

**Methods in Molecular Biology™**

**VOLUME 217**

# **Neurogenetics**

## ***Methods and Protocols***

*Edited by*  
**Nicholas T. Potter**



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# Neurogenetics Methods And Protocols Methods In Molecular Biology

**Nicholas T. Potter**



## **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

**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 *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 **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 **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      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

**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

**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

*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

Peptide Nucleic Acids Peter E. Nielsen, 2008-02-04 Peptide nucleic acids PNAs have now existed for slightly more than ten years with the interest in and applications of this pseudopeptide DNA mimic steadily increasing during the entire period PNAs have rapidly attracted the attention of scientists from a diversity of fields ranging from bio organic and biophysical chemistry to prebiotic evolution and from molecular biology to genetic diagnostics and drug development Many of the applications take advantage of the unique properties of PNA an uncharged pseudopeptide that distinguish this DNA mimic from more traditional DNA analogs Rather than trying to create a comprehensive collection of all published methods and protocols involving PNA many of which have not yet been validated I have decided to concentrate on select protocols that are either very well established by several groups around the world such as PCR clamping and in situ hybridization or on new methods that may have broader future impact Basic methods for PNA oligomer synthesis and analyses have also been included I am very grateful to those friends and colleagues who have enthusiastically contributed their work discussions and writing and thereby made this book possible Peter E Nielsen v Contents Preface v Contributors ix I INTRODUCTION 1 PNA Technology Peter E Nielsen 3 II CHEMISTRY 2 Solid Phase Synthesis of PNA Oligomers Frederik Beck 29 3 Synthesis of PNA Peptide Conjugates Satish Kumar Awasthi and Peter E Nielsen 43 4 Parallel Synthesis of PNA Peptide Conjugate Libraries Satish Kumar Awasthi and Peter E Nielsen

**Oxidants**

**and Antioxidants** Donald Armstrong, 2008-02-02 In our first protocols book Free Radical and Antioxidant Protocols 1 reference to in vivo ex vivo or in situ techniques were few compared to classical biochemical assays and only 6 of the 40 chapters were concerned with these applications In our second book Oxidative Stress Biomarkers and Antioxidant Protocols 2 which is being published concurrently with this third volume Oxidants and Antioxidants Ultrastructure and Molecular Biology Protocols the number of such chapters has increased The literature dealing with histochemical cytochemical and immunohistochemical techniques and staining to identify cellular subcellular sites of oxidative stress has expanded rapidly as has the molecular biology methodology used to analyze free radical and antioxidant AOX reactions as well as the monitoring of living tissue A two way search was performed for each technique listed in Table 1 coupled with oxidative stress using the PUBMED search engine from the National Library of Medicine at NIH Most of the techniques involved in monitoring oxidative stress employ molecular biology or ultrastructural approaches Of these techniques histology polymerase chain reaction and Western blotting are the most widely used Several forms of therapy are now available for patients with increased oxidative stress In addition to standard antioxidant therapy supplementation in vivo and in vitro photodynamic therapy PDT employs excitation of a photon emitting compound delivered systemically for free radical mediated necrosis of affected tissues and stem cells are also being used to induce signaling events or replace antioxidant enzymes

**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

**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

**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

**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

**Cancer Cytogenetics** John Swansbury, 2008-02-05 A collection of key cytogenetic and FISH techniques used by modern clinical laboratories in the genetic analysis of human malignancies The book's practical advice and methods are suitable for use at every level of expertise including fully established laboratories but with a sympathetic bias towards anyone considering setting up a new cytogenetics service Here the reader will find not only elementary tutorials on the fundamentals of human karyotypes and chromosome analysis but also detailed discussions on how laboratories may optimally upgrade their repertoire of capabilities to include such newer complementary techniques as CGH FISH and M FISH

**Single Nucleotide Polymorphisms** Pui-Yan Kwok, 2008-02-04 A comprehensive collection of readily reproducible techniques for the difficult process of single nucleotide polymorphisms SNP discovery and genotyping These cutting edge protocols for mutation SNP detection utilize denaturing high performance liquid chromatography dHPLC single strand conformation polymorphism SSCP conformation sensitive gel electrophoresis CSGE chemical cleavage and direct sequencing Equally powerful and up to date methods are given for genotyping SNPs including molecular beacons the Taqman assay single base extension approaches pyrosequencing ligation the Invader assay and primer extension with mass spectrometry detection

**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

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