

Springer Protocols

Methods in Molecular Biology 586

Cytoskeleton Methods and Protocols

Second Edition

Edited by

Ray H. Gavin

 **Humana Press**

Cytoskeleton Methods And Protocols

Ray H. Gavin



Cytoskeleton Methods And Protocols:

Cytoskeleton Methods and Protocols Ray H. Gavin, 2009-10-02 In this cutting edge book internationally renowned experts present techniques which reflect many of the recent technological advances in experimental tools for cytoskeleton research There is emphasis on animal plant protist and fungal model systems

Cytoskeleton Ray H. Gavin, 2016 The third edition of this volume focuses on experimental models that are useful for investigating various aspects of cytoskeleton structure and function Animal plant protist and fungal models highlight twenty four chapters that provide detailed protocols for live and fixed cell imaging dynamics of cytoskeleton components cell and organelle motility and genetics and proteomics Written in the highly successful Methods in Molecular Biology series format protocols in each chapter are up to date menus organized in a useful step by step format appropriate for novice and established investigators Each chapter is equipped with a valuable notes section that provides a troubleshooting guide and helpful and often unpublished technical information aimed at ensuring success with implementation of the protocols Authoritative and thorough Cytoskeleton Methods and Protocols Third Edition helps researchers expand their understanding of cytoskeleton structure and function

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 result of growth 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

[The Plant Cytoskeleton](#) Patrick J. Hussey, Pengwei Wang, 2023-02-11 This detailed volume explores the development of technologies and protocols that are currently being used to understand the nature and activities of the plant cytoskeleton A focus for many of the chapters is on sample preparation as the quality of plant organ tissue preparation from single to multicellular samples determines the quality of the data Written for the highly successful Methods in Molecular Biology series chapters include

introductions to their respective topics lists of the necessary materials and reagents step by step and readily reproducible laboratory protocols and tips on troubleshooting and avoiding known pitfalls Authoritative and practical The Plant Cytoskeleton Methods and Protocols serves as an ideal guide for researchers interested in or starting to be interested in plant cell and molecular biology research **Methods in Molecular Biology: Cytoskeleton methods and protocols** John M. Walker, 1984 **Cytoskeleton Dynamics** Helder Maiato, 2021-01-10 This volume details comprehensive state of the art methods on actin microfilaments and microtubules and how they work to achieve different cellular functions in different cellular contexts Chapters guide readers through protein purification in vitro reconstitution of several cytoskeleton properties analyses of microtubule and actin based structures functional dissection of post translational modifications and roles in several biological processes Written in the highly successful Methods in Molecular Biology series format chapters include introductions to their respective topics lists of the necessary materials and reagents step by step readily reproducible laboratory protocols and tips on troubleshooting and avoiding known pitfalls Authoritative and cutting edge Cytoskeleton Dynamics Methods and Protocols aims to provide a wide range of experimental approaches and be an invaluable resource for present and future generations of cytoskeleton researchers The chapter Visualization and Functional Analysis of Spindle Actin and Chromosome Segregation in Mammalian Oocytes is available open access under a Creative Commons Attribution 4.0 International License via link.springer.com **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 **Matrix Metalloproteinase Protocols** Ian M. Clark, 2008-02-05 Research in the matrix metalloproteinase field began with the demonstration by Gross and Lapiere in 1962 that resorbing tadpole tail expressed an enzyme that could degrade collagen gels These humble beginnings have led us to the elucidation of around twenty distinct vertebrate MMPs along with a variety of homologs from such diverse organisms as sea urchin plants nematode worm and bacteria This coupled with four known specific inhibitors of MMPs the TIMPs gives a complex picture Part I of Matrix Metalloproteinase Protocols provides the reader with a selective overview of the MMP arena and a chance to come to grips with where the field has been where it is and where it is going I hope that this complements all of the methodology that comes later Part II presents the reader with a diverse set of methods for the expression and purification of MMPs and TIMPs bringing together the long and often hard earned experience of a

number of researchers Part III allows the reader to detect MMPs and TIMPs at both the protein and mRNA level whereas Part IV gives the ability to assay MMP and TIMP activities in a wide variety of circumstances **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 **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 *Biostatistical Methods* Stephen W. Looney, 2008-02-03 Leading biostatisticians and biomedical researchers describe many of the key techniques used to solve commonly occurring data analytic problems in molecular biology and demonstrate how these methods can be used in the development of new markers for exposure to a risk factor or for disease outcomes Major areas of application include microarray analysis proteomic studies image quantitation genetic susceptibility and association evaluation of new biomarkers and power analysis and sample size

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

Genomics Protocols Michael P. Starkey, Ramnath Elavarasu, 2008-02-03 We must unashamedly admit that a large part of the motivation for editing Genomics Protocols was selfish The possibility of assembling in a single volume a unique and comprehensive collection of complete protocols relevant to our work and the work of our colleagues was too good an opportunity to miss We are pleased to report however that the outcome is something of use not only to those who are experienced practitioners in the genomics field but is also valuable to the larger community of researchers who have recognized the potential of genomics research and may themselves be beginning to explore the technologies involved Some of the techniques described in Genomics Protocols are clearly not restricted to the genomics field indeed a prerequisite for many procedures in this discipline is that they require an extremely high throughput beyond the scope of the average investigator However what we have endeavored here to achieve is both to compile a collection of procedures concerned with genome scale investigations and to incorporate the key components of bottom up and top down approaches to gene finding The technologies described extend from those traditionally recognized as coming under the genomics umbrella touch on proteomics the study of the expressed protein complement of the genome through to early therapeutic approaches utilizing the potential of genome programs via gene therapy Chapters 27-30 *Epithelial Cell Culture Protocols* Clare

Wise, 2008-02-05 There have been significant advances in research involving the isolation and culture of epithelial cells in the past decade and many new techniques have been developed Monolayer cultures can be used to evaluate the nature and behavior of cells while the use of epithelial cells in model systems has allowed a deeper understanding of cellular and molecular mechanisms and interactions The aim of this book is to provide a comprehensive step by step guide to many techniques for epithelial cell culture combining in one volume the more commonly used protocols along with many that are more specialized Epithelial Cell Culture Protocols should help those who are new to this field and want to learn the basic culture techniques as well as those needing to use more wide ranging and specific protocols It should be a useful resource on its own and also complement the other volumes that have been written about cell culture in the Methods in Molecular Biology series Epithelial Cell Culture Protocols covers a wide variety of protocols mostly aimed at the researcher but also a few aimed at clinicians The establishment and maintenance of primary cultures derived from many different tissues and different species is covered Particular emphasis has been placed on protocols needed to further analyze and assess epithelial

cells for example by looking at apoptosis and integrins and by measuring membrane capacitance and confluence Using different coculture techniques it is possible also to develop models to investigate many different systems in vitro

DNA-Protein Interactions Tom Moss, 2008-02-02 Dr Tom Moss assembles the new standard collection of cutting edge techniques to identify key protein-DNA interactions and define their components their manner of interaction and their manner of function both in the cell and in the test tube The techniques span a wide range from factor identification to atomic detail and include multiple DNA footprinting analyses including in vivo strategies gel shift EMSA optimization SELEX surface plasmon resonance site specific DNA-protein crosslinking and UV laser crosslinking Comprehensive and broad ranging DNA-Protein Interactions Principles and Protocols 2nd Edition offers a stellar array of over 100 up to date and readily reproducible techniques that biochemists and molecular cellular and developmental biologists can use successfully today to understand DNA-protein interactions

Adipose Tissue Protocols Gérard Ailhaud, 2008-02-03 Adipose tissue is recognized to be exquisitely sensitive to hormone action and is also now recognized as a secretory and endocrine organ required for reproduction and good health Adipocytes are smart cells able within the tissue to communicate with surrounding cells but also with various organs particularly via leptin acting on the central nervous system Brown adipose tissue BAT and white adipose tissue WAT are known to be distinct tissues whereas the heterogeneity of WAT depots is well established Unfortunately excess WAT leads to obesity which is the most common health problem in industrialized countries Therefore from both a scientific and a technical point of view the time has come to create a survey of adipose tissues and their neglected adipocytes In Adipose Tissue Protocols I have attempted to gather together chapters from all areas of adipose tissue research from in vivo to in vitro studies and to provide methods covering a wide variety of techniques including the choice of adipose tissue depot and of morphological techniques for the study of BAT and WAT the isolation subcellular fractionation and transfection of adipocytes where the low density of these cells must be taken into account assays of nutrient and ion fluxes and the metabolic aspects of nutrient uptake assays of lipid related enzymes biopsies and quantification of lipid related mRNAs cultures of adipose precursor cells from WAT and BAT of various species including human tissue measurements of adipose secretory products and assessment of WAT metabolism in vivo

Calcium-Binding Protein Protocols Hans J. Vogel, 2008-02-04 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 of 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 More 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 Protein Structure, Stability, and Folding Kenneth P. Murphy, 2008-02-04 In Protein Structure Stability and Folding Kenneth P Murphy and a panel of internationally recognized investigators describe some of the newest experimental and theoretical methods for investigating these critical events and processes Among the techniques discussed are the many methods for calculating many of protein stability and dynamics from knowledge of the structure and for performing molecular dynamics simulations of protein unfolding New experimental approaches presented include the use of co solvents novel applications of hydrogen exchange techniques temperature jump methods for looking at folding events and new strategies for mutagenesis experiments Unique in its powerful combination of theory and practice Protein Structure Stability and Folding offers protein and biophysical chemists the means to gain a more comprehensive understanding of some of this complex area by detailing many of the major techniques in use today **The ELISA Guidebook** John R. Crowther, 2008-02-04 John R Crowther provides today's premier practical guide to the understanding and application of ELISA Updating and greatly expanding his widely appreciated earlier publication ELISA Theory and Practice 1995 this important work introduces chapters on such major new topics as checkerboard titrations quality control of testing kit production and control novel monoclonal antibodies validation of assays statistical requirements for data examination and epidemiological considerations With its numerous worked examples detailed instructions and extensive illustrations The ELISA Guidebook offers a powerful synthesis of all the basic concepts and practical experimental details investigators need to understand develop and apply the new ELISA methodology successfully in day to day basic and clinical research **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.

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