

Methods in Molecular Biology™

VOLUME 173

Calcium-Binding Protein Protocols

*Volume II
Methods and Techniques*

Edited by

Hans J. Vogel



 **HUMANA PRESS**

Calcium Binding Protein Protocols Volume 2 Methods And Techniques Methods In Molecular Biology

Jeff Braman



Calcium Binding Protein Protocols Volume 2 Methods And Techniques Methods In Molecular Biology:

Calcium-Binding Protein Protocols Hans J. Vogel, 2008-02-05 Calcium plays an important role in a wide variety of biological processes. This divalent metal ion can bind to a large number of proteins by doing so it modifies their biological activity or their stability. Because of its distinct chemical properties, calcium is uniquely suited to act as an on/off switch or as a light dimmer of biological activities. The two books entitled *Calcium Binding Protein Protocols Volumes I and II* focus on modern experimental analyses and methodologies for the study of calcium binding proteins. Both extracellular and intracellular calcium binding proteins are discussed in detail. However, proteins involved in calcium handling, e.g., calcium pumps and calcium channels, fall outside the scope of these two volumes. Also, calcium binding proteins involved in bone deposition will not be discussed as this specific topic has been addressed previously. The focus of these two books is on studies of the calcium binding proteins and their behavior in vitro and in vivo. The primary emphasis is on protein chemistry and biophysical methods. Many of the methods described will also be applicable to proteins that do not bind calcium. *Calcium Binding Protein Protocols* is divided into three main sections. The section entitled *Introduction and Reviews* provides information on the role of calcium in intracellular secondary messenger activation mechanisms. Moreover, unique aspects of calcium chemistry and the utilization of calcium in dairy proteins as well as calcium binding proteins involved in blood clotting are addressed.

Immunotoxin Methods and Protocols Walter A. Hall, 2008-02-02 Immunotoxins represent a new class of human therapeutics that have widespread applications and a potential that has not yet been fully recognized since they were first conceived of by Paul Ehrlich in 1906. The majority of advances in the development and implementation of immunotoxins has occurred over the last 20 years. The reasons for this use of immunotoxins in basic science and clinical research are the powerful concurrent advances in genetic engineering and receptor physiology. Recombinant technology has allowed investigators to produce sufficient quantities of a homogeneous compound that allows clinical trials to be performed. The identification of specific receptors on malignant cell types has enabled scientists to generate immunotoxins that have had positive results in clinical trials. As more cellular targets are identified in coming years, additional trials will be conducted in different disease states affecting still larger patient populations. Modulation of the immune system to decrease the humoral response to immunotoxins may improve their overall efficacy. As increasingly more effective compounds are generated, it will be necessary to decrease the local and systemic toxicity associated with these agents and methods for doing so are presently being developed. The work presented in *Immunotoxin Methods and Protocols* focuses on three specific areas of immunotoxin investigation that are being conducted by experts throughout the world. The first section describes the construction and development of a variety of immunotoxins. **Cytoskeleton Methods and Protocols** Ray H. Gavin, 2008-02-03 Over the past two decades, experimental studies have solidified the interpretation of the cytoskeleton as a highly dynamic network of microtubules, actin, microfilaments, intermediate filaments, and myosin filaments. Rather than a network of disparate fibers

these polymers are often interconnected and display synergy which is the combined action of two or more cytoskeletal polymers to achieve a specific cellular structure or function Cross communication among cytoskeletal polymers is thought to be achieved through cytoskeletal polymer accessory proteins and molecular motors that bind two or more cytoskeletal polymers Development of the modern concept of the cytoskeleton is a direct outgrowth of advances in experimental tools and reagents that are available to cell and molecular biologists Technological advances and refinements in cell imaging have made it possible to selectively image a single cytoskeletal polymer and monitor its dynamics through the use of fluorescence probes in vitro and in vivo Two decades ago cytoskeletal research was limited to a few perturbation reagents that included colchicine and cytochalasin Today the perturbation arsenal has expanded to a highly selective group of reagents that includes Taxol nocodazole benomyl latrunculin jasplakinolide and such endogenous proteins as gelsolin These reagents enable the investigator to selectively perturb or destroy a cytoskeletal polymer while leaving other cytoskeletal polymers intact Site specific monoclonal antibodies that target a specific cytoskeletal polymer have proven to be highly selective affinity tools for cytoskeletal research

Connexin Methods and Protocols Roberto Bruzzone, Christian Giaume, 2008-02-05 Direct cell-cell communication is a common property of multicellular organisms that is achieved through membrane channels which are organized in gap junctions The protein subunits of these intercellular channels the connexins form a multigene family that has been investigated in great detail in recent years It has now become clear that in different tissues connexins speak several languages that control specific cellular functions This progress has been made possible by the availability of new molecular tools and the improvement of basic techniques for the study of membrane channels as well as by the use of genetic approaches to study protein function in vivo More important connexins have gained visibility because mutations in some connexin genes have been found to be linked to human genetic disorders Connexin Methods and Protocols presents in detail a collection of techniques currently used to study the cellular and molecular biology of connexins and their physiological properties The field of gap junctions and connexin research has always been characterized by a multidisciplinary approach combining morphology biochemistry biophysics and cellular and molecular biology This book provides a series of cutting edge protocols and includes a large spectrum of practical methods that are available to investigate the function of connexin channels Connexin Methods and Protocols is divided into three main parts

Transgenesis Techniques Alan R. Clarke, 2008-02-05 The past decade has witnessed a spectacular explosion in both the development and use of transgenic technologies Not only have these been used to aid our fundamental understanding of biologic mechanisms but they have also facilitated the development of a range of disease models that are now truly beginning to impact upon our approach to human disease Some of the most exciting model systems relate to neurodegenerative disease and cancer where the availability of appropriate models is at last allowing radically new therapies to be developed and tested This latter point is of particular significance given the current concerns of the wider public over both the use of animal

models and the merits of using genetically modified organisms Arguably advances of the greatest significance have been made using mammalian systems driven by the advent of embryonic stem cell based strategies and more recently by cloning through nuclear transfer For this reason this new edition of Transgenesis Techniques focuses much more heavily on manipulation of the mammalian genome both in the general discussions and in the provision of specific protocols

Cytokines and Colony Stimulating Factors Dieter Körholz,Wieland Kiess,2008-02-04 The immune system is a complex network in which different cell types and soluble factors interact to efficiently eliminate various kinds of microorganisms as well as aberrant cell clones The roots of immunologic investigations reach far into the past In 430 BC Thucydides reported that survivors of the plague did not present a second time with similar symptoms The first report of a successful immunotherapy was made by Edward Jenner in 1798 who found a protective effect of cowpox vaccination against human pox Since then much knowledge has been accumulated today investigations of the molecular mechanisms of immune regulation are of central research interest The novel insights into gene polymorphisms and gene regulation gathered from this work has improved our knowledge of individual immune reactions and risk factors in overcoming infections Strategies to use the immune system for cancer treatment have been propelled by the discovery of divergent immunoregulatory cytokines and the introduction of new gene therapy strategies to modify immune responses Recently the discovery of various dendritic cells has focused attention on these cell types as central elements of the immune response and to the possibility of dendritic cell expansion maturation and consecutive stimulation with immuno active tumor specific peptides Similarly methods for ex vivo expansion of various stem cell derived cell types have led to an improved therapeutic management of various benign and malignant diseases

In Vitro Mutagenesis Protocols Jeff Braman,2008-02-05 Hands on researchers with proven track records describe in stepwise fashion their advanced mutagenesis techniques The contributors focus on improvements to conventional site directed mutagenesis including a chapter on chemical site directed mutagenesis PCR based mutagenesis and the modifications that allow high throughput mutagenesis experiments and mutagenesis based on gene disruption both in vitro and in situ based Additional methods are provided for in vitro gene evolution for gene disruption based on recombination transposon and cassette mutagenesis and for facilitating the introduction of multiple mutations Time tested and highly practical the protocols in In Vitro Mutagenesis Protocols 2nd Edition offer today s molecular biologists reliable and powerful techniques with which to illuminate the proteome

RT-PCR Protocols Nicola King,Joe O'Connell,2008-02-04 Until the mid 1980s the detection and quantification of a specific mRNA was a difficult task usually only undertaken by a skilled molecular biologist With the advent of PCR it became possible to amplify specific mRNA after first converting the mRNA to cDNA via reverse transcriptase The arrival of this technique termed reverse transcription PCR RT PCR meant that mRNA suddenly became amenable to rapid and sensitive analysis without the need for advanced training in molecular biology This new accessibility of mRNA which has been facilitated by the rapid accumulation of sequence data for human

mRNAs means that every biomedical researcher can now include measurement of specific mRNA expression as a routine component of his/her research plans. In view of the ubiquity of the use of standard RT-PCR, the main objective of RT-PCR Protocols is essentially to provide novel useful applications of RT-PCR. These include some useful adaptations and applications that could be relevant to the wider research community who are already familiar with the basic RT-PCR protocol. For example, a variety of different adaptations are described that have been employed to obtain quantitative data from RT-PCR. Quantitative RT-PCR provides the ability to accurately measure changes in specific mRNA expression between normal and diseased tissues.

Thyroid Hormone Receptors Aria Baniahmad, 2008-02-05 A panel of outstanding investigators surveys and explains the major cutting edge methods used in thyroid receptor (TR) research and explains their practical experimental details. Described in step by step detail to ensure robust experimental results, the techniques presented cover a wide variety of key areas including TR in development and knockout mouse and *Xenopus* transcriptional regulation by TRs in both cell free systems and in living cells and TR mutant analysis of patients. Additional methods provide powerful tools for the isolation of TR regulated protein complexes for studying the oncogene *v-ErbA* in blood cell differentiation and for target gene analysis in the brain. Microarray chip methods are also presented for analyzing the organs of transgenic mice to identify target genes in the liver.

Neurogenetics Nicholas T. Potter, 2008-02-02 The rapid identification and characterization of genes of neurological relevance holds great potential for offering insight into the diagnosis, management and understanding of the pathophysiologic mechanisms of neurological diseases. This volume in the *Methods in Molecular Biology*™ series was conceived to highlight many of the contemporary methodological approaches utilized for the characterization of neurologically relevant gene mutations and their protein products. Although an emphasis has been placed upon descriptions of methodologies with a defined clinical utility, it is hoped that *Neurogenetics: Methods and Protocols* will appeal not only to clinical laboratory diagnosticians but also to clinicians and to biomedical researchers with an interest in advances in disease diagnosis and the functional consequences of neurologically relevant gene mutations. To meet this challenge, more than 60 authors graciously accepted my invitation to contribute to the 32 chapters of this book. Through their collective commitment and diligence, what has emerged is a comprehensive and timely treatise that covers many methodological aspects of mutation detection and screening, including discussions on quantitative PCR, trinucleotide repeat detection, sequence based mutation detection, molecular detection of imprinted genes, fluorescence in situ hybridization (FISH), in vitro protein expression systems and studies of protein expression and function. I would like to take this opportunity to formally thank my colleagues for their effort and dedication to this work.

Mycotoxin Protocols Mary W. Trucksess, Albert E. Pohland, 2008-02-05 Mycotoxins produced by molds are common contaminants of many important crops including wheat, corn, rice and peanuts. Some mycotoxins are found in fruits and vegetables. These contaminants have a broad range of toxic effects including carcinogenicity, neurotoxicity and reproductive and developmental toxicity. The occurrence of mycotoxins in

foods is an unavoidable worldwide problem About 80 countries have imposed regulatory limits to minimize human and animal exposure to mycotoxins Regulatory limits including international standards have tremendous economic impact and must be developed using science based risk assessments The purpose of Mycotoxin Protocols is to provide the scientific and technological basis for analytical methods for use in obtaining the exposure data needed for risk assessments Mycotoxin Protocols is divided into four sections which are interconnected The first section Chapters 1 5 describe the general techniques for mycotoxin analysis with emphasis on the importance of method validation based on statistical parameters sampling procedures for collecting a sample as representative as possible of a bulk lot the isolation of mycotoxins for use as analytical standards or for toxicological studies the evaluation of purity and preparation of standards and the detection and identification of impurities in isolated mycotoxins Sections 2 4 Chapters 6 19 describe the most current chromatographic and immunochemical methods for studies on the major mycotoxins

Neurotrophin Protocols Robert A. Rush, 2008-02-03 The past decade has seen an extraordinary growth in research interest in neurotrophic factors and the study of the neurotrophin family has led this activity Nevertheless this area of research has often struggled as a result of techniques that were either inadequate or just emerging from other research fields and disciplines Neurotrophin Protocols has brought together many leaders in the neurotrophin field who detail their special expertise in a wide variety of techniques Though most procedures are valid across many different fields of research some of those described here have been developed to address particular issues within the neurotrophic factor field The protocols cover a broad range of biochemical histological and biological techniques that are often required by the modern laboratory However all have been written with sufficient detail to allow any laboratory to achieve proficiency without need of reference to other texts Neurotrophin Protocols is divided into four sections dealing with protein RNA recombinant and in vivo techniques Protein techniques have in general been less successfully employed than those dealing with RNA or DNA However procedures that achieve localization and quantification of the neurotrophins are now being used more extensively Their inclusion here should assist further studies at the protein level Transgenic cell lines and animals are commonplace in the scientific research literature but their inclusion in several chapters in this book provide some novel uses that are not readily available elsewhere

Proteoglycan Protocols Renato V. Iozzo, 2008-02-02 Proteoglycans are some of the most elaborate macromolecules of mammalian and lower organisms The covalent attachment of at least five types of glycosaminoglycan side chains to more than forty individual protein cores makes these molecules quite complex and endows them with a multitude of biological functions Proteoglycan Protocols offers a comprehensive and up to date collection of preparative and analytical methods for the in depth analysis of proteoglycans Featuring step by step detailed protocols this book will enable both novice and experienced researchers to isolate intact proteoglycans from tissues and cultured cells to establish the composition of their carbohydrate moieties to generate strategies for prokaryotic and eukaryotic expression to utilize methods for the suppression of specific proteoglycan gene

expression and for the detection of mutant cells and degradation products and to study specific interactions between proteoglycans and extracellular matrix proteins as well as growth factors and their receptors The readers will find concise yet comprehensive techniques carefully drafted by leading experts in the field Each chapter commences with a general Introduction followed by a detailed Materials section and an easy to follow Methods section An asset of each chapter is the extensive notation that includes troubleshooting tips and practical considerations that are often lacking in formal methodology papers The reader will find this section most valuable because it is clearly provided by experienced scientists who have first hand knowledge of the techniques they outline In addition most of the chapters are well illustrated with examples of typical data generated with each method

Recombinant Antibodies for Cancer Therapy Martin

Welschof,Jürgen Krauss,2008-02-05 Since the advent of hybridoma technology more than two decades ago numerous antibodies have entered the clinical setting as potent therapeutic agents Their repeated application in humans however is limited by the development of human antimouse antibodies HAMA in the recipient leading to allergic reactions against the foreign murine protein and rapid neutralization To circumvent these limitations many new antibodies have recently been tailored through recombinant antibody technology The initial clinical data show encouraging results thus demonstrating the potential of these new therapeutic agents The purpose of *Recombinant Antibodies for Cancer Therapy* is to present a collection of detailed protocols in recombinant antibody technology It is primarily addressed to scientists working on recombinant antibodies as well as clinicians involved with antibody based therapies As with other volumes of this series we placed the main focus on providing detailed protocols describing procedures step by step Moreover each protocol supplies a troubleshooting guide containing detailed information on possible problems and hints for potential solutions Antibody technology is a subject of constant and rapid change This volume therefore does not attempt to cover all possible current experimental approaches in the field Rather we present carefully selected protocols written by competent authors who have successfully verified the particular method described Given our own professional backgrounds and interest in oncology we chose to concentrate chiefly on therapeutic agents for cancer patients

DNA Methylation Protocols Ken I. Mills,Bernie H.

Ramsahoye,2008-02-05 *DNA Methylation Protocols* offer a set of readily reproducible protocols of the analysis of DNA methylation and methylases These powerful methods provide the tools necessary for studying methylation at both the global level and the level of sequence and include many techniques for identifying genes that might be aberrantly methylated in cancer and aging Additional methods cover genome wide analysis of abnormal DNA methylation and the isolation and measurement of demethylases and related proteins

Gene Knockout Protocols Martin J. Tymms,Ismail Kola,2008-02-03

As the major task of sequencing the human genome is near completion and full complement of human genes are catalogued attention will be focused on the ultimate goal to understand the normal biological functions of these genes and how alterations lead to disease states In this task there is a severe limitation in working with human material but the mouse has

been adopted as the favored animal model because of the available genetic resources and the highly conserved gene conservation linkage organization. In just of ten years since the first gene targeting experiments were performed in embryonic stem ES cells and mutations transmitted through the mouse germline more than a thousand mouse strains have been created. These achievements have been made possible by pioneering work that showed that ES cells derived from preimplantation mouse embryos could be cultured for prolonged periods without differentiation in culture and that homologous recombination between targeting constructs and endogenous DNA occurred at a frequency sufficient for recombinants to be isolated. In the next few years the mouse genome will be systematically altered and the techniques for achieving manipulations are constantly being streamlined and improved.

Single Nucleotide Polymorphisms Pui-Yan Kwok, 2008-02-04 A comprehensive collection of readily reproducible techniques for the difficult process of single nucleotide polymorphisms SNP discovery and genotyping. These cutting edge protocols for mutation SNP detection utilize denaturing high performance liquid chromatography dHPLC single strand conformation polymorphism SSCP conformation sensitive gel electrophoresis CSGE chemical cleavage and direct sequencing. Equally powerful and up to date methods are given for genotyping SNPs including molecular beacons the Taqman assay single base extension approaches pyrosequencing ligation the Invader assay and primer extension with mass spectrometry detection.

PCR Detection of Microbial Pathogens Konrad Sachse, Joachim Frey, 2003 Hands on laboratory experts present a set of classic PCR based methods for the identification and detection of important animal and food microbial pathogens including several zoonotic agents. These proven techniques can be precisely applied to a wide variety of microbes among them *Campylobacter* spp *Chlamydiae* toxigenic *Clostridia* *Escherichia coli* STEC *Listeria monocytogenes* *Mycoplasmas* *Salmonellae* and *Yersinia enterocolitica*. Additional chapters review the specificity and performance of diagnostic PCR analysis the pre PCR processing of samples the critical aspects of standardizing PCR methods and the general issues involved in using PCR technology for microbial diagnosis.

Transgenic Mouse Methods and Protocols Marten H. Hofker, Jan van Deursen, 2008-02-04 Marten Hofker and Jan van Deursen have assembled a multidisciplinary collection of readily reproducible methods for working with mice and particularly for generating mouse models that will enable us to better understand gene function. Described in step by step detail by highly experienced investigators these proven techniques include new methods for conditional induced knockout and transgenic mice as well as for working with mice in such important research areas as immunology cancer and atherosclerosis. Such alternative strategies as random mutagenesis and viral gene transduction for studying gene function in the mouse are also presented.

Tumor Suppressor Genes Wafik S. El-Deiry, 2008-02-03 It has become clear that tumors result from excessive cell proliferation and a corresponding reduction in cell death caused by the successive accumulation of mutations in key regulatory target genes over time. During the 1980s a number of oncogenes were characterized whereas from the 1990s to the present the emphasis has shifted to tumor suppressor genes TSGs. It has become clear that oncogenes and TSGs function in

the same pathways providing positive and negative growth regulatory activities The signaling pathways controlled by these genes involve virtually every process in cell biology including nuclear events cell cycle cell death cytoskeletal cell membrane angiogenesis and cell adhesion effects Mutations in tumor suppressor genes have been identified in familial cancer syndromes and the same genes in many cases have been found to be mutationally inactivated in sporadically occurring cancers In their normal state TSGs control cancer development and progression as well as contribute to the sensitivity of cancers to a variety of therapeutics Understanding the classes of TSGs the biochemical pathways they function in and how they are regulated provides an essential lesson in cancer biology We cannot hope to advance our current knowledge and to develop new and more effective therapies without understanding the relevant pathways and how they influence the present approaches to therapy Moreover it is important to be able to access not only the powerful tools now available to discover these genes but also their links to cell biology and growth control

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Introduction

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