Methods in Molecular Biology 755

Springer Protocols



Laser Capture Microdissection

Methods and Protocols

Second Edition



Sandra J Rosenthal, David W Wright

Laser Capture Microdissection Graeme I. Murray, Stephanie Curran, 2010-12-09 Expert laboratorians noted for their optimized methods describe in step by step detail how to successfully carry out tissue based laser microdissection in their own laboratory using the different systems available and to apply a wide range of sophisticated molecular technologies to the microdissected tissue samples Highlights include gene expression profiling of primary tumor cell populations using laser capture microdissection RNA transcript amplification and GeneChip microarrays laser capture microdissection and colorectal cancer proteomics and proteomic analysis of human bladder tissue using SELDI approach following microdissection techniques Applications of the microdissected tissue described encompass PCR gene expression studies including microarray analysis and proteomics Phosphodiesterase Methods and Protocols Claire Lugnier, 2008-02-04 Research leaders in the PDE field describe new concepts and techniques for investigating the role of PDEs in orchestrating normal and pathophysiological responses Presented in step by step detail these readily reproducible methods allow the measurement of cyclic nucleotide variations in living cells as well as their visualization in a spatio temporal manner the localization and characterization of their activities in tissues and living cells and the assessment of targeted PDEs in creating specific tools Apoptosis Methods and Protocols Hugh J. M. Brady, 2008-02-05 The most fundamental question facing each and drugs and every cell within an org ism is to survive or to die Cell death is required for normal function some estimates suggest that as many as one million cells undergo cell death every second in the adult human body Almost all cells undergoing physiological or programmed cell death independent of cell type manifest a stereotypic p tern of morphological changes termed apoptosis Typically apoptotic cells d play shrinkage membrane blebbing chromatin condensation and nuclear fragmentation The integrity of the cell membrane is not lost during apoptosis and so avoids eliciting the inflammatory response that would have been caused by the spillage of the cell's contents. This is quite in contrast to the loss of cell contents typical of necrosis The caspases the family of intracellular cysteine proteases associated with apoptosis are responsible for the stereotypical m phological changes Caspases cleave various substrate proteins that act on DNA fragmentation nuclear envelope integrity the cytoskeleton and cell volume regulation Apoptotic cells are cleared in vivo by the process of phagocytosis in which specific phagocytes move to the site of apoptosis engulf the dying cells and digest them Apoptosis has a central role in many physiological processes for example in the immune system Autoreactive cells are deleted via apoptosis to prevent autoimmunity At the end of an immune response activated lymphocytes are removed to maintain homeostasis within the immune system <u>Differential Display Methods and Protocols</u> Peng Liang, Jonathan Meade, Arthur B. Pardee, 2008-02-04 Since the first edition of this book dedicated to differential display DD technology was published in 1997 we have witnessed an explosive interest in studying differential gene expression. The gene hunting euphoria was initially powered by the invention of DD which was gradually overtaken by DNA microarray technology in recent years Then why is

there still the need for second edition of this DD book First of all DD still enjoys a substantial lead over DNA microarrays in the ISI citation data see Table 1 despite the h dreds of millions of dollars spent each year on arrays This may come as a surprise to many but to us it implies that many of the DNA microarray studies went unpublished owing to their unfulfilled promises 1 Second unlike DNA microarrays DD is an open ended gene discovery method that does not depend on prior genome sequence information of the organism being studied As such DD is applicable to the study of all living organisms from bacteria fungi insects fish plants to mammals even when their genomes are not sequenced Second DD is more accessible technically and financially to most cost conscious cottage industry academic laboratories So clearly DD still has its unique place in the modern molecular biological toolbox for gene expression analysis NanoBiotechnology Protocols Sandra J Rosenthal, David W Wright, 2008-02-04 Hands on experts in nanomaterial synthesis and application describe in detail the key experimental techniques currently employed in novel materials synthesis dynamic cellular imaging and biological assays The author's emphasize diverse strategies to synthesize and functionalize the use of nanoparticles for biological applications Additional chapters focus on the use of biological components peptides antibodies and DNA to synthesize and organize nanoparticles to be used a building block in larger assemblies. These new materials make it possible to image cellular processes for longer durations leading to high throughput cellular based screens for drug discovery drug delivery and diagnostic applications Highlights include overview chapters on quantum dots and DNA nanotechnology and cutting edge techniques in the emerging nanobiotachnology arena Forensic DNA Typing Protocols Angel Carracedo, 2008-02-02 A state of the art collection of readily reproducible laboratory methods for DNA identity analysis including Y chromosome haplotyping mtDNA and SNP typing The book offers well tested protocols for DNA quantification using real time PCR on forensic samples and for the determination of the number of amelogenine gene copies For forensic geneticists there are readily reproducible methods for species identification ancient DNA and pharmacogenetics Additional chapters address new applications in the forensic genetics lab such a species identification or typing of CYP polymorphisms for the analysis of adverse to drugs Plant Cell Culture Protocols Víctor M. Loyola-Vargas, Felipe Vázguez-Flota, 2008-02-04 A comprehensive state of the art collection of the most frequently used techniques for plant cell and tissue culture Readily reproducible and extensively annotated the methods range from general methodologies such as culture induction growth and viability evaluation and contamination control to such highly specialized techniques as chloroplast transformation involving the laborious process of protoplast isolation and culture Most of the protocols are currently used in the research programs of the authors or represent important parts of business projects aimed at the generation of improved plant materials Two new appendices explain the principles for formulating culture media and the composition of the eight most commonly used media formulations and list more than 100 very useful internet sites Yeast Protocols Wei Xiao, 2008-02-03 In this second edition of a widely used classic laboratory manual leading experts utilize the tremendous progress and technological advances that

have occurred to create a completely new collection of not only the major basic techniques but also advanced protocols for yeast research and for using yeast as a host to study genes from other organisms. The authors provide detailed methods for the isolation of subcellular components including organelles and macromolecules for the basic cellular and molecular analysis specific for yeast cells and for the creation of conditional mutant phenotypes that lend themselves to powerful genome manipulation Additional protocols offer advanced approaches to study genetic interactions DNA and chromatin metabolism gene expression as well as the foreign genes and gene products in yeast cells **B Cell Protocols** Hua Gu, Klaus Rajewsky, 2008-02-04 B lymphocyte development and function remains an exciting area of research for those interested in the physiology and pathology of the immune system in higher animals While recent advances in genetics and cellular and molecular biology have provided a large spectrum of powerful new experimental tools in this field it is both time consuming and often very difficult for a student or just any bench side worker to identify a reliable experimental protocol in the ocean of the literature The aim of B Cell Protocols is to provide a collection of diverse protocols ranging from the latest inventions and applications to some classic but still frequently used methods in B cell biology The authors of the various chapters are all highly qualified scientists who are either the inventors or expert users of these methods Their extensive experience in mastering a particular method provides not only the step by step details of a reproducible protocol but also useful troubleshooting tips that readers will appreciate in their daily work. We hope that this book will be helpful for both beginning and experienced researchers in the field in designing or modifying an experimental approach and exploring a **Human Retrovirus Protocols** Tuofu Zhu,2008-02-04 A cutting edge collection biological question from multiple angles of basic and state of the art methods optimized for investigating the molecular biology of this class of retrovirus These readily reproducible techniques range from methods for the isolation and detection of human retroviruses to cutting edge methods for exploring the interplay between the viruses and the host Here the researcher will find up to date techniques for the isolation and propagation of HIV HTLV and foamy virus from a variety of sources There are also assays for determining the cell tropism of HIV 1 the coreceptor usage of HIV 1 and human gene expression with HIV 1 infection by microarrays as well as for phenotyping HIV 1 infected monocytes and examining their fitness Highlights include the detection and quantification of HIV 1 in resting CD4 a new cloning system for making recombinent virus cDNA microarrays and the determination of genetic polymorphisms in two recently identified HIV 1 co factors that are critical for HIV 1 infection **Calcium Signaling Protocols** David G. Lambert, 2008-02-04 In the first edition of Calcium Signaling Protocols I began by writing The regula 2 tion of intracellular Ca is a common theme presented in many papers over the last 20 2 or so years and the description of the Ca sensitive indicator dye fura 2 in 1985 resulted in a massive increase in these types of studies This statement is as true in 2005 as it was in 1999 but 20 or so years is now 30 years. There has been some reorganization of the volume such that there are now 22 ch ters including five new ones all written by experts in their field These new chapters 2 include use of the

FlexStation and electrophysiological measurement of Ca channel activity The book is broken into six parts Part I is a general coverage of basic theory and the simplest use of fluorescent indicators Part II covers specialist measurement 2 systems and Part III covers measurement of Ca channel activity Assessment of 2 release of stored Ca is covered in some detail in Part IV with Parts V and VI cover 2 ing specialist measurement techniques and Ca sensitive targets Putting a book like this together even as a second edition takes time and I am again indebted to the individual authors for their help and patience I am also very grateful to Professor John M Walker the series editor for his continued help and advice over the course of this project

Advanced Topics in Forensic DNA Typing: Methodology John M. Butler, 2011-07-27 Intended as a companion to the Fundamentals of Forensic DNA Typing volume published in 2009 Advanced Topics in Forensic DNA Typing Methodology contains 18 chapters with 4 appendices providing up to date coverage of essential topics in this important field and citation to more than 2800 articles and internet resources The book builds upon the previous two editions of John Butler s internationally acclaimed Forensic DNA Typing textbook with forensic DNA analysts as its primary audience This book provides the most detailed information written to date on DNA databases low level DNA validation and numerous other topics including a new chapter on legal aspects of DNA testing to prepare scientists for expert witness testimony Over half of the content is new compared to previous editions A forthcoming companion volume will cover interpretation issues Contains the latest information hot topics and new technologies Well edited attractively laid out and makes productive use of its four color format Author John Butler is ranked as the number one high impact author in legal medicine and forensic science 2001 to **Cytochrome P450 Protocols** Ian R. Phillips, 2008-02-04 For this second edition of their 2011 by ScienceWatch com much praised Cytochrome P450 the editors have collected accounts of the essential core techniques that use the latest methodologies for the investigation of P450s Highlights include protocols for spectral analysis and purification of P450s enzymatic assays of P450s and flavin containing monooxygenases FMOs expression of P450s and FMOs in heterologous systems and the production and use of antipeptide antibodies Additional chapters contain readily reproducible techniques for the transfection of hepatocytes for gene regulation studies P450 reporter gene assays in situ hybridization and analysis of genetic polymorphisms Although the emphasis is on P450s of mammalian origin many of the readily reproducible methods described are suitable for P450s from any source Cell Cycle Control Tim Humphrey, Gavin Brooks, 2008-02-04 The fundamental question of how cells grow and divide has perplexed biologists since the development of the cell theory in the mid 19th century when it was recognized by Virchow and others that all cells come from cells In recent years considerable effort has been applied to the identification of the basic molecules and mechanisms that regulate the cell cycle in a number of different organisms Such studies have led to the elucidation of the central paradigms that underpin eukaryotic cell cycle control for which Lee Hartwell Tim Hunt and Paul Nurse were jointly awarded the Nobel Prize for Medicine and Physiology in 2001 in recognition of their seminal contributions to this field The importance of understanding the fundamental

mechanisms that modulate cell division has been reiterated by relatively recent discoveries of links between cell cycle control and DNA repair growth cellular metabolism development and cell death This new phase of integrated cell cycle research provides further challenges and opportunities to the biological and medical worlds in applying these basic concepts to understanding the etiology of cancer and other proliferative diseases **Receptor Binding Techniques** Anthony P. Davenport, 2008-02-02 A comprehensive collection of readily reproducible methods for studying receptors in silico in vitro and in vivo These cutting edge techniques cover mining from curated databases identifying novel receptors by high throughput screening molecular methods to identify mRNA encoding receptors radioligand binding assays and their analysis quantitative autoradiography and imaging receptors by positron emission tomography PET Highlights include phenotypic characterization of receptors in knockout mice imaging receptors using green fluorescent protein and fluorescent resonance energy transfer and quantitative analysis of receptor mRNA by TagMan PCR These book equips the researcher with techniques for exploring the unprecedented number of new receptor systems now emerging and the so called orphan receptors whose activating ligand has not been identified Chemical Genomics Edward D. Zanders, 2008-02-04 Chemical genomics is an exciting new field that aims to transform biolo cal chemistry into a high throughput industrialized process much in the same way that molecular biology has been transformed by genomics. The intertion of small organic molecules with biological systems mostly proteins underpins drug discovery in the pharmaceutical and biotechnology industries and therefore a volume of laboratory protocols that covers the key aspects of chemical genomics would be of use to biologists and chemists in these orga zations Academic scientists have been exploring the functions of proteins using small molecules as probes for many years and therefore would also be fit from sharing ideas and laboratory procedures Whatever the organizational backgrounds of the scientists involved the challenges of extracting the ma mum human benefit from genome sequencing projects remains considerable and one where it is increasingly recognized that chemical genomics will play an important part Chemical Genomics Reviews and Protocols is divided into two sections the first being a series of reviews to describe what chemical genomics is about and to set the scene for the protocol chapters The subject is introduced by Paul Caron who explains the various flavors of chemical genomics This is f lowed by Lutz Weber and Philip Dean who cover the interaction between organic molecules and protein targets from the different perspectives of la ratory experimentation and in silico design The protocols begin with the me ods developed in Christopher Lowes laboratory Roque et al DNA Repair *Protocols* Daryl S. Henderson, 2008-02-03 The first edition of this book published in 1999 and called DNA Repair Protocols Eukaryotic Systems brought together laboratory based methods for studying DNA damage and repair in diverse eukaryotes namely two kinds of yeast a nematode a fruit fly a toad three different plants and human and murine cells This second edition of DNA Repair Protocols covers mammalian cells only and hence its new subtitle Mammalian Systems There are two reasons for this fresh emphasis both of them pragmatic to cater to the interests of what is now a largely mammalocentric DNA repair

field and to expedite editing and prod tion of this volume Although DNA Repair Protocols Mammalian Systems is a smaller book than its predecessor it actually contains a greater variety of methods Fourteen of the book s thirty two chapters are entirely new and areas of redundancy present in the first edition have been eliminated here for example now just two chapters describe assays for nucleotide excision repair NER rather than seven All eighteen returning chapters have been revised many of them ext sively In order to maintain a coherent arrangement of topics the four part p titioning seen in the first edition was dispensed with and chapters concerned with ionizing radiation damage and DNA strand breakage and repair were re cated to near the front of the book Finally an abstract now heads each chapter Handbook of ELISPOT Alexander E. Kalyuzhny, 2008-02-02 In this first book dedicated entirely to the ELISPOT a critical enzyme linked immunospot assay used widely in biomedical research recognized experts with first hand experience detail how to design perform and analyze these assays The readily reproducible techniques they provide cover a wide variety of topics including the use of membrane backed plates the standardization and validation procedures the removal of cells from ELISPOT plates cell separation techniques and the quantification of ELISPOT data There are also numerous ELISPOT applications involving animal models human cells measles multiple sclerosis immune responses multicytokine detection systems and immunocytochemistry Highlights include dual color and multiplex ELISPOT assays use of the ELISPOT assay on feline lymphocytes standardization of the ELISPOT procedure and combining the ELISPOT assay with immunohistochemistry **Amyloid Proteins** Einar M. Sigurdsson, 2008-02-02 A proven collection of readily reproducible techniques for studying amyloid proteins and their involvement in the etiology pathogenesis diagnosis and therapy of amyloid diseases. The contributors provide methods for the preparation of amyloid and its precursors oligomers and protofibrils in vitro assays and analytical techniques for their study and cell culture models and assays for the production of amyloid proteins Additional chapters present readily reproducible techniques for amyloid extraction from tissue its detection in vitro and in vivo as well as nontransgenic methods for developing amyloid mouse models The protocols follow the successful Methods in Molecular BiologyTM series format each offering step by step laboratory instructions an introduction outlining the principle behind the technique lists of the necessary equipment and reagents and tips on troubleshooting and avoiding known pitfalls In Situ Hybridization **Protocols** Ian A. Darby, Tim D. Hewitson, 2008-02-04 The technique of in situ hybridization in its various forms has been used routinely in many laboratories for a number of years In the post genome era gene arrays and proteomics have allowed us to identify hitherto unknown unrecognized pathways and mechanisms However rather than diminish the importance of in situ hybridization the now widespread use of screening te nologies has increased the need to temporally and spatially localize the dist bution of mRNA expression Our intention in In Situ Hybridization Protocols is to provide ample inf mation for novices planning to set up the in situ hybridization technique and use it in their laboratory for the first time as well as giving updates of recent developments for those laboratories where in situ hybridization techniques are already in use Despite its

widespread significance in situ hybridization has retained a re tation as one of the more difficult and capricious molecular biological te niques This may in part be because of the hybrid nature of the technique which often requires a mixture of molecular biological and histological skills The two techniques are usually taught and acquired in different streams of biolo cal science The step by step and detailed protocols provided in In Situ Hybridization Protocols by researchers active in the field should make it p sible for both the molecular biologist with little experience of histology and the histologist with little experience of molecular biology to use the technique s cessfully in their laboratories

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