

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

Subhash C. Basu, Manju Basu



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 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

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