

Methods in Molecular Biology™

Volume 75

# BASIC CELL CULTURE PROTOCOLS

*Second Edition*

*Edited by*  
**Jeffrey W. Pollard**  
*and John M. Walker*



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# **Basic Cell Culture Protocols Second Edition**

**Jin-Ying Zhang**



## **Basic Cell Culture Protocols Second Edition:**

**Basic Cell Culture Protocols** Jeffrey W. Pollard, John M. Walker, 1997 Now completely revised and updated from the original much acclaimed and bestselling first edition Basic Cell Culture Protocols 2nd ed offers today's most comprehensive collection of easy to follow cutting edge protocols for the culture of a wide range of animal cells Its authoritative contributors provide explicit step by step instructions along with extensive notes and tips that allow both experts and beginners to successfully achieve their desired results Topics range from basic culture methodology to strategies for culturing previously uncultured cell types and hard to culture differentiated cells Methods are also provided for the analysis of living cells by FACS video microscopy and confocal microscopy Like the first edition this book should be in every cell culture laboratory and be of use to all who use cell cultures in research

## **Principles and Practice of Animal Tissue Culture (Second Edition)**

, **Plant Cell Culture Protocols** Víctor M. Loyola-Vargas, Felipe Vázquez-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

**A Manual for Primary Human Cell Culture** Jan-Thorsten Schantz, Kee Woei Ng, 2004-10-14 As part of the boom in biotechnology particularly in tissue engineering primary human cell culture has become a major pillar in academic research and the biopharmaceutical industry Obtaining a viable culture from a tissue sample and maintaining it for experimental diagnostic or therapeutic purposes can be quite a challenge Based on laboratory protocols and practical experience from many years of primary cell culture this manual presents the basic steps necessary for culturing primary human cells Written by students for students the manual serves well as a practical guide to primary human cell culture The authors have left much space for notes and the design of the manual is such that it can be continuously upgraded and extended The content of this manual is by no means exhaustive Protocols for specific cell types out of over 200 different cell types in the human body were selected from major tissue groupings in the body They should serve as a foundation for individual researchers to experiment explore and establish niche protocols for their specific needs Inspired by the practical clinical checklists available to residents and trainees in medicine the authors have chosen a compact physical format that can fit into the pocket of a lab coat

**Human Cell Culture Protocols** Joanna Picot, 2008-02-02 A thoroughly revised and updated collection readily reproducible techniques for culturing human cells This new edition includes a wide range of human cell types relevant to human disease and new chapters on fibroblasts Schwann cells gastric and colonic

epithelial cells and parathyroid cells The protocols follow the successful Methods in Molecular Medicine™ 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 **Xenopus Protocols** X. Johné Liu, 2008-02-02 A collection of standard and cutting edge techniques for using Xenopus oocytes and oocytes egg extracts to reconstitute biological and cellular processes These readily reproducible methods take advantage of the oocyte s impressive protein abundance its striking protein translation capacity and its breathtaking possibilities for the assembly of infectious viral particles by single cell injection of multiple RNAs The authors focus on the versatility of frog oocytes and egg extracts in cell biology and signal transduction and cover all the major uses of oocytes extracts as experimental models

*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 and drugs 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

*Differential Display Methods and Protocols* 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

**Hormone Assays in Biological Fluids** Michael J. Wheeler, William D. Fraser, J. S. Morley Hutchinson, 2008-02-04 Expert researchers who have developed and applied significant new assays describe in step by step detail a variety of methods for measuring a broad variety of hormones related peptides and synthetic steroids in various biological fluids The hormones measured range from glucocorticoids in biological fluids urinary steroids aldosterone in blood and plasma renin activity to gut hormones in plasma melatonin prolactin 6 sulfatoxymelatonin and androgens in blood saliva and hair The emphasis is on noncommercial assays so that investigators can set up novel methods suited to their special needs Commercial assays are also described for comparative purposes Tutorials on radioimmunoassay gas chromatography mass spectrometry high performance liquid chromatography and PCR techniques help the reader to choose the best method for his or her purpose

**RNA Silencing** Gordon Carmichael, 2008-02-04 A collection of readily reproducible methods for the design preparation and use of RNAs for silencing gene expression in cells and organisms The techniques range widely and include methods addressing the biochemical aspects of the silencing machinery RNA silencing in non mammalian organisms and the in vivo delivery of siRNAs and silencing vectors There are also techniques for designing preparing and using RNAs to silence gene expression for fine tuning regulation by targeting specific isoforms of a given gene and for the study and use of microRNAs The protocols follow the successful Methods in Molecular Biology™ 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

**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

**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 production 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 extensively In order to maintain a coherent arrangement of topics the four part partitioning seen in the first edition was dispensed with and chapters concerned with ionizing radiation damage and DNA strand breakage and repair were relocated to near the front of the book Finally an abstract now heads each chapter

**Ubiquitin-Proteasome Protocols** Cam Patterson, Douglas M. Cyr, 2008-02-04 A collection of cutting edge techniques for studying ubiquitin dependent protein degradation via the proteasome The topics covered range broadly from basic biochemistry to cellular assays to discovery techniques using mass spectrometric analysis These biochemical and cellular methods are necessary to explore the ubiquitin proteasome system and ubiquitin proteasome dependent functions State of the art and user friendly Ubiquitin Proteasome Protocols offers novice and experienced bench scientists alike a thorough compendium of readily reproducible techniques that will accelerate discovery enhance productivity and permit manipulation of the system for varied research purposes Animal Cell Culture John M. Davis, 2011-03-21 This is a comprehensive research guide that describes both the key new techniques and more established methods Every chapter discusses the merits and limitations of the various approaches and then provides selected tried and tested protocols as well as a plethora of good practical advice for immediate use at the bench It presents the most accessible and comprehensive introduction available to the culture and experimental manipulation of animal cells Detailed protocols for a wide variety of methods provide the core of each chapter making new methodology easily accessible This book is an essential laboratory manual for all undergraduates and graduates about to embark on a cell culture project It is a book which both experienced researchers and those new to the field will find invaluable

**Plant Tissue Culture Concepts and Laboratory Exercises** Robert N. Trigiano, Dennis J. Gray, 2018-04-27 Alternating between topic discussions and hands on laboratory experiments that range from the in vitro flowering of roses to tissue culture of ferns Plant Tissue Culture Concepts and Laboratory Exercises Second Edition addresses the most current principles and methods in plant tissue culture research The editors use the expertise of some of the top researchers and educators in plant biotechnology to furnish students instructors and researchers with a broad consideration of the field Divided into eight major parts the text covers everything from the history of plant tissue culture and basic methods to propagation techniques crop improvement procedures specialized applications and nutrition of

callus cultures New topic discussions and laboratory exercises in the Second Edition include Micropropagation of *Dieffenbachia* Micropropagation and in vitro flowering of rose Propagation from nonmeristematic tissue organogenesis Variation in culture and Tissue culture of ferns It is the book's extensive laboratory exercises that provide a hands on approach in illustrating various topics of discussion featuring step by step procedures anticipated results and a list of materials needed What's more editors Trigiano and Gray go beyond mere basic principles of plant tissue culture by including chapters on genetic transformation techniques and photographic methods and statistical analysis of data In all *Plant Tissue Culture Concepts and Laboratory Exercises Second Edition* is a veritable harvest of information for the continued study and research in plant tissue culture science

**Chemoinformatics** Jürgen Bajorath, 2008-02-04 In the literature several terms are used synonymously to name the topic of this book chem chemi or chemo informatics A widely recognized definition of this discipline is the one by Frank Brown from 1998 1 who defined chemoinformatics as the combination of all the information resources that a scientist needs to optimize the properties of a ligand to become a drug In Brown's definition two aspects play a fundamentally important role definition support by computational means and drug discovery which distinguishes it from the term chemical informatics that was introduced at least ten years earlier and described as the application of information technology to chemistry not with a specific focus on drug discovery In addition there is of course chemometrics which is generally understood as the application of statistical methods to chemical data and the derivation of relevant statistical models and descriptors 2 The pharmaceutical focus of many developments and efforts in this area and the current popularity of gene to drug or similar paradigms is further reflected by the recent introduction of such terms as discovery informatics 3 which takes into account that gaining knowledge from chemical data alone is not sufficient to be ultimately successful in drug discovery Such insights are well in accord with other views that the boundaries between bio and chemoinformatics are fluid and that these disciplines should be closely combined or merged to significantly impact biotechnology or pharmaceutical research 4

**Plant Virology Protocols** Gary D. Foster, Sally Taylor, 2008-02-03 The aim of *Plant Virology Protocols* is to provide a source of information to guide the reader through the wide range of methods involved in generating transgenic plants that are resistant to plant viruses To this end we have commissioned a wide ranging list of chapters that will cover the methods required for plant virus isolation RNA extraction cloning coat protein genes introduction of the coat protein gene into the plant genome and testing transgenic plants for resistance The book then moves on to treatments of the mechanisms of resistance the problems encountered with field testing and key ethical issues surrounding transgenic technology Although *Plant Virology Protocols* deals with the cloning and expression of the coat protein gene the techniques described can be equally applied to other viral genes and nucleotide sequences many of which have also been shown to afford protection when introduced into plants The coat protein has however been the most widely applied and as such has been selected to illustrate the techniques involved *Plant Virology Protocols* has been divided into six major sections containing 55 chapters in total

*Therapeutic Proteins* C. Mark Smales, David C. James, 2008-02-04 With the recent completion of the sequencing of the human genome it is widely anticipated that the number of potential new protein drugs and targets will escalate at an even greater rate than that observed in recent years However identification of a potential target is only part of the process in developing these new next generation protein based drugs that are increasingly being used to treat human disease Once a potential protein drug has been identified the next rate limiting step on the road to development is the production of sufficient authentic material for testing characterization clinical trials and so on If a protein drug does actually make it through this lengthy and costly process methodology that allows the production of the protein on a scale large enough to meet demand must be implemented Furthermore large scale production must not compromise the authenticity of the final product It is also necessary to have robust methods for the purification characterization viral inactivation and continued testing of the authenticity of the final protein product and to be able to formulate it in a manner that retains both its biological activity and lends itself to easy administration *Therapeutic Proteins Methods and Protocols* covers all aspects of protein drug production downstream of the discovery stage This volume contains contributions from leaders in the field of therapeutic protein expression purification characterization formulation and viral inactivation Pharmacogenomics Federico Innocenti, 2008-02-03 Expert researchers and physician clinicians describe in detail the newest and most commonly used technologies today in this rapidly advancing field The authors provide readily reproducible methods for assessing the functional consequences of a certain polymorphism evaluate the variety of genotyping platforms currently available and discuss the management of pharmacogenomic information Highlights include techniques for making a snapshot of the allele specific variation in human gene expression genome wide analysis of allele specific expression using oligo microarrays in vivo assays with HaploChIP SNP genotyping in DNA pools and PharmGKB the pharmacogenetics and pharmacogenomics knowledge base The methodologies for genotyping include denaturing high performance liquid chromatography pyrosequencing kinetic fluorescence detection mass spectrometry and TaqMan assay for insertion deletions



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## **Table of Contents Basic Cell Culture Protocols Second Edition**

1. Understanding the eBook Basic Cell Culture Protocols Second Edition
  - The Rise of Digital Reading Basic Cell Culture Protocols Second Edition
  - Advantages of eBooks Over Traditional Books
2. Identifying Basic Cell Culture Protocols Second Edition
  - Exploring Different Genres
  - Considering Fiction vs. Non-Fiction
  - Determining Your Reading Goals
3. Choosing the Right eBook Platform
  - Popular eBook Platforms
  - Features to Look for in an Basic Cell Culture Protocols Second Edition
  - User-Friendly Interface
4. Exploring eBook Recommendations from Basic Cell Culture Protocols Second Edition
  - Personalized Recommendations
  - Basic Cell Culture Protocols Second Edition User Reviews and Ratings
  - Basic Cell Culture Protocols Second Edition and Bestseller Lists
5. Accessing Basic Cell Culture Protocols Second Edition Free and Paid eBooks
  - Basic Cell Culture Protocols Second Edition Public Domain eBooks
  - Basic Cell Culture Protocols Second Edition eBook Subscription Services
  - Basic Cell Culture Protocols Second Edition Budget-Friendly Options
6. Navigating Basic Cell Culture Protocols Second Edition eBook Formats

- ePub, PDF, MOBI, and More
- Basic Cell Culture Protocols Second Edition Compatibility with Devices
- Basic Cell Culture Protocols Second Edition Enhanced eBook Features
- 7. Enhancing Your Reading Experience
  - Adjustable Fonts and Text Sizes of Basic Cell Culture Protocols Second Edition
  - Highlighting and Note-Taking Basic Cell Culture Protocols Second Edition
  - Interactive Elements Basic Cell Culture Protocols Second Edition
- 8. Staying Engaged with Basic Cell Culture Protocols Second Edition
  - Joining Online Reading Communities
  - Participating in Virtual Book Clubs
  - Following Authors and Publishers Basic Cell Culture Protocols Second Edition
- 9. Balancing eBooks and Physical Books Basic Cell Culture Protocols Second Edition
  - Benefits of a Digital Library
  - Creating a Diverse Reading Collection Basic Cell Culture Protocols Second Edition
- 10. Overcoming Reading Challenges
  - Dealing with Digital Eye Strain
  - Minimizing Distractions
  - Managing Screen Time
- 11. Cultivating a Reading Routine Basic Cell Culture Protocols Second Edition
  - Setting Reading Goals Basic Cell Culture Protocols Second Edition
  - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Basic Cell Culture Protocols Second Edition
  - Fact-Checking eBook Content of Basic Cell Culture Protocols Second Edition
  - Distinguishing Credible Sources
- 13. Promoting Lifelong Learning
  - Utilizing eBooks for Skill Development
  - Exploring Educational eBooks
- 14. Embracing eBook Trends
  - Integration of Multimedia Elements
  - Interactive and Gamified eBooks

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