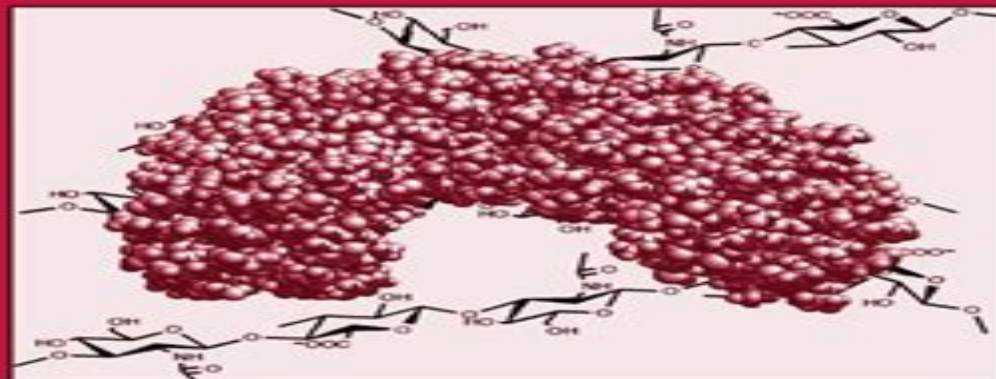


**Methods in Molecular Biology™**

**VOLUME 171**

# **Proteoglycan Protocols**

*Edited by*  
**Renato V. Iozzo, MD**



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# Proteoglycan Protocols Methods In Molecular Biology 171

**Alan R. Clarke**



## **Proteoglycan Protocols Methods In Molecular Biology 171:**

*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 Topoisomerase Protocols* Neil Osheroff, Mary-Ann Bjornsti, 2008-02-05 Beginning with the *Escherichia coli* protein or bacterial DNA topoisomerase I an ever increasing number of enzymes have been identified that catalyze changes in the linkage of DNA strands. DNA topoisomerases are ubiquitous in nature and have been shown to play critical roles in most processes involving DNA including DNA replication, transcription and recombination. These enzymes further constitute the cellular targets of a number of clinically important antibacterial and anticancer agents. Thus further studies of DNA topology and DNA topoisomerases are critical to advance our understanding of the basic biological processes required for cell cycle progression, cell division, genomic stability and development. In addition these studies will continue to provide critical insights into the cytotoxic action of drugs that target DNA topoisomerases. Such mechanistic studies have already played an important role in the development and clinical application of antimicrobial and chemotherapeutic agents. The two volumes of *DNA Topoisomerase Protocols* are designed to help new and established researchers investigate all aspects of DNA topology and the function of these enzymes. The chapters are written by prominent investigators in the field and provide detailed background information and step by step experimental protocols. The topics covered in Part I *DNA Topology and Enzymes* range from detailed methods to analyze various aspects of DNA structure from linking number, knotting, unknotting, site specific recombination and decatenation to the overexpression and purification of bacterial and eukaryotic DNA topoisomerases from a variety of cell systems and tissues.

*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 *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 **Marine Carbohydrates: Fundamentals and Applications, Part A**, 2014-07-29 Marine

Carbohydrates Fundamentals and Applications brings together the diverse range of research in this important area which leads to clinical and industrialized products The volume number 72 focuses on marine carbohydrates in isolation biological and biomedical applications and provides the latest trends and developments on marine carbohydrates Advances in Food and Nutrition Research recognizes the integral relationship between the food and nutritional sciences and brings together outstanding and comprehensive reviews that highlight this relationship Volumes provide those in academia and industry with the latest information on emerging research in these constantly evolving sciences Includes the isolation techniques for the exploration of the marine habitat for novel polysaccharides Discusses biological applications such as antioxidant antiallergic antidiabetic antiobesity and antiviral activity of marine carbohydrates Provides an insight into present trends and approaches for marine carbohydrates Antibody Phage Display Philippa M. O'Brien, Robert Aitken, 2008-02-05 The closing years of the 19th century and the start of the 20th century witnessed the emergence of microbiology and immunology as discrete scientific disciplines and in the work of Roux and Yersin perhaps the first benefits of their synergy immunotherapy against bacterial infection As we advance into the new millennium microbiology and immunology again offer a conceptual leap forward as

antibody phage display gains increasing acceptance as the definitive technology for monoclonal production and unleashes new opportunities in immunotherapy drug discovery and functional genomics In assembling Antibody Phage Display Methods and Protocols we have aimed to produce a resource of real value for scientists who have followed the development of phage display technology over the past decade The founding principles of phage display have always held an elegant simplicity We hope that readers will find similar clarity in the technical guidance offered by the book's contributors In meeting our objectives we have tried to cover the broad scope of the technology and the key areas of library construction screening antibody modification and expression Of course the technology continues to advance apace but we trust that readers will be able to gauge the potential of phage display from our coverage that some of its subtleties will emerge and that our selection of methods will prove appealing We are indebted to all the contributing authors for sharing their expertise with the wider scientific community

**Amino Acid Analysis Protocols** Catherine Cooper, 2008-02-05 A collection of classic and cutting edge techniques of high utility in answering specific biological questions about amino acids Common methods include those based on HPLC or gas chromatography separation and analysis after precolumn derivatization New techniques based on capillary electrophoresis separation high performance anion exchange chromatography and mass spectrometry are also presented Each method is described in step by step detail to ensure successful experimental results and emphasizes sample preparation particularly the collection and storage of bodily fluids Up to date and highly practical Amino Acid Analysis Protocols offers analytical and clinical chemists as well as a broad range of biological and biomedical investigators a rich compendium of laboratory tools for the productive analysis of both common and uncommon amino acids

**GTPase Protocols** Ed Manser, Thomas Leung, 2008-02-03 In the last 10 years researchers have firmly established key roles for Ras related GTPases in almost every aspect of cell biology In the 1980s the proto-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 understood in the appropriate biochemical and cellular contexts In the process of trying to uncover the role of these versatile proteins a variety of novel techniques and methodologies has been developed These now enable investigators 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 presently 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 principles 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

**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 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 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

**Capillary Electrophoresis of Nucleic Acids** Keith R. Mitchelson, Jing Cheng, 2008-02-05 The development of PCR which enables extremely small amounts of DNA to be amplified led to the rapid development of a multiplicity of analytical procedures that permit use of this new resource for the analysis of genetic variation and for the detection of disease causing mutations The advent of capillary electrophoresis CE with its power to separate and analyze very small amounts of DNA has also stimulated researchers to develop analytical procedures for the CE format The advantages of CE in terms of speed and reproducibility of analyses are manifold Furthermore the high sensitivity of detection and the ability to increase sample throughput with parallel analysis has led to the creation of a full range of analysis of DNA molecules from modified DNA adducts and single strand oligonucleotides through PCR amplified DNA fragments and whole chromosomes Capillary Electrophoresis of Nucleic Acids focuses on analytical protocols that can be used for detection and analysis of mutations and modification from precise DNA loci through entire genomes of organisms Important practical considerations for CE such as the choice of separation media electrophoresis conditions and the influence of buffer additives and dyes on DNA mobility are discussed in several key chapters and within particular applications

**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

*PCR Mutation Detection Protocols* Bimal D. M. Theophilus, Ralph Rapley, 2008-02-02 Bimal D Theophilus and Ralph Rapley provide biological and clinical investigators with a comprehensive collection of new recent and updated PCR based screening methods suitable for detecting the presence of both known and novel mutations The methods cover point mutations e.g. ASO PCR SSCP DGGE chemical cleavage deletions multiplex PCR FISH blotting non sense mutations PTT and more The new and exciting techniques of DNA array analysis along with such recently developed experimental methods as conformation sensitive gel electrophoresis are also included Each chapter explains the basic theory behind the technique and provides valuable notes essential for its successful execution

**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

Telomeres and Telomerase John A. Double, Michael J. Thompson, 2008-02-02 The fundamental problem that dividing cells have to overcome is that of end replication Chromosomes shorten by many bases during DNA replication and so this presents a major hurdle that a cell has to overcome both to enable it to proliferate and for the larger organism to survive and reproduce The enzyme telomerase provides a mechanism to ensure chromosome stability in both normal and neoplastic cells The demonstration of telomerase expression in a majority of tumors and the realization of the potential role of telomerase in aging has opened up the potential for telomerase to be used as a target for therapeutic intervention There is therefore great interest in the expression and activity of telomerase in a wide range of biological disciplines Telomeres and Telomerase

Methods and Protocols has been produced as a tool for the many researchers in different areas of cell biology who are interested in following research in the area of telomerase and telomere maintenance either in the area of fundamental mechanisms or perhaps in the area of more applied drug discovery work      *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      *Neural Stem Cells* Tanja Zigova, Paul R. Sanberg, Juan R. Sanchez-Ramos, 2008-02-03 Over the last decade neural stem cell research has provided penetrating insights into the plasticity and regenerative potential of the brain Stem cells have been isolated from embryonic as well as adult central nervous system CNS Many non CNS mammalian tissues also contain stem cells with a more limited repertoire the replacement of tissue specific cells throughout the life time of the organism Progress has been made in understanding fundamental stem cell properties that depend on the interplay of extrinsic signaling factors with intrinsic genetic programs within critical time frames With this growing knowledge scientists have been able to change a neural stem cell's fate under certain conditions neural stem cells have been induced to differentiate into cells outside the expected neural lineage and conversely stem cells from nonneural tissue have been shown to transdifferentiate into cells with distinct neural phenotypes At the moment there is an accelerated effort to identify a readily available socially acceptable stem cell that can be induced to proliferate in an undifferentiated state and that can be manipulated at will to generate diverse cell types We are on the threshold of a great new therapeutic era of cellular therapy that has as great if not greater potential as the current pharmacologic era glorified by antibiotics anesthetics pain killers immunosuppressants and psychotropics      *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      *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 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 **Proteoglycans** Nikos K. Karamanos, 2023-01-20 This volume is a comprehensive and up to date collection of strategies reproducible methods and protocols for the in depth analysis of Proteoglycans PGs and their glycan part the GAGs Chapters are divided into three parts detailing GAGs in biological specimens protocols for the evaluation of the in vitro and in vivo effects of PGs GAGs and protocols for compounds related with the metabolic enzymes epigenetic regulation and PGs GAGs based inhibitors Written in the format of the highly successful Methods in Molecular Biology series each chapter includes an introduction to the topic lists necessary materials and methods includes tips on troubleshooting and known pitfalls and step by step readily reproducible protocols Cutting edge and thorough Proteoglycans Methods and Protocols aims to provide information on the elucidated the structural and functional aspects of the complex matrix macromolecules such as the proteoglycans and glycosaminoglycans

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