

Methods in Molecular Biology™

VOLUME 217

Neurogenetics

Methods and Protocols

Edited by
Nicholas T. Potter



HUMANA PRESS

Neurogenetics Methods And Protocols Methods In Molecular Biology

John M. Walker



Neurogenetics Methods And Protocols Methods In Molecular Biology:

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 **Methods in Molecular Biology: Neurogenetics: methods and protocols** John M.

Walker, 1984 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 **Neurogenetics** Nicholas T. Potter, 2003 An international panel of recognized academic physicians researchers and clinical laboratory diagnosticians describe their best methods for characterizing neurologically relevant genes their mutations and their proteins Providing detailed step by step instructions to assure successful experimental results these experts cover the key methods for mutation detection and screening including discussions of quantitative PCR trinucleotide repeat detection sequence based mutation detection fluorescence in situ hybridization FISH in vitro protein expression systems and studies of protein expression and function *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 peptides 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 *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 *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 *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 1 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

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

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

Protein Kinase C Protocols Alexandra C. Newton, 2008-02-03 Since the discovery that protein kinase C PKC transduces the abundance of signals that result in phospholipid hydrolysis this enzyme has been at the forefront of research in signal transduction Protein Kinase C Protocols covers fundamental methods for studying the structure function regulation

subcellular localization and macromolecular interactions of PKC Protein Kinase C Protocols is divided into 11 sections representing the major aspects of PKC regulation and function Part I contains an introduction and a historical perspective on the discovery of PKC by Drs Yasutomi Nishizuka and Ushio Kikkawa Part II describes methods to purify PKC Part III describes the standard methods for measuring PKC activity its enzymatic activity and its stimulus dependent translocation from the cytosol to the membrane Part IV describes methods for measuring the membrane interaction of PKC in vivo and in vitro Part V provides methodologies and techniques for measuring the phosphorylation state of PKC including a protocol for measuring the activity of PKC's upstream kinase PDK 1 Novel methods for identifying substrates are described in Part VI Part VII presents protocols for expressing and analyzing the membrane targeting domains of PKC Part VIII provides a comprehensive compilation of methods used to identify binding partners for PKC Part IX describes pharmacological probes used to study PKC The book ends with a presentation of genetic approaches to study PKC Part X and a discussion of approaches used to study PKC in disease Part XI

Lentivirus Gene Engineering Protocols Maurizio Federico, 2008-02-03 Cell gene engineering is emerging as a field with outstanding impact not only in medicine biology but also and perhaps most importantly in agriculture and in all those food sciences involved in the fight against world hunger Lentivirus vector based technologies represent the last frontier in the development of powerful and reliable methods for both in vitro and in vivo gene transfer in eukaryotic animal cells Although the design of lentivirus vectors is closely reminiscent of those already successfully applied to the construction of oncoretroviral vectors some unique features e.g. the efficiency in transducing both postmitotic and stem cells render the use of lentivirus vectors invaluable It has been a great pleasure to edit *Lentivirus Gene Engineering Protocols* owing in part to the high level of enthusiasm that the authors demonstrated in contributing to this book The fact that so many outstanding scientists engaged in lentivirus vector research have provided articles renders it something more than a technical handbook In addition to detailed descriptions of the most innovative methodologies the reader may find very informative overviews concerning both theoretical and practical aspects of the origin and the development of diverse lentivirus vector types This in my opinion represents a unique added value of this volume which should help our work resist the passage of time to which books such as this are particularly sensitive 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.

Protein Sequencing Protocols Bryan John Smith, 2008-02-02 Determination of the protein sequence is as important today as it was a half century ago even though the techniques and purposes have changed over time. Mass spectrometry has continued its recent rapid development to find notable application in the characterization of small amounts of protein for example in the field of proteomics. The traditional chemical N terminal sequencing is still of great value in quality assurance of the increasing number of biopharmaceuticals that are to be found in the clinic checking processing events of recombinant proteins and so on. It is joined in the armory of methods of protein analysis by such techniques as C terminal sequencing and amino acid analysis. These methods are continually developing. The first edition of Protein Sequencing Protocols was a snapshot of methods in use in protein biochemistry laboratories at the time and this the second edition is likewise. Methods have evolved in the intervening period and the content of this book has similarly changed the content of some chapters having been superseded and replaced by other approaches. Thus in this edition there is inclusion of approaches to validation of methods for quality assurance work reflecting the current importance of biopharmaceuticals and also a guide to further analysis of protein sequence information acknowledging the importance of bioinformatics.

Atomic Force Microscopy Pier Carlo Braga, Davide Ricci, 2008-02-02 The natural biological medical and related sciences would not be what they are today without the microscope. After the introduction of the optical microscope a second breakthrough in morphostructural surface analysis occurred in the 1940s with the development of the scanning electron microscope SEM which instead of light i.e. photons and glass lenses uses electrons and electromagnetic lenses magnetic coils. Optical and scanning or transmission electron microscopes are called far field microscopes because of the long distance between the sample and the point at which the image is obtained in comparison with the wavelengths of the photons or electrons involved. In this case the image is a diffraction pattern and its resolution is wavelength limited. In 1986 a completely new type of microscopy was proposed which without the use of lenses photons or electrons directly explores the sample surface by means of mechanical scanning thus opening up unexpected possibilities for the morphostructural and mechanical analysis of biological specimens. These new scanning probe microscopes are based on the concept of near field microscopy which overcomes the problem of the limited diffraction related resolution inherent in conventional microscopes. Located in the immediate vicinity of the sample itself usually within a few nanometers the probe records the intensity rather

than the interference signal thus significantly improving resolution Since the most we known microscopes of this type operate using atomic forces they are frequently referred to as atomic force microscopes AFMs

Mammalian Artificial Chromosomes Vittorio Sgaramella, Sandro Eridani, 2008-02-02 In 1996 we organized a workshop inter alia at the National Research Council in Milan under the generous sponsorship of the European Science Foundation On that occasion a small group of investigators convened from many countries and presented early evidence of the possibility of assembling basic units of mammalian chromosomes into artificial constructs or indeed rejoining the relevant components to more manageable dimensions and defined constitution Progress in the following years has been slow but steady Many scientists who took part in the workshop have since been engaged in active and productive research It goes to the credit of Humana Press to have realized the need for a book on artificial chromosomes that aims to provide better tools to all scientists committed to this field who are confronted with very difficult technical problems We have strived to cover in Mammalian Artificial Chromosomes Methods and Protocols all relevant areas of artificial chromosome research from basic genetics to daring attempts to build new tools for genetic therapy We are of course grateful to the authors who have accepted the task of describing the technical steps and pitfalls that can be encountered in their research Rarely has a very delicate methodology been presented with such meticulous care We have been helped in this enterprise by the excellent librarian of the LITA Institute in Segrate Italy Ms Claudia Piergigli whom we thank warmly

Cytokine Protocols Marc De Ley, 2008-02-03 A collection of biochemical cellular and molecular techniques for unraveling and quantifying the events occurring between the initial contact of a cytokine at the membrane receptor and the eventual activation of gene transcription The techniques used include the generation of transfectants the immunohistochemical detection of cytokines in tissue sections and optimized staining for cytoplasmic detection Highlights include RT-PCR of small amounts of mRNA in situ hybridization biosensor analysis measurement of biological activities and standardization immunohistochemical and single cell detection and receptor isolation characterization and crystallization Enjoy a quick and smooth introduction to the key methods used in cytokine research Use readily reproducible techniques that ensure successful experimental results Employ antisense RNA RT-PCR of small amounts of mRNA and in situ hybridization

Tumor Suppressor Genes Wafik S. El-Deiry, 2008-02-03 It has become clear that tumors arise from excessive cell proliferation and a corresponding reduction in cell death Tumors result from the successive accumulation of mutations in key regulatory target genes over time During the 1980s a number of oncogenes were characterized whereas from the 1990s to the present the emphasis shifted to tumor suppressor genes TSGs It has become clear that oncogenes and tumor suppressor genes function in the same pathways providing positive and negative growth regulatory activities The signaling pathways controlled by these genes involve virtually every process in cell biology including nuclear events cell cycle cell death cytoskeletal cell membrane angiogenesis and cell adhesion effects Tumor suppressor genes are mutated in hereditary cancer syndromes as well as somatically in nonhereditary cancers In their

normal state TSGs control cancer development and progression as well as contribute to the sensitivity of cancers to a variety of therapeutics Understanding the classes of TSGs the biochemical pathways they function in and how they are regulated provides an essential lesson in cancer biology We cannot hope to advance our current knowledge and to develop new and more effective therapies without understanding the relevant pathways and how they influence the present approaches to therapy Moreover it is important to be able to access the powerful tools now available to discover these genes as well as their links to cell biology and growth control

E. coli Plasmid Vectors Nicola Casali, Andrew Preston, 2008-02-03 A comprehensive collection of readily reproducible techniques for the manipulation of recombinant plasmids using the bacterial host E coli The authors describe proven methods for cloning DNA into plasmid vectors transforming plasmids into E coli and analyzing recombinant clones They also include protocols for the construction and screening of libraries as well as specific techniques for specialized cloning vehicles such as cosmids bacterial artificial chromosomes l vectors and phagemids Common downstream applications such as mutagenesis of plasmids recombinant protein expression and the use of reporter genes are also described

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

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