# Advances in Electrophoresis

Edited by A. Chrambach,
M. J. Dunn, B. J. Radola

Volume 4

| | |
|---|---|
| Gemmill | Pulsed Field Gel Electrophoresis |
| Gersten and Zapolski | Detection and Quantification of DNA in Gels and Blots |
| Aebersold | Sequence Analysis of Proteins |
| Riesner et al. | Temperature-Gradient Gel Electrophoresis |
| Rothe | Pore Gradient Gel Electrophoresis of Enzymes |
| Giometti and Taylor | Two-Dimensional Electrophoresis in Mutation Studies |
| Takagi | Electrophoresis of Membrane Proteins |

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**Volume 1**

*M. J. Dunn,* Two-dimensional polyacrylamide gel electrophoresis
*C. R. Merril,* Detection of proteins separated by electrophoresis
*J. M. Gershoni,* Protein blotting: a tool for the analytical biochemist
*N. C. Stellwagen,* Electrophoresis of DNA in agarose and polyacrylamide gels
*K. Takeo,* Affinity electrophoresis
*P. Gebauer et al.,* Recent trends in capillary isotachophoresis
*R. Horuk,* Preparative polyacrylamide gel electrophoresis of proteins
*J. G. Sutton,* Red cell enzyme markers in forensic science: methods of separation and some important applications

**Volume 2**

*A. Görg et al.,* The current state of electrofocusing in immobilized pH gradients
*W. Thormann* and *R. A. Mosher,* Theory of electrophoretic transport and separations: the study of electrophoretic boundaries and fundamental separation principles by computer simulation
*D. Tietz,* Evaluation of mobility data obtained from gel electrophoresis: strategies in the computation of particle and gel properties on the basis of the extended Ogston model
*R. J. Cooke,* Electrophoresis in plant testing and breeding
*C. Damerval et al.,* Two-dimensional electrophoresis in plant biology
*S. M. Hanash,* Contribution of protein electrophoretic analysis to cancer research
*G. Unteregger,* Two-dimensional electrophoresis of nonhistone chromosomal proteins with emphasis on protein prefractionation

**Volume 3**

*J. E. Celis et al.,* Protein databases derived from the analysis of two-dimensional gels
*M. J. Miller,* Computer-assisted analysis of two-dimensional gel electrophoretograms
*K. E. Willard-Gallo,* Immunological applications of two-dimensional polyacrylamide gel electrophoresis
*F. Foret* and *P. Boček,* Capillary electrophoresis
*A. W. Preece* and *K. A. Brown,* Recent trends in particle electrophoresis
*P. Gill,* The forensic application of DNA profiling

# Preface

It seems appropriate to reflect on the reasons behind the production of an annual review series on electrophoresis. The monthly journal *Electrophoresis* provides space for reviews and so do many other methodological journals. Moreover, the meetings of the international and some national electrophoresis societies produce proceedings also containing review articles. The purpose of *Advances in Electrophoresis* is to assemble these multiple sources into a central "review bank" that is readily available to everyone using electrophoretic methods.

A central review bank should provide a forum for the authoritative voices in each specialized field of electrophoresis, thereby helping to resolve problems created by discordant advice at different levels of expertise. It should serve to unify research areas whose results are published in a wide range of journals, for example, those of the two most challenging classes of substances – proteins and nucleic acids. Rather than summarizing all available information, the reviews in *Advances in Electrophoresis* present the essence of each topic and demonstrate its potential. The reviews are directed to the great many readers who already use electrophoretic techniques but do not follow their development in the original literature. Also, the reviews should be indispensible to those interested in the application of a new technique or entering a field requiring the use of electrophoresis. Ideally, the reviews will be the key references for the following years in a particular area.

*Advances in Electrophoresis* contains reviews dealing either with selected techniques or important areas of application of electrophoresis. We have already alluded to the need for reviews on methodological progress. However, we consider it equally essential to provide reviews on important areas of application. Electrophoresis is not an esoteric method employed by only a small group of experts. On the contrary, its range of applications is increasing at an astonishing pace and, in many areas, it is already established as an indispensible tool. By publishing in *Advances in Electrophoresis* a balanced blend of reviews covering applications and techniques we expect a crossfertilizing effect which should stimulate further developments in the field of electrophoresis.

In order to accomplish these aims, we should like to encourage our readers to send us their comments, criticisms and suggestions for important topics to be included in forthcoming volumes. Finally, we wish to thank the authors for the sacrifice they have made in filling these pages and thereby in providing the field of electrophoresis with its first centralized retrieval bank.

October 1990

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# Contents

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# PULSED FIELD GEL ELECTROPHORESIS

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**Abbreviations: CHEF**, contour clamped homogeneous electric field; **EEO**, electroendosmosis; **FIGE**, field inversion gel electrophoresis; **kb**, kilobase pairs; **Mb**, megabase pairs; **OFAGE**, orthogonal field alteration gel electrophoresis; **PACE**, programmable autonomously-controlled electrode; **PFG**, pulsed field gel; **PFGGE**, pulsed field gradient gel electrophoresis; **PHOGE**, pulsed homogeneous orthogonal field gel electrophoresis; **TAFE**, transverse alternating field electrophoresis; **YAC**, yeast artificial chromosome

# 1 Introduction

The development of recombinant DNA technologies over nearly the last 20 years has resulted in an explosion of information about gene structure and function that continues unabated today. Size separation of DNA molecules using agarose and polyacrylamide gel electrophoresis is a critical component of these techniques. It is therefore surprising that our theoretical understanding of DNA electrophoresis, both on the macro and molecular scales, is so inadequate. Recent direct observations of DNA molecular motions during electrophoresis [1–3] underscore the inadequacy. The puzzling inability of standard (single field) gel electrophoresis to fractionate DNA molecules greater than about 30–50 kilobases (kb) in size led to the idea that DNA molecules above this size must orient in the electric field resulting in migration at rates independent of size. This limitation was overcome in spectacular fashion by the development of pulsed field gel (PFG) electrophoresis [4]. This novel technique is capable of resolving DNA molecules in excess of 5 000 000 bp (5 megabases or Mb), and the upper limit is still unknown. This represents at least a 100-fold increase in the maximum size of resolvable DNA molecules and has led to a revolution in molecular genetics. For example, it is now possible to electrophoretically separate the chromosomes of many lower eukaryotes, including the yeasts, and to rapidly examine very large chromosomal regions in mammalian genomes. PFG electrophoresis has been rapidly adopted in laboratories studying the molecular genetics of diverse organisms in spite of the inadequate understanding of how the method achieves separations. Increased study of the pulsed field phenomenon by theoretical and applied biophysicists should provide the foundation so necessary to effective utilization by other investigators.

Electrophoresis is normally performed with a uniform electric field that does not change strength or direction over time. In contrast, PFG electrophoresis utilizes at least two electric fields that are alternately activated and that act at an angle to the direction of migration. These fields, in some versions of PFG, may be of different strengths or durations. The reptation model [5] was invoked to explain why large DNA fragments migrate at a rate independent of size and thus are not resolved by single field electrophoresis. Still larger DNA fragments typically cannot even enter the gel matrix and remain trapped at the origin. How can two alternately activated electric fields, applied in different directions, achieve size-dependent migration of large DNA molecules? First, the alternate fields may force large DNA to undergo cyclical orientation and reorientation with each field change. This could achieve separations because larger stretched molecules require longer times for reorienta-

tion in a changed electric field [4]. Second, the fields may force molecules to retrace all or a portion of their previous migration paths [6]. Longer molecules would spend more time than shorter molecules retracing previous paths. Third, alternate fields could promote disentanglement of very large DNA molecules trapped on the gel fiber [1]. Models incorporating some of these notions have been proposed and will be reviewed in Section 7.

Many parameters are known to affect pulsed field separations including pulse time, electric field strength, field angle and shape, agarose type and concentration, ionic strength and temperature. Reorientation, retracing of migration paths and disentanglement could readily be affected by all of these parameters. Thus, careful attention to and control of all parameters is essential for development of a more accurate understanding of the PFG phenomenon.

Since the seminal experiments demonstrating the pulsed field effect were reported by Schwartz and Cantor [4], a wide variety of methods have been reported that achieve the same or similar separations [7, 8] on various types of apparatus (Section 2). Clearly each apparatus reported operates on the same fundamental principle, *i.e.*, cyclical application of two alternating electric fields during the electrophoretic separation. Each of these reports has provided further insight into the applications of PFG separations and has added to our understanding of how this separation technique works. Unfortunately, the variable parameters that affect PFG separations are not unequivocally defined by all of the methods (see below) and even when so defined, have not been reported in consistent ways. This frequently prevents rigorous comparisons between the methods. While it is therefore possible, at this time, to select separation conditions and apparatus to achieve a particular separation similar to those already reported, it is difficult as yet to derive the optimal conditions for new separation problems from general principles derived from the separation conditions and the apparati used to date. An attempt is made in this review to pose the relevant questions that can lead us toward predicting and optimizing conditions of PGF electrophoresis in the future.

This review is intended to provide those with a theoretical or applied interest in electrophoresis with an overview of PFG technology and applications. It will cover designs for PFG apparatus, parameters known to affect separations of large DNA, the separations achieved with such gels and theories proposed to explain these separations. Direct observations of individual DNA molecules undergoing migration have now been reported and modeled [3, 19, 27]. These observations will be summarized and used to illustrate the inade-

quacies of earlier theories attempting to explain DNA electrophoresis and PFG separations. Finally, there is an overview of the applications and impact of PFG-separations on molecular genetics. A number of methods' papers [9–11] are available to permit the novice to begin using this technique [10] and several companies offer commercial versions of PFG equipment. Olson [12] has recently published an excellent review of pulsed field gel electrophoresis to which the readers' attention is directed. Other reviews have appeared [7, 13, 14] in the past. Although some duplication here is unavoidable, every effort will be made to provide a different viewpoint toward this complex field than has been presented previously.

## 2 The field geometries of pulsed field gel electrophoretic devices

The original description of size-dependent separations achievable with pulsed electric fields appeared over six years ago [4, 15]. Since that time a rather confusing diversity of devices that employ the pulsed electric field concept have been reported. It is now possible to make several generalizations regarding these devices and the separations they achieve. First, it is clear that uniform electric fields provide the best conditions for separation and that distortion inherent in many devices were the result of distortions in the electric field. Second, all PFG separations are achieved for the same physico-chemical reasons, even though these reasons are only poorly understood.

Development of PFG technology occurred simultaneously with development of methods to handle large DNA molecules without shear damage. The immobilization of large DNA in low melting point agarose will be described briefly followed by a definition for electric field angles and interaction angles essential to discussion of each PFG design. This is followed by a description of each reported PFG device. It is hoped this will untangle the confusing array of names that have been applied to those systems and help the reader see the fundamental similarities of each (see also [12]).

### 2.1 Size standards

Observations on large DNA separations could only be achieved given a method to preserve the intact size of such molecules. Immobilization of living cells

and whole viral particles in low gelling temperature agarose [4] followed by detergent lysis and proteinase K digestion has been found to yield very high molecular weight, intact DNA in a form that can be analyzed on pulsed field gels. Moreover, such preparations can be readily treated with restriction endonucleases to achieve site specific cleavage.

A set of molecular weight markers has proven extremely valuable for comparisons of separations achieved using different PFG apparati under varying operational parameters. Markers have included the chromosomes of the yeasts, *Saccharomyces cerevisiae* (200 to 2000 kb) [16] and *Schizosaccharomyces pombe* (3 chromosomes of 3.5, 4.5 and 5.7 Mb) [17]. Linear concatemers of lambda phage DNA (monomer = 48.5 kb) have provided standards that typically entend above 1 Mb. Different lambda strains with different genome sizes have been used to generate concatemers with different repeat distances. This has demonstrated that PFG separations are internally consistent and yield band positions reflective of expected physical size with little or no sequence specific effects [18]. Additional molecular weight standards have included the genomes of T4 (167 kb), T5 (116 kb), T7 (40 kb) and G phages (760 kb) [16, 19].

## 2.2 Field angle

PFG separations all utilize at least two alternating electric fields that do not coincide. The angle between the two electric fields is an important parameter that is known to affect PFG separations. To simplify the discussion of each device that follows, it is important to define the angles between the applied fields, and between these fields and the direction of net sample migration down the gel. The field angle $\alpha$ or $\beta$ is defined as the angle between the DNA migration path and each of the separate electric fields (Fig. 1). The sum of the field angles determines the direction of migration along the gel. The sum of the absolute values of the two field angles is designated here as the interaction angle, $\theta$. The sideways components of the electric field vectors cancel leading to net migration along a path defined by the bisector of $\theta$.

## 2.3 Pulsed field gradient gel electrophoresis

Schwartz and Cantor's [4] design for their pulsed field gradient gel electrophoresis (PFGGE) chamber is shown schematically in Fig. 2A. The apparatus contained arrays of vertically mounted platinum electrodes ("point elec-