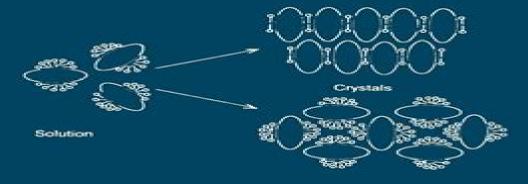
# **Methods in Molecular Biology**

Volume 27

# BIOMEMBRANE PROTOCOLS

II. Architecture and Function

Edited by John Graham and Joan Higgins



Jac A. Nickoloff

Methods in Molecular Biology: Biomembrane protocols: II. Architecture and function John M. Walker, 1984 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 facili tated 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 el troporation 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 inter est 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 **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 pro teins 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 tech nologies 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 laborato ries 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 newcom ers 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 Immunocytochemical Methods and Protocols Lorette C. Javois, 2008-02-02 The principle that antibodies can be used as cytochemical agents provided they are tagged with suitable markers has been

evident for over 50 years During this time the use of immunocytochemical methods has spread to a wide array of biological disciplines Early applications focused on the detection of microbial antigens in tissues while more recent applications have used monoclonal antibodies to study cell differentiation during embryonic development For a select few disci plines volumes have been published focusing on the specific application of immunocytochemical techniques to that discipline What distinguishes the present book Immunocytochemical Meth ods and Protocols from earlier books is its broad appeal to researchers in all disciplines including those in both research and clinical settings. The methods and protocols presented here are designed to be general in their application and the accompanying Notes provide invaluable assistance in adapting or troubleshooting the protocols Interspersed throughout the book are chapters providing overviews of select topics related to immunocytochemistry Capillary Electrophoresis Guidebook Kevin D. Altria, 2008-02-02 This book is intended to be a working guide to the operation of capillary electrophoresis CE instrumentation Since CE is still a rap idly maturing technique detailed validated protocols are not widely established Therefore extensive experimental procedures are not pro vided for individual analyses The intention is to provide general guide lines on the principles and practice of CE and to give an overview of the specific technologies and important application areas Part I provides operating instructions for standard commercially available instruments Guidelines are included for activities such as changing capillaries method development quantitative procedures optimization of precision and sensitivity and the validation of meth ods fraction collection and troubleshooting as well as a quick guide to running a separation The application range of CE is possibly the most diverse of all analytical techniques and ranges from large complex macromolecules such as proteins and nucleic acids to small solutes such as organic drugs and inorganic anions and cations 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 Chromosome Analysis Protocols John R. Gosden, 2008-02-02 Chromosomes as the genetic vehicles provide the basic material for a large proportion of genetic investigations from the construction of gene maps and models of chromosome organization to the inves tigation of gene function and dysfunction The study of chromosomes has developed in parallel with other aspects of molecular genetics beginning with the first preparations of chromosomes from animal cells through the development of banding techniques which permitted the unequivocal identification of each chromosome in a karyotype to the present

analytical methods of molecular cytogenetics Although some of these techniques have been in use for many years and can be learned relatively easily most published scientific reports as a result of pressure on space from editors and the response to that pressure by authors contain little in the way of technical detail and thus are rarely adequate for a researcher hoping to find all the necessary information to embark on a method from scratch A new user needs not only a detailed description of the methods but also some help with problem solving and sorting out the difficulties en countered in handling any biological system This was the require ment to which the series Methods in Molecular Biology is addressed and Chromosome Analysis Protocols forms a part of this series Plant Cell Electroporation And Electrofusion Protocols Jac A. Nickoloff, 2008-02-02 Gene transfer is an essential technology for improving our under standing of gene structure and function Although there are many meth ods 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 medi ated DNA transfer soon prompted researchers to investigate electroporation for transferring other types of molecules into cells in cluding 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 alterna tives to electric field based methods citing the advantages and limitations of the various methods for achieving specific goals 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 Computer Analysis of Sequence Data Part II Annette M. Griffin, Hugh G. Griffin, 2008-02-02 DNA sequencing has become increasingly efficient over the years resulting in an enormous increase in the amount of data gener ated In recent years the focus of sequencing has shifted from being the endpoint of a project to being a starting point This is especially true for such major initiatives as the human genome project

where vast tracts of DNA of unknown function are sequenced This sheer volume of available data makes advanced computer methods essential to analysis and a familiarity with computers and sequence analysis software a vital requirement for the researcher involved with DNA sequencing Even for nonsequencers a familiarity with sequence analysis software can be important For instance gene sequences already present in the databases can be extremely useful in the design of cloning and genetic manipulation experiments This two part work on Computer Analysis of Sequence Data is designed to be a practical aid to the researcher who uses computers for the acquisition storage or analysis of nucleic acid and or pro tein sequences Each chapter is written such that a competent scien tist with basic computer literacy can carry out the procedure successfully at the first attempt by simply following the detailed prac tical instructions that have been described by the author A Notes section which is included at the end of each chapter provides advice on overcoming the common problems and pitfalls sometimes encoun tered by users of the sequence analysis software **Cryopreservation and Freeze-Drying Protocols** John G. Day, Mark R. McLellan, 2008-02-02 The storage of biological material for regular or future use is a fundamental requirement in many biological and medical sciences Cryopreservation and freeze drying are the preferred techniques for achieving long term storage and have been applied to a diverse range of biological materials Though the basis for many methodologies is common laboratories frequently lack expertise with the correct storage procedures so that many apply outdated or inappropriate protocols for storing their samples or cultures Cryopreservation and Freeze Drying Protocols is a compilation of the many and varied methodologies that have been developed in expert laboratories. The protocols are reproducible robust and in most instances have been transferred quite successfully to other laboratories Our intended readers are those proposing to establish or improve biostorage systems in their own laboratories or units whether concerned with culture collections animal husbandry aguaculture or human fertilization programs Because the emphasis of Cryopreservation and Freeze Drying Protocols is on methodology it is our intention to provide readers with the tools to make practical progress without reference to other sources Each chapter deals with an organelle cell or tissue type a short int duction on the status of its biostorage development is followed by a detailed description of the materials required and a methodological p tocol to be followed with explanatory notes This is very much a first edition we hope and trust that future editions will contain cryopreservation and freeze drying protocols for ceils tissues and organs that are at present still recalcitrant to succe ful preservation **Gene Isolation and Mapping Protocols** Jacqueline Boultwood, 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 In Vitro Transcription and Translation Protocols Martin J. Tymms, 2008-02-02 Most laboratories conducting studies that use molecular biology techniques employ in vitro transcription and translation systems as a routine part of their day to day research The commercial availability of purified bacterial RNA polymerase and the availability of robust tra lation systems has made in vitro systems attractive not only as an alt native to the in vivo expression of genes but also as good model systems for studying specific aspects of transcription and translation Although fairly efficient eukaryotic translation systems have been established for a number of years reconstitution of transcription in vitro has proved to be more difficult Recent improvements in fractionation techniques and the cloning of proteins involved in transcription have made this a fast moving area of research Considerable progress has also been made in recent years in developing in vitro systems to study transcription and translation in chloroplasts and mitochondria together with systems for the study of protein import In Vitro Transcription and Translation Protocols provides many detailed experimental procedures for prokaryotic transcription and translation systems together with protocols for many key techniques used in the analysis of eukaryotic transcription In keeping with the successful format of preceding volumes of the Methods in Molecular Biology series step by step instructions are provided together with extensive notes that cover troubleshooting and special tips considered important Signal Transduction Protocols David A. Kendall, Stephen J. Hill, 2008-02-02 As our understanding of the biological sciences expands the bou aries between traditional disciplines tend to blur at the edges Physio gists and pharmacologists for instance now need to embrace techniques that until recently were the strict preserves of biochemists and mole lar biologists However the acquisition of new technologies can be a time consuming and frustrating business and unless an expert is on hand to give instruction precious hours can be spent poring over half described Methods sections with no guarantee of eventual success The aim of Signal Transduction Protocols has been to get experts with hands on experience in particular techniques to give detailed accounts of experimental protocols in a recipe type format which we hope will circumvent the problems of ambiguity often encountered when reading the literature The techniques described in Signal Transduction Protocols are those that we think will be most useful in addressing questions in the area of receptor mediated cell signaling with particular regard to those receptors that are part of the G protein linked superfamily To keep it to a manageable size we have omitted any reference to electrophysi ogy and have instead concentrated on more biochemical ICN ICN Pharmaceuticals, inc, 1999 **Electroporation Protocols for Microorganisms** Jac A. approaches Nickoloff, 2008-02-02 Electroporation is one of the most widespread techniques used in modern molecular genetics It is most commonly used to introduce DNA into cells for investigations of gene structure and function and in this regard electroporation is both highly versatile being effective with nearly all species and cell types and highly efficient For many cell types electroporation is either the most efficient or the only means known to effect gene transfer However exposure of cells to brief hi intensity electric fields has found broad application in other aspects of biological research and is now routinely

used to introduce other types of biological and analytic molecules into cells to induce cell cell fusion and to transfer DNA directly between different species The first seven chapters of Electroporation Protocols for Micro organisms describe the underlying theory of electroporation the com mercially available instrumentation and a number of specialized electroporation applications such as cDNA library construction and interspecies DNA electrotransfer Each of the remaining chapters pre sents a well developed method for electrotransformation of a particular bacterial fungal or protist species These chapters also serve to intro duce those new to the field the important research questions that are currently being addressed with particular organisms highlighting both the major advantages and limitations of each species as a model organ ism and explaining the roles that electroporation has played in the development of the molecular genetic systems currently in use

cDNA Library Protocols Ian G. Cowell, Caroline A. Austin, 2008-02-02 The first libraries of complementary DNA cDNA clones were con structed in the mid to late 1970s using RNA dependent DNA polymerase reverse transcriptase to convert poly A mRNA into double stranded cDNA suitable for insertion into prokaryotic vectors Since then cDNA technology has become a fundamental tool for the molecular biologist and at the same time some very significant advances have occurred in the methods for con structing and screening cDNA libraries It is not the aim of cDNA Library Protocols to give a comprehensive review of all cDNA library based methodologies instead we present a series of up to date protocols that together should give a good grounding of proce dures associated with the construction and use of cDNA libraries In deciding what to include we endeavored to combine up to date versions of some of the most widely used protocols with some very usefiil newer techniques cDNA Library Protocols should therefore be especially useful to the investigator who is new to the use of cDNA libraries but should also be of value to the more experienced worker Chapters 1 5 concentrate on cDNA library construction and manipula tion Chapters 6 and 7 describe means of cloning difficult to obtain ends of cDNAs Chapters 8 18 give various approaches to the screening of cDNA libraries and the remaining chapters present methods of analysis of cDNA clones including details of how to analyze cDNA sequence data and how to make use of the wealth of cDNA data emerging DNA'Protein Interactions G. Geoff Kneale, 2008-02-02 The study of protein nucleic acid from the human genome project interactions is currently one of the most rapidly growing areas of molecular biology DNA binding proteins are at the very heart of the regulation and control of gene expression replication and recombination Enzymes that recognize and either modify or cleave specific DNA sequences are equally important to the cell Some of the techniques reported in this volume can be used to identify previously unknown DNA binding proteins from crude cell extracts Virtually all are capable of giving direct information on the molecular basis of the interaction the location of the DNA binding site the strength and specificity of binding the identities of individual groups on specific bases involved in binding the specific amino acid residues of the protein that interact with the DNA or the effects of protein binding on gross conformation and local structure of DNA The recognition of DNA sequences by proteins is a complex phenomenon involving specific hydrogen bonding contacts to the

DNA bases direct readout and or interactions with the sugar phos phate backbone indirect readout The latter interactions can also be highly specific because of sequence dependent conformational changes in the DNA In addition intercalation of planar aromatic amino acid side chains between the DNA bases can occur most notably with single stranded DNA binding proteins Furthermore when bound many DNA binding proteins induce drastic structural changes in the DNA as an integral Membrane Analysis Dr John Graham, Joan Higgins, 2020-08-13 Membrane Analysis provides a comprehensive review of laboratory methods for membrane study with an emphasis on isolating membranes analysing their composition and architecture and investigating membrane function **Diagnostic Bacteriology Protocols** Jenny Howard, David M. Whitcombe, 2008-02-03 Enhanced public awareness of microbiological issues and increased governmental regulation have ensured that microbiology and bacteriology remain important from the academic laboratory to a broad range of indus trial environments Since the techniques used in bacteriology vary widely and have advanced rapidly of late it has become increasingly desirable to gather a collection of up to date protocols from a comprehensive range of disciplines into a single volume Diagnostic Bacteriology Protocols thus aims to provide a wide variety of up to date protocols for the identification and differentiation of bacteria from pure or mixed cultures and for the study of bacteria at the genus through to subspecies levels In addition we aim to provide methods covering a range of complexities some requiring little or no special equipment others exploiting recent advances in sophisticated instrumentation or novel nucleic acid based technology. We also present techniques to study a variety of aspects of bacteria from the features of whole cells and their growth through their enzyme activities and structural components to nucleic ac based analysis

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