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Transgenic Mouse

Methods and Protocols

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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.

Transgenic Mouse Methods and Protocols Marten H. Hofker, Jan van Deursen, 2016-08-23 The generation of genetically modified mice is absolutely crucial to gene function studies today primarily because mice are genetically similar to man and because gene function studies in mice are in the context of a whole organism making them particularly useful. In *Transgenic Mouse Methods and Protocols* Second Edition expert researchers explore current advances in the field through detailed laboratory protocols. Chapters provide a general introduction outlining how to deal with mice and how to generate transgenic mouse models, explore the generation of conditional and induced knockout and transgenic mice and offer alternative routes to studying gene function in mice. Composed in the highly successful *Methods in Molecular Biology*™ series format each chapter contains a brief introduction, step by step methods, a list of necessary materials and a Notes section which shares tips on troubleshooting and avoiding known pitfalls. Comprehensive and state of the art *Transgenic Mouse Methods and Protocols* second Edition is the ideal guide for all researchers interested in the latest information about the production and analysis of transgenic and knockout mice.

[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 *Superantigen Protocols* Teresa Krakauer, 2008-02-05 Leading researchers in the biological chemical and physical investigation of superantigens describe in step by step detail their best experimental techniques to assess the physical characteristics and biological effects of superantigens Their protocols range from those for investigating the interactions of superantigens with cellular receptors to those for the analysis of their immunological and biological effects including methods for using BIOcore to determine binding kinetics and establishing various lymphocyte cell culture systems There are also accounts of such methods as the RNase protection assay cytokine ELISA FACS analysis and cytokine production at the single cell level **Molecular Cytogenetics** Yao-Shan Fan, 2008-02-05 The new techniques of molecular cytogenetics mainly fluorescence in situ hybridization FISH of DNA probes to metaphase chromosomes or interphase nuclei have been developed in the past two decades Many FISH techniques have been implemented for diagnostic services whereas some others are mainly used for investigational purposes Several hundreds of FISH probes and hybridization kits are now commercially available and the list is growing rapidly FISH has been widely used as a powerful diagnostic tool in many areas of medicine including pediatrics medical genetics maternal fetal medicine reproductive medicine pathology hematology and oncology Frequently a physician may be puzzled by the variety of FISH techniques and wonder what test to order It is not uncommon that a sample is referred to a laboratory for FISH without indicating a specific test On the other hand a cytogeneticist or a technologist in a laboratory needs from case to case to determine which procedure to perform and which probe to use for an informative result To obtain the best results one must use the right DNA probes and have reliable protocols and measures of quality assurance in place Also one must have sufficient knowledge in both traditional and molecular cytogenetics as well as the particular areas of medicine for which the test is used in order to appropriately interpret the FISH results and to correlate them with clinical diagnosis treatment and prognosis

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

PCR Protocols John M. S. Bartlett, David Stirling, 2008-02-03 In this new edition the editors have thoroughly updated and dramatically expanded the number of protocols to take advantage of the newest technologies used in all branches of research and clinical medicine today These proven methods include real time PCR SNP analysis nested PCR direct PCR and long range PCR Among the highlights are chapters on genome profiling by SAGE differential display and chip technologies the amplification of whole genome DNA by random degenerate oligonucleotide PCR and the refinement of PCR methods for the analysis of fragmented DNA from fixed tissues Each fully tested protocol is described in step by step detail by an established expert in the field and includes a background introduction outlining the principle behind the technique equipment and reagent lists tips on trouble shooting and avoiding known pitfalls and where needed a discussion of the interpretation and use of results

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

E. coli Gene Expression Protocols Peter E. Vaillancourt, 2008-02-02 Peter E Vaillancourt presents a collection of popular and emerging methodologies that take advantage of E coli's ability to quickly and inexpensively express recombinant proteins The authors focus on two areas of interest the use of E coli vectors and strains for production of pure functional protein and the use of E coli as host for the functional screening of large collections of proteins and peptides Among the cutting edge techniques demonstrated are those for rapid high level expression and purification of soluble and functional recombinant protein and those essential to functional genomics proteomics and protein engineering

p53 Protocols Sumitra Deb, Swati Palit Deb, 2008-02-02 Since the discovery of p53 as a tumor suppressor numerous methods have evolved to reveal the unique structural features and biochemical functions of this protein Several unique properties of p53 posed a

challenge to understanding its normal function in the initial phase of its research. The low levels of p53 in normal cells, its stabilization under situations of genotoxic stress, induction of growth arrest and apoptosis with stabilization of the protein, obstructed the visibility of its normal unmutated function. The property of p53 that can sense a promoter and transactivate or inhibit is still not well understood. It is still not known whether it is the absence of the protein that causes tumorigenesis or if its mutants have a dominant role in inducing cancer.

p53 Protocols comprises eighteen chapters for the study of the diverse properties of p53 and related proteins. The methods included are invaluable for delineating the function of other proteins that may function as tumor suppressors or growth suppressors. The chapters are not presented in any schematic order for the importance and diversity of the functions of p53 make it impossible to organize them suitably. We have made a sincere effort to collect the methods most useful to those investigators working on tumor suppressors or growth suppressors. The purpose of **p53 Protocols** is not only to provide investigators with methods to analyze similar biochemical functions but also to familiarize them with the associated problems that arose during the course of investigations.

Mitochondrial DNA William C. Copeland, 2008-02-04

Peptide Research Protocols Janet J. Maguire, Anthony P. Davenport, 2008-02-04

A panel of multidisciplinary experts describes in detail readily reproducible methods to investigate all aspects of the endothelin system from its synthesis and metabolism to its function in health and disease. These methods use state of the art molecular techniques to quantify the expression of mRNA for both endothelin receptors and the endothelin converting enzymes. They show how peptide precursors, receptors and synthetic enzymes can be localized and quantified in plasma, culture supernatants, tissue homogenate and tissue sections using antibodies. Several in vivo protocols illustrate the role of the endothelin peptides in healthy human individuals and describe animal models that can be used to predict the therapeutic potential of cardiovascular drugs that manipulate endothelin synthesis or function.

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

MHC Protocols Stephen H. Powis, Robert W. Vaughan, 2008-02-05 The aim of MHC Protocols is to document protocols that can be used for the analysis of genetic variation within the human major histocompatibility complex MHC HLA region The human MHC encompasses approximately 4 million base pairs on the short arm of chromosome 6 at cytogenetic location 6p21.3 The region is divided into three subregions The telomeric class I region contains the genes that encode the HLA class I molecules HLA A B and C The centromeric class II region contains the genes encoding the HLA class II molecules HLA DR DQ and DP In between is the class III region originally identified because it contains genes encoding components of the complement pathway The entire human MHC has recently been sequenced and each subregion is now known to contain many other genes a number of which have immunological functions The study of polymorphism within the MHC is well established because the region contains the highly polymorphic HLA genes HLA polymorphism has been used extensively in solid organ and bone marrow transplantation to match donors and recipients As a result large numbers of HLA alleles have been identified a process that has been further driven by recent interest in HLA gene diversity in ethnic populations The extreme genetic variation in HLA genes is believed to have been driven by the evolutionary response to infectious agents but relatively few studies have analyzed associations between HLA genetic variation and infectious disease which has been difficult to demonstrate

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 immunotherapy 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 immunologically 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

RT-PCR Protocols Nicola King, Joe O'Connell, 2008-02-04 Until the mid 1980s the detection and quantification of a specific mRNA was a difficult

task usually only undertaken by a skilled molecular biologist. With the advent of PCR it became possible to amplify specific mRNA after first converting the mRNA to cDNA via reverse transcriptase. The arrival of this technique termed reverse transcription PCR (RT-PCR) meant that mRNA suddenly became amenable to rapid and sensitive analysis without the need for advanced training in molecular biology. This new accessibility of mRNA which has been facilitated by the rapid accumulation of sequence data for human mRNAs means that every biomedical researcher can now include measurement of specific mRNA expression as a routine component of his/her research plans. In view of the ubiquity of the use of standard RT-PCR the main objective of RT-PCR Protocols is essentially to provide novel useful applications of RT-PCR. These include some useful adaptations and applications that could be relevant to the wider research community who are already familiar with the basic RT-PCR protocol. For example a variety of different adaptations are described that have been employed to obtain quantitative data from RT-PCR. Quantitative RT-PCR provides the ability to accurately measure changes in mRNA expression between normal and diseased tissues.

Thyroid Hormone Receptors Aria Baniahmad, 2008-02-05 A panel of outstanding investigators surveys and explains the major cutting edge methods used in thyroid receptor (TR) research and explains their practical experimental details. Described in step by step detail to ensure robust experimental results the techniques presented cover a wide variety of key areas including TR in development and knockout mouse and Xenopus transcriptional regulation by TRs in both cell free systems and in living cells and TR mutant analysis of patients. Additional methods provide powerful tools for the isolation of TR regulated protein complexes for studying the oncogene v-ErbA in blood cell differentiation and for target gene analysis in the brain. Microarray chip methods are also presented for analyzing the organs of transgenic mice to identify target genes in the liver.

PCR Detection of Microbial Pathogens Konrad Sachse, Joachim Frey, 2003 Hands on laboratory experts present a set of classic PCR based methods for the identification and detection of important animal and food microbial pathogens including several zoonotic agents. These proven techniques can be precisely applied to a wide variety of microbes among them *Campylobacter* spp, *Chlamydiae*, toxigenic *Clostridia*, *Escherichia coli* STEC, *Listeria monocytogenes*, *Mycoplasmas*, *Salmonellae* and *Yersinia enterocolitica*. Additional chapters review the specificity and performance of diagnostic PCR analysis, the pre-PCR processing of samples, the critical aspects of standardizing PCR methods and the general issues involved in using PCR technology for microbial diagnosis.

PCR Cloning Protocols Bing-Yuan Chen, Harry W. Janes, 2008-02-05 PCR Cloning Protocols Second Edition updates and expands Bruce White's best selling PCR Cloning Protocols 1997 with the newest procedures for DNA cloning and mutagenesis. Here the researcher will find readily reproducible methods for all the major aspects of PCR use including PCR optimization, computer programs for PCR primer design and analysis and novel variations for cloning genes of special characteristics or origin with emphasis on long distance PCR and GC rich template amplification. Also included are both conventional and novel enzyme free and restriction site free procedures to clone PCR products into a range of vectors as well as state of the art

protocols to facilitate DNA mutagenesis and recombination and to clone the challenging uncharacterized DNA flanking a known DNA fragment *Posttranslational Modification of Proteins* Christoph Kannicht, 2008-02-04 Christoph Kannicht and a panel of highly experienced researchers describe readily reproducible methods for detecting and analyzing the posttranslational modifications of protein particularly with regard to protein function proteome research and the characterization of pharmaceutical proteins

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