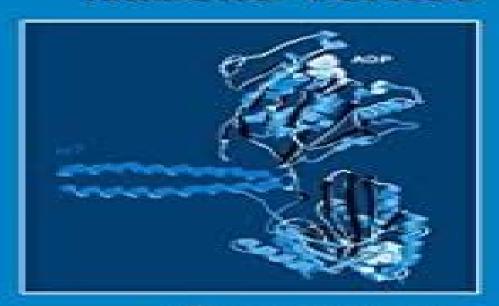
**VOLUME 164** 

# Kinesin Protocols

Edited by Isabelle Vernos



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# **Kinesin Protocols Methods In Molecular Biology**

Jeffrey D. Zahn

#### **Kinesin Protocols Methods In Molecular Biology:**

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 c pound 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 sociated with these agents and methods for doing so are presently being veloped 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 int pretation 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 commu cation 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 o 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 po mer 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 te niques 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 c bining 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 fu tion of connexin channels Connexin Methods and Protocols is divided into three main parts Nuclease Methods and Protocols Catherine H. Schein, 2008-02-03 Nucleases enzymes that restructure or degrade nucleic acid polymers are vital to the control of every area of metabolism They range from housekeeping enzymes with broad substrate ranges to extremely specific tools 1 Many types of nucleases are used in lab protocols and their commercial and clinical uses are expanding The purpose of Nuclease Methods and Protocols is to introduce the reader to some we characterized protein nucleases and the methods used to determine their activity structure interaction with other molecules and physiological role Each chapter begins with a mini review on a specific nuclease or a nuclease related theme Although many chapters cover several topics they were arbitrarily divided into five parts Part I Characterizing Nuclease Activity includes protocols and assays to determine general processive distributive or specific mechanisms Methods to assay nuclease products identify cloned nucleases and determine their physiological role are also included here Part II Inhibitors and Activators of Nucleases summarizes assays for measuring the effects of other proteins and small molecules Many of these inhibitors have clinical relevance Part III Relating Nuclease Structure and Function provides an overview of methods to determine or model the 3 D structure of nucleases and their complexes with substrates and inhibitors A 3 D structure can greatly aid the rational design of nucleases and inhibitors for specific purposes Part IV Nucleases in the Clinic summarizes assays and protocols suitable for use with t sues and for nuclease based therapeutics **Liposome Methods and Protocols** Subhash C. Basu, Manju Basu, 2008-02-04 In vitro utilization of liposomes is now recognized as a powerful tool in many bioscience investigations and their associated clinical studies e g liposomes in drug targeting liposomes in gene transport across plasma and nuclear membranes liposomes in enzyme therapy in patients with genetic disorders However before these areas can be effectively explored many basic areas in liposome research require elucidation including a attachment of liposomes to cell surfaces b permeation of liposomes through the plasma membranes and c stability of liposomes in cell or nuclear matrices None of

these areas have been exhaustively explored and liposome researchers have ample opportunities to contribute to our knowledge The aim of Liposome Methods and Protocols is to bring together a wide range of detailed laboratory protocols covering different aspects of liposome biology in order to assist researchers in those rapidly advancing medical fields mentioned earlier With this goal in mind in each protocol chapter we have detailed the materials to be used followed by a step by step protocol The Notes section of each protocol is also certain to prove particularly useful since the authors include troubleshooting tips straight from their benchtops valuable information that is seldom given in restricted methods sections of standard research journals For this reason we feel that the book will prove especially useful for all researchers in the **Neurotrophin Protocols** Robert A. Rush, 2008-02-03 The past decade has seen an extraordinary growth liposome field 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 diff ent 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 p tein 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 inc sion in several chapters in this book provide some novel uses <u>Proteoglycan Protocols</u> Renato V. Iozzo, 2008-02-02 Proteoglycans are some of that are not readily available elsewhere the most elaborate macromolecules of mammalian and lower organisms. The covalent attachment of at least five types of glycosami glycan 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 prepa tive and analytical methods for the in depth analysis of proteoglycans Featuring st 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 **Neuropeptide Y Protocols** Ambikaipakan Balasubramaniam, 2008-02-05 The observation that neuropeptide Y NPY is the most abundant peptide present in the mammalian nervous system and the finding that it elicits the most powerful or exigenic signal have led to active investigations of the properties of the NPY family of hormones including peptide YY PYY and pancreatic polypeptide PP Nearly two decades of research have led to the identification of several NPY receptor subtypes and the development of useful receptor selective ligands Moreover these investigations have imp cated NPY in the pathophysiology of a number of diseases including feeding disorders seizures memory loss anxiety depression and heart failure Vigorous efforts are therefore continuing not only to understand the bioche cal aspects of NPY actions but also toward developing NPY based treatments for a variety of disorders To facilitate these efforts it was decided to produce the first handbook on NPY research techniques as part of the Methods in Molecular Biology Series In compiling Neuropeptide Y Protocols I have gathered contributions on techniques considered critical for the advancement of the NPY field from experts in various disciplines Each chapter starts with a brief introduction with Materials and Methods sections following The latter sections are presented in an easy to follow step by step format The last section of the chapter Notes highlights pitfalls and the maneuvers employed to overcome them This information not usually disseminated in standard research pub cations may prove extremely useful for investigators employing these te nigues in NYP research 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 interc nected 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 imputies in

isolated mycotoxins Sections 2 4 Chapters 6 19 describe the most current chromatographic and immunochemical methods for studies on the major mycotoxins 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 p formed 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 rec bination between targeting constructs and endogenous DNA occurred at a f quency sufficient for recombinants to be isolated In the next few years the mouse genome will be systematically altered and the techniques for achi ing manipulations are constantly being streamlined and improved Methods in Bioengineering Jeffrey D. Zahn, 2010 Written and edited by recognized experts in the field the new Artech House Methods in Bioengineering series offers detailed guidance on authoritative methods for addressing specific bioengineering challenges Offering a highly practical presentation of each topic each book provides research engineers scientists and students with step by step procedures clear examples and effective ways to overcome problems that may be encountered This unique volume presents leading edge microfluidics methods used to handle manipulate and analyze cells particles biological components e g proteins and DNA for microdiagnostics 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 casette 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 **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 Matrix Metalloproteinase Protocols Ian M. Clark,2008-02-05 Research in the matrix metalloproteinase field began with the demonstration by Gross and Lapi re 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

GTPase Protocols Ed Manser, Thomas Leung, 2008-02-03 In the last 10 years researchers have firmly established key roles for R related GTPases in almost every aspect of cell biology In the 1980s the pro oncogene Ras itself was the focus of interest though in the 1990s this shifted to the increasing variety of Ras related proteins In this new decade much yet needs to be done to establish the role for all the small GTPases now uncovered by the human genome project In particular these GTPases need to be und stood in the appropriate biochemical and cellular contexts In the process of trying to uncover the role of these versatile proteins a variety of novel te niques and methodologies has been developed These now enable investi tors to move easily within a diversity of fields ranging from structural studies to real time in vivo analysis of a GTPase In recognition of the need for access to key background methodologies GTPase Protocols The Ras Superfamily is devoted to techniques that are prently widely used and that will continue to be the standard for researchers worldwide Each chapter is aimed at supplying detailed methodologies to allow reproduction in any laboratory while also providing the general pr ciples on which the methods are based Some of the techniques grouped in the first section apply broadly to small GTPases whereas others in Part II are more applicable within each GTPase subfamily 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 **Genomics Protocols** Michael P. Starkey, Ramnath Elaswarapu, 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 geno 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 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 Transgenesis Techniques Alan R. Clarke, 2008-02-05 The past decade has witnessed a spectacular explosion in both the devel ment and use of transgenic technologies Not only have these been used to aid our fundamental understanding of biologic mechanisms but they have also faci tated 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 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

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