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Shohreh Amini  
Martyn K. White *Editors*

# Neuronal Cell Culture

Methods and Protocols

 Humana Press

# Neuronal Cell Culture Methods And Protocols Methods In Molecular Biology

**ICN Biomedicals, Inc**



## **Neuronal Cell Culture Methods And Protocols Methods In Molecular Biology:**

*Neuronal Cell Culture* Shohreh Amini, Martyn K. White, 2021 This second edition volume details the latest aspects of neural cells covering the practical and theoretical considerations of each techniques involved Chapters guide readers through a general overview of the neuronal culturing principles cell line models for neural cells the isolation and propagation of primary cultures stem cells transfection and transduction of neural cultures and other more advanced techniques 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 tips on troubleshooting and avoiding known pitfalls Practical and easy to use *Neuronal Cell Culture Methods and Protocols* Second Edition aims to be of interest to scientists at all levels studying cell culture models for neuroscientific studies

*Epithelial Cell Culture Protocols* Clare Wise, 2008-02-05 There have been significant advances in research involving the isolation and culture of epithelial cells in the past decade and many new techniques have been developed Monolayer cultures can be used to evaluate the nature and behavior of cells while the use of epithelial cells in model systems has allowed a deeper understanding of cellular and molecular mechanisms and interactions The aim of this book is to provide a comprehensive step by step guide to many techniques for epithelial cell culture combining in one volume the more commonly used protocols along with many that are more specialized *Epithelial Cell Culture Protocols* should help those who are new to this field and want to learn the basic culture techniques as well as those needing to use more wide ranging and specific protocols It should be a useful resource on its own and also complement the other volumes that have been written about cell culture in the Methods in Molecular Biology series *Epithelial Cell Culture Protocols* covers a wide variety of protocols mostly aimed at the researcher but also a few aimed at clinicians The establishment and maintenance of primary cultures derived from many different tissues and different species is covered Particular emphasis has been placed on protocols needed to further analyze and assess epithelial cells for example by looking at apoptosis and integrins and by measuring membrane capacitance and confluence Using different coculture techniques it is possible also to develop models to investigate many different systems in vitro

*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

**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

**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

**In Vitro Mutagenesis Protocols** Jeff Braman, 2008-02-05 Hands on researchers with proven track records describe in stepwise fashion their advanced mutagenesis techniques The contributors focus on improvements to conventional site directed mutagenesis including a chapter on chemical site directed mutagenesis PCR based mutagenesis and the modifications that allow high throughput mutagenesis experiments and mutagenesis based on gene disruption both in vitro and in situ based Additional methods are provided for in vitro gene evolution for gene disruption based on recombination transposon and cassette mutagenesis and for facilitating the introduction of multiple mutations Time tested and highly practical the protocols in In

Vitro Mutagenesis Protocols 2nd Edition offer today's molecular biologists reliable and powerful techniques with which to illuminate the proteome

**Neural Stem Cells** Tanja Zigova, Paul R. Sanberg, Juan R. Sanchez-Ramos, 2008-02-03 Over the last decade neural stem cell research has provided penetrating insights into the plasticity and regenerative potential of the brain Stem cells have been isolated from embryonic as well as adult central nervous system CNS Many non CNS mammalian tissues also contain stem cells with a more limited repertoire the replacement of tissue specific cells throughout the lifetime of the organism Progress has been made in understanding fundamental stem cell properties that depend on the interplay of extrinsic signaling factors with intrinsic genetic programs within critical time frames With this growing knowledge scientists have been able to change a neural stem cell's fate under certain conditions neural stem cells have been induced to differentiate into cells outside the expected neural lineage and conversely stem cells from nonneural tissue have been shown to transdifferentiate into cells with distinct neural phenotypes At the moment there is an accelerated effort to identify a readily available socially acceptable stem cell that can be induced to proliferate in an undifferentiated state and that can be manipulated at will to generate diverse cell types We are on the threshold of a great new therapeutic era of cellular therapy that has as great if not greater potential as the current pharmacologic era glorified by antibiotics anesthetics pain killers immunosuppressants and psychotropics

**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

**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      **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      **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      *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      **Functional Genomics** Michael J. Brownstein, Arkady Khodursky, 2008-02-03 This collection of robust readily reproducible methods for microarray based studies includes expert guidance in the optimal data analysis and informatics On the methods side are proven techniques for monitoring subcellular RNA localization en masse for mapping chromosomes at the resolution of a single gene and for surveying the steady state genome wide distribution of DNA binding proteins in vivo For those workers dealing with massive data sets the book discusses the methodological aspects of data analysis and informatics in the design of microarray experiments the choice of test statistic and the assessment of observational significance data reduction and clustering

**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      **Laboratory Methods in Cell**

**Biology: Imaging** , 2013-01-03 Cell biology spans among the widest diversity of methods in the biological sciences From physical chemistry to microscopy cells have given up with secrets only when the questions are asked in the right way This new volume of Methods in Cell Biology covers laboratory methods in cell biology and includes methods that are among the most important and elucidating in the discipline such as bioluminescent imaging of gene expressions confocal imaging and electron microscopy of bone Covers the most important laboratory methods in cell biology Chapters written by experts in their fields      *DNA Methylation Protocols* Ken I. Mills, Bernie H. Ramsahoye, 2008-02-05 DNA Methylation Protocols offer a set of readily reproducible protocols of the analysis of DNA methylation and methylases These powerful methods provide the tools necessary for studying methylation at both the global level and the level of sequence and include many techniques for identifying genes that might be aberrantly methylated in cancer and aging Additional methods cover genome wide analysis of abnormal DNA methylation and the isolation and measurement of demethylases and related proteins      **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.

*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 bioorganic 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 distinguishes 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  
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**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 continues 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.

**Calcium-Binding Protein Protocols** Hans J. Vogel, 2008-02-05 Calcium plays an important role in a wide variety of biological processes. This divalent metal ion can bind to a large number of proteins by doing so it modifies their biological activity or their stability. Because of its distinct chemical properties calcium is uniquely suited to act as an on/off switch or as a light dimmer of biological activities. The two books entitled Calcium Binding Protein Protocols Volumes I and II focus on modern experimental analyses and methodologies for the study of calcium binding proteins. Both extracellular and intracellular calcium binding proteins are discussed in detail. However proteins involved in calcium handling e.g. calcium pumps and calcium channels fall outside of the scope of these two volumes. Also calcium binding proteins involved in bone deposition will not be discussed as this specific topic has been addressed previously. The focus of these two books is on studies of the calcium binding proteins and their behavior in vitro and in vivo. The primary emphasis is on protein chemistry and biophysical methods. Many of the methods described will also be applicable to proteins that do not bind calcium. Calcium Binding Protein Protocols is divided into three main sections. The section entitled Introduction and Reviews provides information on the role of calcium in intracellular secondary messenger activation mechanisms. Moreover unique aspects of calcium chemistry and the utilization of calcium in dairy proteins as well as calcium binding proteins involved in blood clotting are addressed.

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