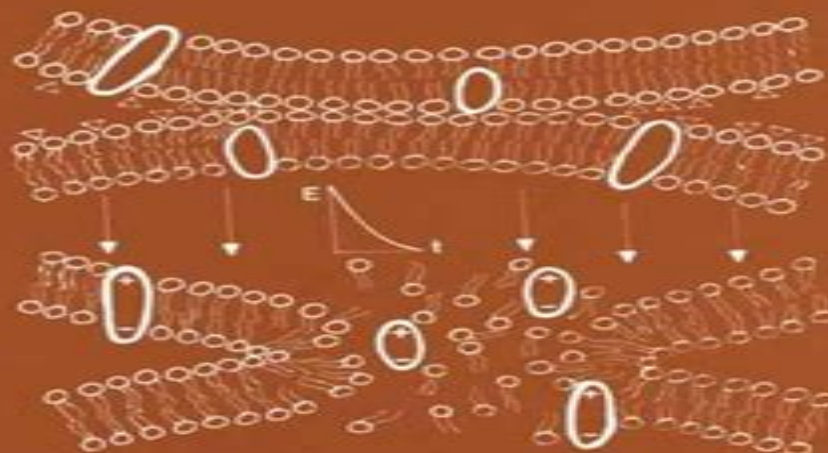


Methods in Molecular Biology™

Volume 48

ANIMAL CELL ELECTROPORATION & ELECTROFUSION PROTOCOLS

Edited by
Jac A. Nickoloff



Animal Cell Electroporation And Electrofusion Protocols Methods In Molecular Biology

John M. Walker



Animal Cell Electroporation And Electrofusion Protocols Methods In Molecular Biology:

Animal Cell Electroporation and Electrofusion Protocols Jac A. Nickoloff, 2008-02-03 The ability to introduce macromolecules into animal cells including DNA RNA proteins and other bioactive compounds has facilitated a broad range of biological studies from biochemistry and biophysics to molecular biology cell biology and whole animal studies Gene transfer technology in particular will continue to play an essential role in studies aimed at improving our understanding of the relationships between the gene structure and function and it has important practical applications in both biotechnology and biomedicine as evidenced by the current intense interest in gene therapy Although DNA and other macromolecules may be introduced into cells by a variety of methods including chemical treatments and microinjection electroporation has proven to be simpler to perform more efficient and effective with a wider variety of cell types than other techniques The early and broad success of electric field mediated DNA transfer soon prompted researchers to investigate electroporation for transferring other types of molecules into cells including RNA enzymes antibodies and analytic dyes **Animal Cell Electroporation and Electrofusion Protocols** begins with three chapters that describe the theoretical and practical aspects of electroporation including a review of the commercially available instrumentation These introductory chapters will be of particular interest to those new to electric field technologies and to those developing protocols for as yet untested species or cell types Nineteen chapters follow that present well tested protocols for electroporation of proteins and DNA into insect fish and mammalian cells

Methods in Molecular Biology: Animal cell electroporation and electrofusion protocols

John M. Walker, 1984

Animal Cell Electroporation and Electrofusion Protocols. Methods in Molecular Biology

Jac A. Nickoloff, 1995 This superb collection of well tested protocols for the electroporation of proteins and DNA into insect fish and mammalian cells also includes proven animal cell electrofusion techniques for studies of somatic cell genetics and development and for generating monoclonal antibodies It is distinguished by its coverage of important model cell types from many organisms and tissue types and its detailed instructions for the growth and preparation of specific cells to achieve optimum transfection Its extensive reference lists citations of alternative transfer methods advice on pitfalls to avoid and descriptions of expected results ensure readily reproducible results even for beginners **Plant Cell Electroporation And Electrofusion Protocols**

Jac A. Nickoloff, 2008-02-02 Gene transfer is an essential technology for improving our understanding of gene structure and function Although there are many methods by which DNA may be introduced into cells including heat and chemical treatments and microinjection electroporation has been found to be the most versatile gene transfer technique Electroporation is effective with a wide variety of cell types including those that are difficult to transform by other means For many cell types electroporation is either the most efficient or the only means known to effect gene transfer The early and broad success of electric field mediated DNA transfer soon prompted researchers to investigate electroporation for transferring other types of molecules into cells including RNA enzymes antibodies and analytic dyes The

first section of Plant Cell Electroporation and Electrofusion Protocols includes two chapters that serve as a guide to theoretical and practical aspects of electroporation and will be of particular interest to those developing protocols for as yet untested species or cell types and a third chapter that describes commercially available electroporation instruments The remaining chapters describe well tested protocols for DNA electrotransfection electroporation of other biomolecules or cell electrofusion These chapters also include brief discussions of alternatives to electric field based methods citing the advantages and limitations of the various methods for achieving specific goals Handbook of Membrane Separations Anil Kumar Pabby, Syed S.H. Rizvi, Ana Maria Sastre Requena, 2008-07-07 The Handbook of Membrane Separations Chemical Pharmaceutical and Biotechnological Applications provides detailed information on membrane separation technologies as they have evolved over the past decades To provide a basic understanding of membrane technology this book documents the developments dealing with these technologies It explores T Cell Protocols Kelly P. Kearse, 2008-02-02 The purpose of T Cell Protocols Development and Activation is to collect a series of protocols particularly those that have been developed within the past few years to help investigators master new techniques or improve existing ones for the study of T cell Biology Invariably in putting together a book like this it is difficult to decide which methods to include and which to leave out To this end methods were selected from a variety of disciplines including cellular immunology biochemistry and molecular biology to try to provide something of interest for everyone who works on T cell development and activation I would like to mention that my primary reason for agreeing to put this book together is that when I was a graduate student I purchased a copy of Selected Methods in Cellular Immunology by Mishell and Shigii which proved a tremendous help in learning the basics of one and two dimensional gel techniques and other methods The cover has long since fallen off but it still remains one of my most valued reference books for the laboratory It is my hope that T Cell Protocols Development and Activation will prove similarly useful to current and future scientists wishing to learn new methods for exploring the development and activation of T cells

Immunochemical Protocols John Pound, 2008-02-03 This much anticipated second edition provides a user friendly up to date handbook of reliable immunochemical techniques optimized for molecular biologists It covers the breadth of relevant established methods from protein blotting and immunoassays through to visualization of cellular antigens and in situ hybridization each with their latest refinements Protocols for the production and purification of important classes of immunochemical reagents are also provided including conventional and recombinant antibodies fusion proteins and their various conjugates This book will open the door to a new generation of immunochemical reagents with exciting possibilities

Glycoanalysis Protocols Elizabeth F. Hounsell, 2008-02-02 Glycoanalysis Protocols Second Edition makes available to all protein scientists and particularly those working in today's pharmaceuticals industry the most advanced and reproducible glycoanalysis techniques These detailed up to date and proven analytical methods cover the areas of glycoprotein macromolecular structural analysis oligosaccharide profiling lipid conjugate characterization microorganism structure

determination and proteoglycan function They also include advanced analytical techniques in biotechnology during the production of recombinant glycoproteins and other therapeutics These protocols will well serve anyone starting work on the analysis of glycoproteins as well as experienced investigators seeking to carry their expertise to higher levels of accomplishment

Plant Virology Protocols Gary D. Foster, Sally Taylor, 2008-02-03 The aim of Plant Virology Protocols is to provide a source of information to guide the reader through the wide range of methods involved in generating transgenic plants that are resistant to plant viruses To this end we have commissioned a wide ranging list of chapters that will cover the methods required for plant virus isolation RNA extraction cloning coat protein genes introduction of the coat protein gene into the plant genome and testing transgenic plants for resistance The book then moves on to treatments of the mechanisms of resistance the problems encountered with field testing and key ethical issues surrounding transgenic technology Although Plant Virology Protocols deals with the cloning and expression of the coat protein gene the techniques described can be equally applied to other viral genes and nucleotide sequences many of which have also been shown to afford protection when introduced into plants The coat protein has however been the most widely applied and as such has been selected to illustrate the techniques involved Plant Virology Protocols has been divided into six major sections containing 55 chapters in total

Gene Isolation and Mapping Protocols Jacqueline Boulton, 2008-02-02 An unprecedented collection of all the most up to date techniques for gene isolation and mapping including the latest methods for gene characterization using database analyses This collection of thoroughly tested recipes also includes chapters for the computational analysis of novel cDNA sequences with up to the minute information on basic sequence analysis sequence similarity searches exon detection and similarity searches and the prediction of gene function Its state of the art methods constitute indispensable tools for all scientists engaged in the search for specific disease genes or in the general advancement of the human genome project

Antibody Engineering Protocols Sudhir Paul, 2008-02-02 This comprehensive collection of recently developed methods for producing new antibody reagents by immunization and recombinant DNA techniques contains ready to use protocols that illuminate current areas of research on antibody structure functions and applications The methods can be applied in basic immunological studies involving antibody specificity catalysis and evolution and in the isolation of rare antibodies by phage display technology and the engineering of new antibodies by mutagenesis They offer insight into new ways of developing clinically useful antibody reagents Antibody Engineering Protocols constitutes a single source volume for laboratory investigators who want to minimize extensive literature and methodology searches and to work productively in their fields with reproducible step by step protocols

Protein Purification Protocols Shawn Doonan, 2008-02-02 Hans Neurath has written that this is the second golden era of enzymology Protein Science 1994 vol 3 pp 1734 1739 he could with justice have been more general and referred to the second golden age of protein chemistry The last two decades have seen enormous advances in our understanding of the structures and functions of proteins arising on the one hand from improvements and

developments in analytical techniques see the companion volume Basic Protein and Peptide Protocols in this series and on the other hand from the technologies of molecular genetics Far from turning the focus away from protein science the ability to isolate analyze and express genes has increased interest in proteins as gene products Hence many laboratories are now getting involved in protein isolation for the first time either as an essential adjunct to their work in molecular genetics or because of a curiosity to know more about the products of the genes that they have been studying Protein Purification Protocols is aimed mainly at these newcomers to protein purification but it is hoped that it will also be of value to established practitioners who may find here techniques that they have not tried but which might well be most applicable in their work With the exception mainly of the first and last chapters the format of the contributions to the present book conform to the established format of the Methods in Molecular Biology series

Polyamine Protocols David M. L. Morgan, 2008-02-02 A unique collection of hands on enzyme assay techniques to study polyamines and their function The techniques range from assay methods for enzymes of polyamine biosynthesis and catabolism to measurements of polyamines polyamine transport and polyamine effects on cell growth The methods are presented by leading researchers who have perfected them to a high art and include clear step by step instructions with numerous hints and tips to ensure readily reproducible results

Handbook of Biochemistry and Molecular Biology, 2010-05-21 Edited by renowned protein scientist and bestselling author Roger L Lundblad with the assistance of Fiona M Macdonald of CRC Press this fourth edition of the Handbook of Biochemistry and Molecular Biology represents a dramatic revision the first in two decades of one of biochemistry's most referenced works This edition gathers a wealth of information not easily obtained including information not found on the web Offering a molecular perspective not available 20 years ago it provides physical and chemical data on proteins nucleic acids lipids and carbohydrates Presented in an organized concise and simple to use format this popular reference allows quick access to the most frequently used data Covering a wide range of topics from classical biochemistry to proteomics and genomics it also details the properties of commonly used biochemicals laboratory solvents and reagents Just a small sampling of the wealth of information found inside the handbook Buffers and buffer solutions Heat capacities and combustion levels Reagents for the chemical modification of proteins Comprehensive classification system for lipids Biological characteristics of vitamins A huge variety of UV data Recommendations for nomenclature and tables in biochemical thermodynamics Guidelines for NMR measurements for determination of high and low pKa values Viscosity and density tables Chemical and physical properties of various commercial plastics Generic source based nomenclature for polymers Therapeutic enzymes About the Editors Roger L Lundblad Ph D Roger L Lundblad is a native of San Francisco California He received his undergraduate education at Pacific Lutheran University and his PhD degree in biochemistry at the University of Washington After postdoctoral work in the laboratories of Stanford Moore and William Stein at the Rockefeller University he joined the faculty of the University of North Carolina at Chapel Hill He joined the Hyland Division of Baxter Healthcare in 1990

Currently Dr Lundblad is an independent consultant and writer in biotechnology in Chapel Hill North Carolina He is an adjunct Professor of Pathology at the University of North Carolina at Chapel Hill and Editor in Chief of the Internet Journal of Genomics and Proteomics Fiona M Macdonald Ph D F R S C Fiona M Macdonald received her BSc in chemistry from Durham University UK She obtained her PhD in inorganic biochemistry at Birkbeck College University of London studying under Peter Sadler Having spent most of her career in scientific publishing she is now at Taylor and Francis and is involved in developing chemical information products

Basic Cell Culture Protocols Jeffrey W. Pollard, John M. Walker, 1997 Now completely revised and updated from the original much acclaimed and bestselling first edition Basic Cell Culture Protocols 2nd ed offers today's most comprehensive collection of easy to follow cutting edge protocols for the culture of a wide range of animal cells Its authoritative contributors provide explicit step by step instructions along with extensive notes and tips that allow both experts and beginners to successfully achieve their desired results Topics range from basic culture methodology to strategies for culturing previously uncultured cell types and hard to culture differentiated cells Methods are also provided for the analysis of living cells by FACS video microscopy and confocal microscopy Like the first edition this book should be in every cell culture laboratory and be of use to all who use cell cultures in research

Protein Targeting Protocols Roger A. Clegg, 2008-02-04 It is by no means a revelation that proteins are not uniformly distributed throughout the cell As a result the idea that protein molecules because of the specificity with which they can engage in interactions with other proteins may be aimed via these interactions at a restricted target is a fundamental one in contemporary molecular life sciences The target may be variously conceived as a specific molecule a group of molecules a structure or a more generic type of intracellular environment Because the concept of protein targeting is intuitive rather than explicitly defined it has been variously used by different groups of researchers in cell biology biochemistry and molecular biology For those working in the field of intracellular signaling an influential introduction to the topic was the seminal article by Hubbard Cohen TIBS 1993 18 172-177 which was based on the work of Cohen's laboratory on protein phosphatases Subsequently the ideas that they discussed have been further developed and extended by many workers to other key intermediaries in intracellular signaling including protein kinases and a great variety of modulator and adaptor proteins

2-D Proteome Analysis Protocols Andrew J. Link, 2008-02-02 With the completion of sequencing projects and the advancement of analytical tools for protein identification proteomics the study of the expressed part of the genome has become a major region of the burgeoning field of functional genomics High resolution 2-D gels can reveal virtually all proteins present in a cell or tissue at any given time including posttranslationally modified proteins Changes in the expression and structure of most cellular proteins caused by differentiation or external stimuli can be displayed and eventually identified using 2-D protein gels 2-D Proteome Analysis Protocols covers all aspects of the use of 2-D protein electrophoresis for the analysis of biological problems The contributors include many of the leaders in the fields of biochemistry and analytical chemistry who were instrumental in the development

of high resolution 2 D gels immobilized pH gradients computer analysis and mass spectrometry based protein identification methodologies This book is intended as a benchtop manual and guide both for novices to 2 D gels and for those aficionados who wish to try the newer techniques Any group using protein biochemistry especially in the fields of molecular biology biochemistry microbiology and cell biology should find this book eminently useful 2 D Proteome Analysis Protocols takes the researcher through the complete process of working with 2 D protein gels from making the protein tract to finally identifying the proteins of interest It includes protocols for generating 2 D protein extracts from most of the standard model organisms including bacteria yeast nematode Drosophila plants mouse and human Advanced Electroporation Techniques in Biology and Medicine Andrei G. Pakhomov, Damijan Miklavcic, Marko S. Markov, 2010-06-02 A reflection of the intense study of the effects of electromagnetic fields on living tissues that has taken place during the last decades Advanced Electroporation Techniques in Biology and Medicine summarizes most recent experimental findings and theories related to permeabilization of biomembranes by pulsed electric fields Edited by experts and **Combinatorial Peptide Library Protocols** Shmuel Cabilly, 2008-02-02 During the course of evolution an imbalance was created between the rate of vertebrate genetic adaptation and that of the lower forms of living organisms such as bacteria and viruses This imbalance has given the latter the advantage of generating relatively quickly molecules with unexpected structures and features that carry a threat to vertebrates To compensate for their weakness vertebrates have accelerated their own evolutionary processes not at the level of whole organism but in specialized cells containing the genes that code for antibody molecules or for T cell receptors That is when an immediate requirement for molecules capable of specific interactions arose nature has preferred to speed up the mode of Darwinian evolution in preference to any other approach such as the use of X ray diffraction studies and computergraphic analysis Recently Darwinian rules have been adapted for test tube research and the concept of selecting molecules having particular characteristics from random pools has been realized in the form of various chemical and biological combinatorial libraries While working with these libraries we noticed the interesting fact that when combinatorial libraries of oligopeptides were allowed to interact with different selector proteins only the actual binding sites of these proteins showed binding properties whereas the rest of the protein surface seemed inert This seemingly common feature of protein having no extra potential binding sites was probably selected during evolution in order to minimize nonspecific interactions with the surrounding milieu **Forensic DNA Profiling Protocols** Patrick J. Lincoln, Jim Thomson, 1998-01-22 This state of the art collection of easily reproducible methods includes all of the major techniques of DNA analysis currently used in forensic identity testing The methods include the recovery of DNA from a large range of sample types analysis of DNA as single and multi locus VNTR probes PCR amplification of STR and other loci and mitochondrial sequencing The expert scientists writing here many from laboratories around the world also discuss how to interpret the results in cases of unknown identity and disputed parentage Covers all steps from extraction of human DNA through to analysis and interpretation Takes advantage of

new methodologies such as capillary electrophoresis Clear step by step instructions ensure unfailing reproducibility

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