

Methods in Molecular Biology™

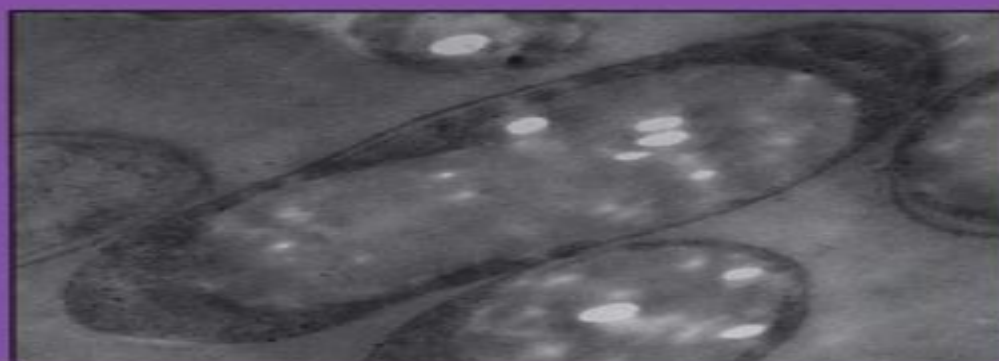
Volume 63

RECOMBINANT PROTEIN PROTOCOLS

Detection and Isolation

Edited by

Rocky S. Tuan



Humana Press

Recombinant Protein Protocols

John E. Coligan



Recombinant Protein Protocols:

Recombinant Protein Protocols Rocky S. Tuan, 2013-08-23 A major success story of modern molecular biology is the development of technologies to clone and express specific genes. Current applications of recombinant gene products cover a wide spectrum including gene therapy, production of bioactive pharmaceuticals, synthesis of novel biopolymers, agriculture and animal husbandry and so on. Inherent in bringing these applications to fruition is the need to design expression constructs that will permit the ready and specific detection and isolation of the defined recombinant gene products. *Recombinant Protein Protocols* grows out of the need for a laboratory manual on the detection and isolation of recombinantly expressed genes that covers both the background information and the practical laboratory recipes for these analyses. In this book, detailed and contemporary protocols are collected to provide the reader with a wide ranging number of methodologies to enhance the detection and isolation of their gene products of interest. A large number of molecular tags and labels and their usage are described including enzymes, ligand binding moieties, immunodetectable molecules as well as methods to detect interactive proteins and gene expression mediated alterations in cellular activity. Chapters on in situ detection of gene expression deal with technologies that are currently being applied to the study of gene function and activity. Highlights of applications for recombinant gene expression technologies are provided to give readers exciting perspectives on the future of such technologies.

Recombinant Proteins in Plants Stefan Schillberg, Holger Spiegel, 2022-05-27 This volume provided methods and protocols on recombinant protein production in different plant systems, downstream processing and strategies to optimize protein expression. Chapters guide readers through recombinant protein production in important plant systems, protein recovery and purification, different strategies to optimise productivity, cloning and fusion protein approaches and the regulation and freedom to operate analysis of plant produced proteins. 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. Authoritative and cutting edge, *Recombinant Proteins in Plants: Methods and Protocols* aims to be useful to newcomers and experienced researchers interested in expanding their expertise in the field of plant based protein production. Chapters 6, 8 and 17 are available open access under a Creative Commons Attribution 4.0 International License via link.springer.com.

Recombinant Protein Expression in Mammalian Cells David L. Hacker, 2024-06-26 This fully updated volume explores notable developments in the field of mammalian cell based recombinant protein production. Beginning with methods for transient recombinant protein production, the book continues with methods for stable cell pool generation, protein production using stable clonal cell lines as well as high throughput screening technologies for characterizing transient cell surface protein ectodomain expression and for identifying host genes involved in protein production. Written for the highly successful *Methods in Molecular Biology* series, chapters include introductions to their respective topics, lists of the necessary

materials and reagents step by step and readily reproducible laboratory protocols and tips on troubleshooting and avoiding known pitfalls Authoritative and practical Recombinant Protein Expression in Mammalian Cells Methods and Protocols Second Edition serves as an ideal guide for researchers investigating protein structure and function and accelerating the discovery of new therapeutic proteins *The Protein Protocols Handbook* John M. Walker, 2007-10-02 The Protein Protocols Handbook Second Edition aims to provide a cross section of analytical techniques commonly used for proteins and peptides thus providing a benchtop manual and guide for those who are new to the protein chemistry laboratory and for those more established workers who wish to use a technique for the first time All chapters are written in the same format as that used in the Methods in Molecular Biology™ series Each chapter opens with a description of the basic theory behind the method being described The Materials section lists all the chemicals reagents buffers and other materials necessary for carrying out the protocol Since the principal goal of the book is to provide experimentalists with a full account of the practical steps necessary for carrying out each protocol successfully the Methods section contains detailed step by step descriptions of every protocol that should result in the successful execution of each method The Notes section complements the Methods material by indicating how best to deal with any problem or difficulty that may arise when using a given technique and how to go about making the widest variety of modifications or alterations to the protocol Since the first edition of this book was published in 1996 there have of course been significant developments in the field of protein chemistry **Short Protocols in Protein Science** John E. Coligan, 2003-10-24 Short Protocols in Protein Science provides condensed descriptions of more than 500 protocols compiled from Current Protocols in Protein Science Drawing from both the original core manual as well as the quarterly update service this compendium includes all step by step descriptions of the principal methods covered in Current Protocols in Protein Science *Recombinant Protein Production in Yeast* Roslyn M. Bill, 2016-05-01 This book reviews preparation of expression vectors generation of high yielding clones scale up disruption of yeast cells to enable isolation of recombinant protein prior to purification and more in the popular Methods in Molecular Biology format **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 **Methods in Molecular Biology: Recombinant protein protocols** John M. Walker, 1984 Insoluble Proteins Elena García-Fruitós, 2014-12-02 With insolubility proving to be one of the most crippling bottlenecks in the protein production and purification process this volume serves to aid researchers working in the recombinant protein production field by describing a wide number of protocols and

examples Insoluble Proteins Methods and Protocols includes chapters that describe not only the recombinant protein production in different expression systems but also different purification and characterization methods to finally obtain these difficult to obtain proteins Beginning with protein production methods using both prokaryotic and eukaryotic expression systems the book continues with purification protocols using insoluble proteins the characterization of insoluble proteins as well as a general overview of interesting applications of insoluble proteins 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 Comprehensive and practical Insoluble Proteins Methods and Protocols aims to provide the scientific community with detailed and reliable state of the art protocols that are used in order to successfully produce and purify recombinant proteins prone to aggregate

Pichia Protocols James M Cregg, 2007-08-08 This book focuses on recent developments of *Pichia pastoris* as a recombinant protein production system Highlighted topics include a discussion on the use of fermentors to grow *Pichia pastoris* information on the O and N linked glycosylation methods for labeling *Pichia pastoris* expressed proteins for structural studies and the introduction of mutations in *Pichia pastoris* genes by the methods of restriction enzyme mediated integration REMI Each chapter presents cutting edge and cornerstone protocols for utilizing *P. pastoris* as a model recombinant protein production system This volume fully updates and expands upon the first edition

Protein Purification Protocols Paul Cutler, 2008-02-02 The first edition of Protein Purification Protocols 1996 edited by Professor Shawn Doonan rapidly became very successful Professor Doonan achieved his aims of producing a list of protocols that were invaluable to newcomers in protein purification and of significant benefit to established practitioners Each chapter was written by an experienced expert in the field In the intervening time a number of advances have warranted a second edition However in attempting to encompass the recent developments in several areas the intention has been to expand on the original format retaining the concepts that made the initial edition so successful This is reflected in the structure of this second edition I am indebted to Professor Doonan for his involvement in this new edition and the continuity that this brings Each chapter that appeared in the original volume has been reviewed and updated to reflect advances and bring the topic into the 21st century In many cases this reflects new applications or new matrices available from vendors Many of these have increased the performance and or scope of the given method Several new chapters have been introduced including chapters on all the currently used protein fractionation and chromatographic techniques They introduce the theory and background for each method providing lists of the equipment and reagents required for their successful execution as well as a detailed description of how each is performed

Protein Chromatography Sinéad T Loughran, John Joseph Milne, 2023-08-30 This third edition expands on the previous editions with updated and new chapters on protein chromatography Chapters detail protein stability and storage avoiding proteolysis protein quantitation methods generation and purification of recombinant proteins

recombinant antibody production and the tagging of proteins Written in the format of the highly successful Methods in Molecular Biology series each chapter includes an introduction to the topic lists necessary materials and reagents includes tips on troubleshooting and known pitfalls and step by step readily reproducible protocols Authoritative and cutting edge Protein Chromatography Methods and Protocols Third Edition aims to provide commonly used methods and new approaches to help both new researchers and experts expand their knowledge

Calcium-Binding Protein Protocols Hans J. Vogel, 2008-02-04 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

The Nucleic Acid Protocols Handbook Ralph Rapley, 2008-06-29 A comprehensive treasury of all the key molecular biology methods ranging from DNA extraction to gene localization in situ needed to function effectively in the modern laboratory Each of the 120 highly successful techniques follows the format of the much acclaimed Methods in Molecular Biology series providing an introduction to the scientific basis of each technique a complete listing of all the necessary materials and reagents and clear step by step instruction to permit error free execution Included for each technique are notes about pitfalls to avoid troubleshooting tips alternate methods and explanations of the reasons for certain steps all key elements contributing significantly to success or failure in the lab The Nucleic Acid Protocols Handbook constitutes today's most comprehensive collection of all the key classic and cutting edge techniques for the successful isolation analysis and manipulation of nucleic acids by both experienced researchers and those new to the field

Production Technology of Recombinant Therapeutic Proteins Chiranjib Chakraborty, 2004 An Increasing Number Of Recombinant Therapeutic Proteins Are Currently Being Developed Tested In Clinical Trials And Marketed For Use Most Of The Recombinant Therapeutic Proteins Are Being Successfully Produced Into Escherichia Coli And Pichia Pastoris Expression System These Two Expression Systems Are Very Much Efficient And Cost

Effective This Book Takes A Close Look Of These Two Expression Systems And Fermentation Conditions Purification Strategies Of Different Recombinant Proteins This Book Also Discusses The Market Size And Cost Analysis For The Production Of Different Therapeutic Proteins And Some General Experimental Protocols For Production Contents Part I Recombinant Protein Expression Into Escherichia Coli And Fermentation Conditions Chapter 1 Introduction Chapter 2 Construction Of Efficient Expression Vector Plasmid Chapter 3 Factors Affecting Transcription Promoters Upstream Elements Transcriptional Terminators Transcriptional Antitermin Tightlly Regulated Expression Systems Chapter 4 Mrna Stability Chapter 5 Factors Affecting Translation Mrna Translational Initiator Translational Enhancers Translational Termination Chapter 6 Expression Of Target Protein And The Compartments Of Expression Cytoplasmic Expression Periplasmic Expression Extracellular Secretion Chapter 7 Fusion Proteins Chapter 8 Post Translational Protein Folding Chapter 8 Codon Usage Chapter 10 Protein Degradation Chapter 11 Fermentation Conditions For High Density Cell Cultivation Hdcc Growth Medium Efficient Production Of Recombinant Protein In Hdcc Nutrient Feeding Strategy In Hdcc Chapter 12 One Examples Of Protein Production Using E Coli Expression System Chapter 13 Conclusion Part Ii Recombinant Protein Expression Into Yeast Pichia Pastoris And Fermentation Conditions Chapter 1 Introduction Chapter 2 Why P Pastoris Chapter 3 Construction Of Expression Strains Expression Vectors Alternative Promoters Host Strains Methanol Utilisation Phenotype Protease Reduced Host Strains Integration Of Expression Vectors Into The P Pastoris Genome Generating Multicopy Strains Chapter 4 Post Translational Modifications Of Secreted Proteins Secretion Signal Selection N Linked Glycosylation Chapter 5 Production Of Recombinant Proteins In Fermenter Cultures Of The Yeast Pichia Pastoris Conceptual Basis For The P Pastoris Expression System High Level Expression In Fermenter Cultures Protein Specific Adjustments To Improve Yield Glycosylation Of Recombinant Proteins Secretion Signals Chapter 6 One Examples Of Protein Producing Using P Pastoris Expression System Chapter 7 Conclusion Part Iii Purification Strategies For Recombinant Proteins Chapter 1 Purification Of Proteins Chapter 2 Conventional Chromatography Ion Exchange Chromatography Reversed Phase Chromatography Gel Permeation Chromatography Affinity Chromatography Affinity Tags Cleavage Conclusion Part Iv Market Size And Cost Analysis For The Production Of Therapeutic Proteins Chapter 1 Market Size Of Therapeutic Proteins Chapter 2 Outline Structure Of A Productin Unit And Cost Analysis For The Production Of Three Therapeutic Proteins Part V General Experimental Protocols Chapter 1 Different Experimental Protocols Preparation Of Genome Dna For E Coli A Differnt Method For Preparation Of Genomic Dna From Bacteria Preparation Of Proteins From Periplasm Osmotic Shock Method Preparation Of Proteins From Outer Membrane Transformation Of Plasmid Dna Into E Coli Calcium Chloride Heat Shock Method Transformation Of Plasmid Dna Into E Coli Electroporation Sds Page For Large Proteins Sds Page For Small Peptide Pcr Amplification Of Dna Protein Quantification Brandford Method Trans Blotting For Protein Restriction Enzyme Digestion Of Dna Phenol Chloroform Extraction Of Dna Ethanol Precipitation Of Dna Agarose Gel Electrophoresis Transformation Of E

Coli By Electroporation Alternative Method Wizard Tm Pcr Preps Dna Purification System For Rapid Purification Of Dna Fragments Alternate Method For Purifying Dna From Agarose Gels Southern Blotting Rt Pcr Protocol Using Superscript Reverse Transcriptase Preparation Of Sequencing Gels Isolation Of Rna From Mammalian Cells Using RnazolTm Teltest Preparation For Yeast Transformation Yeast Transformation Digesting Prsq Ura3 With Bamhi Genomic Dna Preparation Of Yeast Ligation Circularisation Of Genomic Dna Fragments E Coli Transformation Alternate Method Dna Miniprep From E Coli Alternate Method Basic Plasmid Dna Isolation Protocol Identification And Determination Of Amount Rec Hum Proteins Via An Immunoenzymatic Test Elisa Determination Of Host Dna Contaminant Into R Hu Protein Through Dot Blot Method Protocols For Down Stream Processing Membrane Protein Protocols Barry S. Selinsky, 2008-02-03 Knowledge of the three dimensional structure of a protein is absolutely required for the complete understanding of its function The spatial orientation of amino acids in the active site of an enzyme demonstrates how substrate specificity is defined and assists the medicinal chemist in the design of specific tight binding inhibitors The shape and contour of a protein surface hints at its interaction with other proteins and with its environment Structural analysis of multiprotein complexes helps to define the role and interaction of each individual component and can predict the consequences of protein mutation or conditions that promote dissociation and rearrangement of the complex Determining the three dimensional structure of a protein requires milligram quantities of pure material Such quantities are required to refine crystallization conditions for X ray analysis or to overcome the sensitivity limitations of NMR spectroscopy Historically structural determination of proteins was limited to those expressed naturally in large amounts or derived from a tissue or cell source inexpensive enough to warrant the use of large quantities of cells However with the advent of the techniques of modern gene expression many proteins that are constitutively expressed in minute amounts can become accessible to large scale purification and structural analysis

Recombinant Proteins from Plants Jacqueline MacDonald, Igor Kolotilin, Rima Menassa, 2015-11-27 This volume provides up to date scientific achievements from the world's top researchers Recombinant Proteins from Plants Methods and Protocols Second Edition guides readers through protocols for use with a variety of plant expression systems Various aspects of production are covered including vector selection and cloning product improvements for stability glycosylation and antibiotic free selection extraction and scale up and analysis of transgenic plants and their recombinant proteins Written for the Methods in Molecular Biology series 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 Authoritative and practical Recombinant Proteins from Plants Methods and Protocols Second Edition is an ideal reference for those who are interested in plant molecular biology and molecular farming Protocols in Advanced Genomics and Allied Techniques Aruna Pal, 2021-11-14 This laboratory manual includes the latest tools and techniques involved in genomic research It starts with an introductory chapter on genomics and the various tools and applications

involved The initial chapters present protocols for basic techniques such as DNA isolation electrophoresis PCR cDNA synthesis etc The book then goes on to describe more advanced techniques such as next generation sequencing exome sequencing use of RNAi RNAseq genome editing single cell genomics etc Each topic includes a brief description information on the principles involved materials methods protocol and expected results with diagrams and graphs All protocols are presented in a very lucid and precise way to make it easy for readers to follow and replicate them **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 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

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