

Methods in Molecular Biology™

VOLUME 177

Two-Hybrid Systems

Methods and Protocols

Edited by

Paul N. MacDonald

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Two Hybrid Systems Methods And Protocols Methods In Molecular Biology

Hugh J. M. Brady



Two Hybrid Systems Methods And Protocols Methods In Molecular Biology:

Two-Hybrid Systems Paul N. MacDonald, 2008-02-03 Paul N MacDonald has assembled a collection of powerful molecular tools for examining and characterizing protein protein protein DNA and protein RNA interactions The techniques range from the most basic introducing plasmids into yeasts interaction assays and recovering the plasmids from yeast to the most advanced alternative strategies involving one hybrid split two hybrid three hybrid membrane recruitment systems and mammalian systems Methods are also provided for dealing with the well known problem of artifacts and false positives and for identifying the interacting partners in important biological systems including the SMAD and nuclear receptor pathways To ensure ready reproducibility and robust results each technique is described in step by step detail by researchers who employ it regularly **Methods in Molecular Biology: Two-hybrid systems: Methods and protocols** John M.

Walker, 1984 **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 tissues 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 liposome field.

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.

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

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.

Apoptosis Methods and Protocols Hugh J. M. Brady, 2008-02-05 The most fundamental question facing each and every cell within an organism is to survive or to die. Cell death is required for normal function; some estimates suggest that as many as one million cells undergo cell death every second in the adult human body. Almost all cells undergoing physiological or programmed cell death, independent of cell type, manifest a stereotypic pattern of morphological changes termed apoptosis. Typically, apoptotic cells display shrinkage, membrane blebbing, chromatin condensation, and nuclear fragmentation. The integrity of the cell membrane is not lost during apoptosis and so avoids eliciting the inflammatory response that would have been caused by the spillage of the cell's contents. This is quite in contrast to the loss of cell contents typical of necrosis. The caspases, the family of intracellular cysteine proteases associated with apoptosis, are responsible for the stereotypical morphological changes. Caspases cleave various substrate proteins that act on DNA fragmentation, nuclear envelope integrity, the cytoskeleton, and cell volume regulation. Apoptotic cells are cleared in vivo by the process of phagocytosis, in which specific phagocytes move to the site of apoptosis, engulf the dying cells, and digest them. Apoptosis has a central role in many physiological processes; for example, in the immune system, autoreactive cells are deleted via apoptosis to prevent autoimmunity. At the end of an immune response, activated lymphocytes are removed to maintain homeostasis within the immune system.

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

Combinatorial Library Lisa B. English, 2008-02-04 The continued successes of large and small scale genome sequencing projects are increasing the number of genomic targets available for drug discovery at an exponential rate In addition a better understanding of molecular mechanisms such as apoptosis signal transduction telomere control of chromosomes cytoskeletal development modulation of stress related proteins and cell surface display of antigens by the major histocompatibility complex molecules has improved the probability of identifying the most promising genomic targets to counteract disease As a result developing and optimizing lead candidates for these targets and rapidly moving them into clinical trials is now a critical juncture in pharmaceutical research Recent advances in combinatorial library synthesis purification and analysis techniques are not only increasing the numbers of compounds that can be tested against each specific genomic target but are also speeding and improving the overall processes of lead discovery and optimization There are two main approaches to combinatorial library production parallel chemical synthesis and split and mix chemical synthesis These approaches can utilize solid or solution based synthetic methods alone or in combination although the majority of combinatorial library synthesis is still done on solid support In a parallel synthesis all the products are assembled separately in their own reaction vessels or microtiter plates The array of rows and columns enables researchers to organize the building blocks to be combined and provides an easy way to identify compounds in a particular well

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

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 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

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

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

continue 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

Bacterial Secretion Systems Laure Journet, Eric Cascales, 2023-11-06 This second edition details new and updated protocols that cover techniques used to study secretion systems Chapters focus on identifying and localizing the different subunits defining interactions within subunits monitoring conformational changes purifying and imaging of large complexes defining the assembly pathway by fluorescence microscopy and the role of energy during assembly and or secretion identifying secreted effectors as well as using reporters to follow effector transport 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 key tips on troubleshooting and avoiding known pitfalls Authoritative and cutting edge Bacterial Secretion Systems Methods and Protocols Second Edition aims to be a useful and practical guide to new researchers and experts looking to expand their knowledge

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 immu therapy 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

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

Capillary Electrophoresis of Carbohydrates Pierre Thibault, Susumu Honda, 2008-02-03 A collection of cutting edge techniques for using capillary electrophoresis CE to analyze complex carbohydrates These readily reproducible protocols provide methods for sample preparation analysis of mono and oligosaccharides glycoproteins and glycoconjugates A useful appendix describes the structures of the most commonly encountered carbohydrate residues and oligosaccharides from mammalian and bacterial origins Each protocol contains detailed information on reagents apparatus notes comments and tips on procedures

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

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

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