unresolved band with anomalously high mobility. Although larger fragments (up to 750 kb) have been resolved by this technique (2), the gels used are extremely fragile due to the very low agarose concentrations, and the separation is not adequate for most applications. The separation of DNA molecules by other techniques is time-consuming. The need for the analysis of large DNA molecules is in large-scale mapping (3).

In 1982, Schwartz et al. (4) introduced the concept that DNA molecules larger than 50 kb can be separated by using two alternating electric fields (i.e. PFGE). Since that time, a number of instruments based on this principle have been developed, and the value of using pulsed fields has been demonstrated for separating DNAs from a few kb to over 10 megabase pairs (Mb).

The development of PFGE has increased by two orders of magnitude the size of DNA molecules that can be routinely fractionated and analyzed. This increase is of major importance in molecular biology because it simplifies many previously laborious investigations and makes possible many new ones. Its range of application spans all organisms (2) from bacteria and viruses to mammals (5). PFGE has shown excellent ability to separate small, natural linear chromosomal DNAs ranging in size from 50-kb parasite microchromosomes to multimillion-bp yeast chromosomes. However, intact human chromosomes range in size from 50 million to 250 million bp (Mb), too large for direct PFGE separations (6). PFGE provides the means for the routine separation of fragments exceeding 6,000 kb (2, 7, 8, 9). Therefore, PFGE separates DNAs from a few kilobase (kb) to over 10 megabase pairs (Mb) (10).

The new technique of PFGE takes advantage of the elongated and oriented configuration of large DNA molecules in agarose gels at finite field strengths. An important bonus of this technique is the ease with which the genome size can be measured, a parameter that was previously subject to considerable error when measured by other techniques. One important outcome of the use of PFGE and restriction endonuclease digestion is the construction of a physical map. General applications of PFGE can be in the separation of whole chromosomes,

the large - scale restriction mapping of chromosome regions and in using DNA fragment purification as an aid in cloning. PFGE will greatly facilitate the precise selection of very large fragments for cloning, and it provides rapid analysis of a large chromosomal region. PFGE has proven extremely powerful in the analysis of large DNA molecules from a variety of sources, including specifically fragmented genomes of bacteria (11), mammals (5), parasite protozoa (12-15) and intact chromosomal DNAs from fungi (16-18). The introduction of PFGE techniques for separating large DNA molecules has had an invigorating effect on the study of chromosomal DNA molecules, genome structure and electrophoretical theory.

In this review, the use of PFGE in molecular biology the general characteristics of PFGE, different types of PFGE and factors affecting PFGE are introduced.

Types of PFGE

PFGE size resolves DNA molecules of almost a millimeter in length through the use of pulsed-field electric fields, which selectively modulate mobilities in a size-dependent fashion. The pulsed electrophoresis effect has been utilized by a variety of instruments (FIGE, TAFE, CHEF, OFAGE, PACE and rotating electrode gel) to increase the size resolution of both large and small DNA molecules (1). It is important when choosing a PFGE system to evaluate cost and performance in the light of projected use. There are different types of PFGE. These are:

1. Field-Inversion Gel Electrophoresis (FIGE): In 1986, Carle, Frank and Olson developed a simpler system, FIGE, in which the two fields were 180° apart (19). Electrode polarity was reversed at intervals, with a longer forward than reverse pulse time to generate a net forward sample migration. Net forward migration is achieved by increasing the ratio of forward to reverse pulse times to 3:1. To improve the resolution of the bands by FIGE, the duration of pulse times is increased progressively during a run. This is called “switch time ramping”. By changing pulse durations continually during the course of an experiment, FIGE has the advantages of straight lanes and simple equipment. All that is needed are standard gel boxes and a pulse controller. Today, FIGE is very popular for smaller fragment separations. FIGE provides acceptable resolution up to 800 kilobases (600-750 kb).

2. Transverse-Alternating Field Gel Electrophoresis (TAFE): This form of PFGE allows separation of large DNA fragments in a simple, convenient format without the drawbacks of earlier pulsed-field techniques. In TAFE, the gel is oriented vertically and a simple four-electrode array is placed not in the plane of the gel, but in front and at the back of it. Sample molecules are forced to zigzag through the thickness of the gel, and all lanes experience the same effects, so the bands remain straight (20). As the molecules move down the gel, they are subjected to continual variations in field strength and reorientation angle, but to all lanes equally. However, the angle between the electric fields varies from the top of the gel (115°) to the bottom (approximately 165°) and hence molecules still do not move at a constant velocity over the

length of the gel. TAFE technology, with regular and sharp separation of DNA bands, will be of special advantage in the study of genetics of many pathogenic protozoans, where such analysis was impossible before (20). TAFE has been used for the separation of fragments up to 1,600 kilobase fragments.

3. Contour-Clamped Homogeneous Electric Fields (CHEF): CHEF is the most widely used apparatus. The CHEF apparatus provides a more sophisticated solution to the distorting effects of both the edges of the chamber and the passive electrodes. CHEF has twenty-four point electrodes equally spaced around the hexagonal contour. In the CHEF system, there are no "passive" electrodes. All the electrodes are connected to the power supply via an external loop of resistors, all of which have the same resistance. This loop is responsible for setting the voltages of all the electrodes around the hexagonal contour to values appropriate to the generation of uniform fields in each of the alternate switching positions. The CHEF system sets the voltages at these 24 points. This apparatus produces electric fields that are sufficiently uniform so that all lanes of a gel run straight. CHEF uses an angle of reorientation of 120° with gradiations of electropotential radiating from the positive to the negative pores. Molecules up to 7,000 kb can be separated by CHEF (10).

4. Orthogonal-Field Alternation Gel Electrophoresis (OFAGE): A similar apparatus that used two nonhomogeneous electric fields was reported by Carle and Olson (17) in 1984. The major drawbacks of these apparatuses were that because the electric fields were not uniform, and the angle between the electric field varied across the gel, DNA molecules migrated at different rates depending on their location in the gel. This is especially problematic in mammalian genome mapping, where a continuous distribution of fragment sizes is generated. Lane-to-lane comparisions and size estimations for digested genomic DNA are less straightforward when fewer discrete bands are being separated, as with the chromosomes of lower organisms like yeast. The angle between the electric fields varies from less than 180° and the more than 90°. DNA molecules from 1,000 to 2,000 kb can be separated in OFAGE (17, 21).

5. Rotating Gel Electrophoresis (RGE): In England in 1987, Southern (22) described a novel PFGE system that rotates the gel between two set angles while the electrodes are off. In RGE, the electric field is uniform and bands are straight because only one set of electrodes is used. RGE makes it easy to perform time and voltage ramping. It also enables users to study the effects of different angles, and even to vary these, during an experiment-angle ramping. RGE uses a single homogeneous field and changes the orientation of the electric field in relation to the gel by discontinuously and periodically rotating the gel. Switch times are too long in RGE. The DNA molecules migrate in straight lanes, due to the homogeneous fields, and DNA molecules from 50 kb to 6,000 kb can be separated by adjusting the frequency of the gel rotation. In addition, the angle of reorientation can be easily altered simply by changing the angle of rotation (2, 23).

6. Programmable Autonomously-Controlled Electrodes (PACE): The PACE electrophoresis system offers precise control over all electric field parameters by the independent regulation of the voltages on 24 electrodes arranged in a closed contour. The flexibility of the PACE system derives from its ability to generate an unlimited number of electric fields of controlled homogeneity, voltage gradient, orientation and duration. The PACE system can perform all previous pulsed field switching regimens (i.e. FIGE, OFAGE, PHOGE, unidirectional pulsing), as well as generate voltage clamped homogeneous static fields. The PACE system separates DNA fragments from 100 bp to over 6 Mb. The ability to alter the reorientation angle between the alternating fields permits an increased speed of separation for large DNA molecules. A computer-driven system known as PACE, designed by Lai et al. (1) may be the ultimate PFGE device. It is an extremely useful tool for studying variables such as pulse time, temperature, agarose concentration, voltage and angles between fields affecting DNA migration in PFGE (24).

7. Pulsed-Homogeneous Orthogonal Field Gel Electrophoresis (PHOGE): The major difference between this instrument and other gel boxes with homogeneous electric fields is that the field reorientation angle is 90°. PHOGE uses a 90° reorientation angle, but the DNA molecules undergo four reorientations per cycle instead of two. The DNA lanes in PHOGE do not run straight, a phenomenon which has been described for gel runs involving multiple electric fields in this manner. This system separates DNA fragments of up to 1 Mb (23).

PFGE Equipment

The basic components of a PFGE system consist of a gel box with some means of temperature regulation, a switching unit for controlling the electric fields, a cooler and a power supply (1).

Gel Box

The basic design of PFGE boxes consists of an immobilized gel within an array of electrodes and a means of circulating the electrophoresis buffer. Voltage gradients of 10 volts/cm are commonly used in PFGE. Voltage gradients as high as 15 volts/cm have been used in field inversion separations of cosmid clones (1). The temperature of the buffer is controlled by a heat-exchange mechanism. Generally, the buffer is recirculated throughout the gel box using inlet and outlet ports (17, 24).

High Voltage Power Supply

Precise control of the electric field gradient is necessary to obtain consistent PFGE separations. The output ratings of the power supply should therefore be high enough to meet

both the voltage and current requirements of the gel box. A typical PFGE gel box has electrodes that are 25 to 50 cm apart. To achieve the commonly used range of voltage gradients of 1.5 to 15 volts/cm requires a power supply with a maximum voltage rating of 750 volts. The current drawn at this voltage in most PFGE boxes is about 0.5 amperes at 14°C using 0.5x TBE (1x TBE is 89 mM Tris pH: 7. 89 mM Boric acid and 2 mM EDTA) as running buffer (1).

Switch Unit

The ability to reproducibly control the switch interval is critical for the separation (24). The limited speed at which relays can switch will not accommodate the fast switching necessary for the PFGE separation of small DNA molecules (2-50 kb). The relays are usually controlled by a computer. To overcome the drawbacks of electromechanical relays, high-voltage solid-state electronics has supplanted electromechanical relays in recently designed commercial PFGE systems. These switching units are commonly based on the use of metal oxide semiconductor field effect transistors (MOSFETs) in both switching and electrode voltage control circuits. These designs offer the advantages of improved reliability, the capability of high speed switching (0.1 ms) and ample voltage (750 V) and current (0.5 amperes) ratings (1). These apparatuses have the ability to control the reorientation angles between electric fields. However, these instruments cannot provide fast enough switching for the improvement of the separation of DNA molecules smaller than 50 kb.

Computer Program

Careful control of the switch interval is crucial in controlling the resolution in PFGE. A versatile switching unit should have software with the same characteristics. The algorithm should be fast enough so that switch times at least as short as 1 ms can be achieved and switch interval increments should have at least 1 ms resolution. Linear switch interval ramping has been the most commonly used procedure because of its simple implementation. The maximum run time should be about two weeks to allow for the separation of very large DNA molecules. This is controlled by a computer program (1).

Cooler

Buffer recirculation is an important factor, as it eliminates temperature variations within the gel so as to alleviate buffer breakdown due to electrolysis. DNA molecule migration is sensitive to temperature, and thus a uniform temperature across the gel is needed to ensure even migration in each of the lanes. Buffer is recirculated through the gel chamber by a reciprocating solenoid pump at a rate of about 450 ml/min. The buffer is chilled in its reservoir tank by cold water (5°C) circulated through a glass tubing heat exchanger. Buffer temperature is thus maintained at 13-15°C throughout a typical run (17, 24).

Running Conditions for PFGE

PFGE separations are sensitive to a variety of different molecular and environmental variables. The principle significant variables are the molecular properties of the DNA, the pulse time, the electrical field shape, the electrical field strength, the gel composition, the sample concentration and the temperature.

1. Pulse Time: In PFGE, DNA is subjected alternately to two electrical fields at different angles for a time called the pulse time. The molecules must presumably change direction prior to net translational motion. Each time the field is switched, larger molecules take longer to change direction and have less time to move during each pulse, so they migrate slower than smaller molecules. Molecules so small that their reorientation time is short compared to the pulse time will spend most of the pulse duration in conventional electrophoretic motion where size resolution is quite limited. As a result of this, resolution in PFGE is likely to be optimal for molecules with reorientation times comparable to the pulse time. At applied field strengths of about 10 V/cm, 0.1 s pulse times resolve DNA optimally in the 5-kb size range, while pulse times of 1,000 s at 3 V/cm are used to resolve 3-7 Mb molecules. Pulse times are selected so that DNA molecules of a targeted size spend most of the duration of the pulse reorienting rather than moving through the gel, which accounts for the long periods of time, usually days or weeks, needed to fractionate large DNA molecules. The chromosomal DNA molecules of *Saccharomyces cerevisiae* in the 10 Mb range requires langer electrophoresis of approximately one week (25).

2. Electrical Field Shape: A number of different electrical field configurations were employed in early PFGE experiments. It was apparent that certain aspects at the field shape were critical in achieving high-resolution PFGE separations. Electrical field strength can be adjusted to tune the size range of effective PFGE resolution. The resolution of PFGE is affected by the number and configuration of the electrodes used, because these alter the shape of the applied electrical fields. The most critical variable appears to be the angle between the alternate electrical fields. The most effective electrode configurations yield angles of more than 110°. A continually increasing angle between the fields produces band sharpening that greatly enhances the resolution. The angle between the alternate fields is always greater than 90° where good resolution is observed. In cases of excellent resolution, field angles typically range from 120° to 150°. In contrast, where poor resolution was seen, the field angles ranged typically from 110° to 150° (25). Angles of 90° or smaller are not effective, probably because the DNA molecules easily become oriented midway between the two applied fields. Angles larger than 90° are more effective (25). While more complete studies on optimum angles are needed, it is clear that angles in the range of 120°-150° provide very high resolution (25). Field strengths that decrease, or angles that increase, progressively along the direction of the net DNA motion produce band sharpening because the molecules at the front of each DNA zone always migrate more slowly than those at the rear.

3. Electrical Field Strength: Electrophoretic mobility is defined as the velocity per unit field. In most ordinary electrophoresis, the mobility is independent of field strength. This independence is expected if the properties of the molecules are not directly altered by a separation process. The field strength affects mobility in two ways. The mobilities of 100-500 kb DNA show an approximately linear dependence on field strength. The field strength affects the DNA size of the transition between the two zones of resolution (25).

4. Reorientation Angle: The widening of the reorientation angle should yield sharper bands and better resolution. The separation of yeast chromosomes is nearly identical for those chromosomes separated with reorientation angles of 110° and 165°. However, when reorientation angles from 105° to 165° are used to separate molecules in the size range of *S. cerevisiae* (200-3,000 kb), there is a 4-fold difference among the DNA velocities observed with these different angles (1). The increase in mobility obtained with smaller reorientation angles is even more pronounced when separating larger molecules. Most commercially available pulsed-field gel boxes use a fixed angle of 120° between the alternating fields.

5. Voltage: As with switch time, the choice of the voltage used in PFGE must also be varied with the size of the DNA to be separated. While voltage gradients of 6-10 V/cm can be used to separate molecules up to 1 Mb, resolving molecules larger than this in pulsed field gels requires a reduction in voltage gradient (14, 24). Separation of chromosomes from the yeast *S. pombe* (3 Mb, 5 Mb and 6 Mb) requires that voltage gradients do not exceed 2 V/cm. The even larger chromosomes of *N. crassa* (larger than 12 Mb) were separated at 1.5 V/cm (15). The practical effect is to increase the run times for larger DNA molecules. Thus, electrophoretic separation of *N. crassa* chromosomes required up to 7 days. When the voltage gradient is reduced to separate large DNAs, switching intervals must be lengthened (15).

6. Temperature: In conventional gel electrophoresis, DNA molecules were run at room temperature but, in PFGE, DNA was run at a low temperature (between 4°C and 15°C). Temperature has a dramatic effect on DNA mobility in PFGE. Temperatures between 14°C and 22°C are generally regarded as the best compromise between speed and resolution while gels can be run at room temperature, it is usually necessary to circulate the buffer through a heat exchanger to dissipate the heat generated by the voltage gradients used during most pulsed field runs (2, 25). The velocity of lambda DNA at 34°C is twice that at 4°C. However, gels run at temperatures as high as 34°C show diminished resolution (1).

7. Switch interval: The single most important determinant of mobility in PFGE is the interval at which the direction of the electric field is switched. If the switching interval is increased beyond the time required for a fragment to reorient, then the fragment will spend a large portion of the gel run migrating, as in conventional electrophoresis, with a resulting loss in resolution. The choice of an appropriate switching interval for PFGE must reflect the size range of the fragments to be resolved. Birren et al. (24) have measured the velocity of DNA molecules from 50 to 1,000 kb in PFGE with switch times of from 5 to 300 s. The highest

resolution for molecules of a given size is obtained by using the shortest switch intervals which permit separation of the complete size range of the fragments (26).

8. Agarose Concentration: The agarose concentration will affect the separation obtained with PFGE. Faster DNA migration occurs in gels of lower agarose concentration. The λ DNA monomer (48.5 kb) migrates 50% faster in PFGE of 0.6% agarose compared to a 1% gel. The DNA bands which are quite diffuse in the 0.7% gel become increasingly sharp as the agarose concentration is raised in 1.4% and 1.8% gels over identical times. The distance migrated by the identical samples demonstrated the decrease in velocity as the agarose concentration is increased (24).

9. Restriction Enzymes: The ability of restriction enzymes (REs) to cut DNA at a specific sequence of bases has greatly stimulated the growth of recombinant DNA technology. Over 1,900 REs are known, and of there 275 are available from companies based around the world (27). The common restriction enzymes, *EcoR* I and *Hind* III, digest bacterial and mammalian DNA to fragments averaging approximately 4 kb in sizes much too small for PFGE. For this reason, it is advisable to use enzymes which have relatively few sites and give larger fragments from the target DNA in PFGE (27). Any enzyme producing a large number of small fragments (smaller than 10 kb) is unlikely to be useful for PFGE and mapping. A major factor in selecting suitable restriction enzymes is the base composition (%G+C content) of the target DNA. Analysis by PFGE and rare-restriction enzymes have been useful for obtaining relevant information on genome size, characterizing various strains at the DNA level (28), following the genetic history of a particular strain, constructing physical and genetic maps of bacterial chromosomes and studying chromosomal dynamics among bacteria (29-31). Enzymes that recognize sequences larger than 6 bp are potentially useful in genome mapping because they generate large fragments (27). At present, there are nine restriction enzymes commercially available with an 8-bp recognition sequence. Of these, *Not I, Sfi I, Srf I, Ase I, Pac I* and *Swa I* are rare-cutters in genomes with a G+C content of about 35-55%. Below and above these margins the number of fragments becomes too small and too large, respectively. *Pac I* (AATTAATT), *Swa I* (ATTTAAAT), *Pme I* (GTTTAAAC) and *Sse* 83871 (CCTGCAGG) are new on the market (32). On the other hand, *Pac I* and *Swa I* enzymes should be useful especially for genomes with a G+C contents in the range of 45-65% (32). The 4-bp pair sequence 5'-CTAG-3' seems to be selected against in most bacterial genomes (33). This tetranucleotide is part of the recognition sequence of *Spe I* (A/CTAGT), *Xba I* (T/CTAGA), *Nhe I* (G/CTAGC) and *Avr II* (C/CTAGG). CTAG is found infrequently in most prokaryotes and restriction endonucleases that include this sequence in their recognition sequence cut bacterial genomes infrequently (32). PFGE of large fragments of DNA generated using infrequently cutting *Swa* I and *Pac* I restriction endonucleases were used in genome analysis of *Xanthomonas axonopodis* pv. *vesicatoria*, a causal agent of leaf spot disease of pepper and tomato, and optimal conditions for digestion in the genome analysis were determined (26, 34-36).

Pulsed-Field Applications

Full understanding of a biological system requires knowledge of the structure and function of the genes and their arrangement on the chromosome. PFGE has been used to separate DNA molecules as large as 12 Mb. The ability to analyze such large fragments will greatly facilitate the construction of physical maps (1).

The ability to separate, isolate and analyze megabase size fragments of DNA is already providing insights into the genome organization of organisms as diverse as bacteria and humans (2).

PFGE is used in the following areas:

1. The advent of PFGE techniques for the resolution of large DNA molecules has provided a new analysis approach for bacterial genomes (37). The PFGE of DNA fragments obtained using different enzymes is a powerful technique for quick resolution of the bacterial genome into a small number of large fragments.

2. The PFGE of DNA fragments obtained by using endonucleases produce a discrete pattern of bands useful for the fingerprinting and physical mapping of the chromosome (38).

3. The PFGE technique is useful to establish the degree of relatedness among different strains of the same species (38).

4. PFGE has proved to be an efficient method for genome size estimation and the construction of chromosomal maps, as well as being useful for the characterization of bacterial species (39-41). PFGE technology has proven invaluable for the accurate estimation of genome size and in the construction of physical maps of a diverse range of prokaryotic organisms (42,43).

5. PFGE is a powerful tool for genome characterization and has led to the construction of the physical map of more than 180 bacterial chromosomes (44).

6. PFGE has proven extremely powerful in the analysis of large DNA molecules from a variety of sources including intact chromosomal DNAs from fungi (16), parasitic protozoa (45) and specifically fragmented genomes of bacteria (26, 38) and mammals (5, 6).

7. PFGE simplifies many previously laborious investigations and makes possible many new investigations. This technique has been used extensively in application to all organisms from bacteria to viruses (2).

8. Yeast Artificial Chromosome (YAC) libraries have been constructed by PFGE (2).

9. PFGE experiments are used in the construction of transgenic mice (2).

10. PFGE has also shown itself useful in the study of radiation-induced DNA damage and repair, size organization and variation in mammalian centromers (2, 46).

11. Many investigations directly involve studies of genome organization. For example, in determining the physical proximity of two genes, while determining the size of very large genes or when identifying the location of chromosomal breaks (2, 46).

12. PFGE will play a major role in the mapping of the human genome (47). The physical mapping of a human chromosome involves ordering and measuring the distances between a set of DNA markers that are unique to that chromosome. Given the large sizes of the chromosomes involved (50,000-300,000 kb), PFGE is the method to pursue (47).

13. PFGE is used to determine the order of markers more precisely than is possible with genetic linkage analysis, and with a PFGE map available, new markers can be quickly localized within that region (32).

14. A new mutation can be mapped by cloning the gene, followed by restriction analysis and hybridization, to a set of ordered restriction fragments by PFGE (47).

15. PFGE will greatly facilitate the precise selection of large DNA fragments for cloning. REs which are specific for cutting infrequently occurring sequences, are used to create large DNA fragments which are then separated by PFGE. By blotting and hybridization the fragments containing the desired gene are determined. This region is recovered from the gel and cloned (2, 23).

16. PFGE allows for easy isolation of the individual restriction fragments for further restriction mapping, gene insertion and functional gene mapping (13).

Results

The developments in PFGE over the past decade have led to an ingenious collection of workable designs. Today's instruments resolve DNA several orders of magnitude larger than was possible by conventional electrophoresis, permitting the direct study of intact chromosomes from yeasts and human parasites. Investigations now center on a better understanding of these techniques, which will support the design of new generations of devices to separate even larger fragments and, eventually, entire human genomes.

The analysis of entire genomes represents a revolutionary approach to genetics, and the availability of vast amounts of information from these projects will allow new conceptual approaches in many areas of biological research. It is clear that at the present time, no single technique or strategy is sufficient for preparing a map of an intact human chromosome. However, the combined use of many powerful new techniques brings the analysis of mammalian genomes within reach.

PFGE has made possible the development of the YAC cloning system and will play a major role in the mapping of the human genome. The PFGE technique will be useful for establishing the degree of relatedness among different strains of the same species.

Future applications for PFGE techniques may include protein separations and nucleic acid sequencing and studies of DNA topology. PFGE allows physical-map construction for virtually any organisms. PFGE techniques should soon provide physical maps and their applications for a wide number of microorganism. As physical mapping methods are rapidly displacing genetic methods for chromosome assignment, it is essential to understand the behavior of circular DNA species on pulsed-field gels.

Analysis with PFGE can be used to establish long-range maps based on anonymous markers that have been shown to be genetically linked. Ideally, the targeted region is saturated with markers, allowing the construction of several overlapping maps. However, for plant genomes, markers are rarely clustered with sufficient density to allow the immediate construction of detailed maps based on several markers.

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