

Methods in Molecular Biology

Volume 2

Nucleic Acids

Edited by

John M. Walker

Nucleic Acid Vol 2 Methods In Molecular Biology

Ken I. Mills, Bernie H. Ramsahoye



Nucleic Acid Vol 2 Methods In Molecular Biology:

Nucleic Acid Probes Robert H. Symons, 1989-03-31 With each chapter written by a distinguished expert in the biochemistry field this comprehensive volume describes the preparation and use of a variety of radioactive and non radioactive probes in situations ranging from research laboratories to routine diagnosis laboratories The enzymatic and chemical techniques for labeling nucleic acid probes with radioisotopes and with non radioactive ligands and haptens are discussed Additionally the associated methods for their detection the use of these probes in the diagnosis of human and microbial pathogens of plant viruses and viroids and of human genetic disorders as well as in the detection of nucleic acids in tissues and cells by in situ hybridization are presented

Capillary Electrophoresis of Nucleic Acids Keith R. Mitchelson, Jing Cheng, 2008-02-05 The development of PCR which enables extremely small amounts of DNA to be amplified led to the rapid development of a multiplicity of analytical procedures that permit use of this new resource for the analysis of genetic variation and for the detection of disease causing mutations The advent of capillary electrophoresis CE with its power to separate and analyze very small amounts of DNA has also stimulated researchers to develop analytical procedures for the CE format The advantages of CE in terms of speed and reproducibility of analyses are manifold Furthermore the high sensitivity of detection and the ability to increase sample throughput with parallel analysis has led to the creation of a full range of analysis of DNA molecules from modified DNA adducts and single strand oligonucleotides through PCR amplified DNA fragments and whole chromosomes Capillary Electrophoresis of Nucleic Acids focuses on analytical protocols that can be used for detection and analysis of mutations and modification from precise DNA loci through entire genomes of organisms Important practical considerations for CE such as the choice of separation media electrophoresis conditions and the influence of buffer additives and dyes on DNA mobility are discussed in several key chapters and within particular applications

Capillary Electrophoresis Philippe Schmitt-Kopplin, 2008-02-25 This book presents a selection of current capillary electrophoresis methods used to separate representative types of molecules and particles and in combination with different detection techniques It includes practical details which are hard to find elsewhere The volume is intended for beginners in the field and provides an overview of the technique and a starting point for the exploration of the defined literature on different application topics

The ELISA Guidebook John R. Crowther, 2008-02-04 John R Crowther provides today's premier practical guide to the understanding and application of ELISA Updating and greatly expanding his widely appreciated earlier publication ELISA Theory and Practice 1995 this important work introduces chapters on such major new topics as checkerboard titrations quality control of testing kit production and control novel monoclonal antibodies validation of assays statistical requirements for data examination and epidemiological considerations With its numerous worked examples detailed instructions and extensive illustrations The ELISA Guidebook offers a powerful synthesis of all the basic concepts and practical experimental details investigators need to understand develop and apply the new ELISA methodology

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

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

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

DNA Topoisomerase Protocols Neil Osheroff, Mary-Ann Bjornsti, 2008-02-05 Beginning with the Escherichia coli protein or bacterial DNA topoisomerase I an ever increasing number of enzymes have been identified that catalyze changes in the linkage of DNA strands DNA topoisomerases are ubiquitous in nature and have been shown to play critical roles in most processes involving DNA including DNA replication transcription and recombination These enzymes further constitute the cellular targets of a number of clinically important antibacterial and anticancer agents Thus further studies of DNA topology and DNA topoisomerases are critical to advance our understanding of the basic biological processes required for cell cycle progression cell division genomic stability and development In addition these studies will continue to provide critical insights into the cytotoxic action of drugs that target DNA topoisomerases Such mechanistic studies have already played an important role in the development and clinical application of antimicrobial and chemotherapeutic agents The two volumes of DNA Topoisomerase Protocols are designed to help new and established researchers investigate all aspects of DNA topology and the function of these enzymes The chapters are written by prominent investigators in the field and provide detailed background information and step by step experimental protocols The topics covered in Part I DNA Topology and Enzymes range from detailed methods to analyze various aspects of DNA structure from linking number knotting unknotting site specific recombination and decatenation to the overexpression and purification of bacterial and eukaryotic DNA topoisomerases from a

variety of cell systems and tissues Quantitative Trait Loci Nicola J. Camp, Angela Cox, 2008-02-03 In *Quantitative Trait Loci Methods and Protocols* a panel of highly experienced statistical geneticists demonstrate in a step by step fashion how to successfully analyze quantitative trait data using a variety of methods and software for the detection and fine mapping of quantitative trait loci QTL Writing for the nonmathematician these experts guide the investigator from the design stage of a project onwards providing detailed explanations of how best to proceed with each specific analysis to find and use appropriate software and to interpret results Worked examples citations to key papers and variations in method ease the way to understanding and successful studies Among the cutting edge techniques presented are QTDT methods variance components methods and the Markov Chain Monte Carlo method for joint linkage and segregation analysis 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* Oao 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 **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 **Peptide Research Protocols** Janet J. Maguire, Anthony P.

Davenport,2008-02-04 A panel of multidisciplinary experts describes in detail readily reproducible methods to investigate all aspects of the endothelin system from its synthesis and metabolism to its function in health and disease These methods use state of the art molecular techniques to quantify the expression of mRNA for both endothelin receptors and the endothelin converting enzymes They show how peptides precursors receptors and synthetic enzymes can be localized and quantified in plasma culture supernatants tissue homogenate and tissue sections using antibodies Several in vivo protocols illustrate the role of the endothelin peptides in healthy human individuals and describe animal models that can be used to predict the therapeutic potential of cardiovascular drugs that manipulate endothelin synthesis or function **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 **GTPase Protocols** Ed Manser,Thomas

Leung,2008-02-03 In the last 10 years researchers have firmly established key roles for R related GTPases in almost every aspect of cell biology In the 1980s the pro oncogene Ras itself was the focus of interest though in the 1990s this shifted to the increasing variety of Ras related proteins In this new decade much yet needs to be done to establish the role for all the small GTPases now uncovered by the human genome project In particular these GTPases need to be understood in the appropriate biochemical and cellular contexts In the process of trying to uncover the role of these versatile proteins a variety of novel techniques and methodologies has been developed These now enable investigators to move easily within a diversity of fields ranging from structural studies to real time in vivo analysis of a GTPase In recognition of the need for access to key background methodologies GTPase Protocols The Ras Superfamily is devoted to techniques that are presently widely used and that will continue to be the standard for researchers worldwide Each chapter is aimed at supplying detailed methodologies to allow reproduction in any laboratory while also providing the general principles on which the methods are based Some of the techniques grouped in the first section apply broadly to small GTPases whereas others in Part II are more applicable within each GTPase subfamily **PCR Cloning Protocols** Bing-Yuan Chen,Harry W. Janes,2008-02-05 PCR Cloning Protocols Second

Edition updates and expands Bruce White s best selling PCR Cloning Protocols 1997 with the newest procedures for DNA cloning and mutagenesis Here the researcher will find readily reproducible methods for all the major aspects of PCR use including PCR optimization computer programs for PCR primer design and analysis and novel variations for cloning genes of special characteristics or origin with emphasis on long distance PCR and GC rich template amplification Also included are both conventional and novel enzyme free and restriction site free procedures to clone PCR products into a range of vectors as

well as state of the art protocols to facilitate DNA mutagenesis and recombination and to clone the challenging uncharacterized DNA flanking a known DNA fragment Laboratory Methods in Enzymology: RNA Jon Lorsch, 2013-09-11

Methods in Enzymology volumes provide an indispensable tool for the researcher Each volume is carefully written and edited by experts to contain state of the art reviews and step by step protocols In this volume we have brought together a number of core protocols concentrating on RNA complementing the traditional content that is found in past present and future Methods in Enzymology volumes Indispensable tool for the researcher Carefully written and edited by experts to contain step by step protocols In this volume we have brought together a number of core protocols concentrating on RNA **Genomics Protocols** Michael P. Starkey, Ramnath Elaswarapu, 2008-02-03 We must unashamedly admit that a large part of the motivation for editing Genomics Protocols was selfish The possibility of assembling in a single volume a unique and comprehensive collection of complete protocols relevant to our work and the work of our colleagues was too good an opportunity to miss We are pleased to report however that the outcome is something of use not only to those who are experienced practitioners in the genomics field but is also valuable to the larger community of researchers who have recognized the potential of genomics research and may themselves be beginning to explore the technologies involved Some of the techniques described in Genomics Protocols are clearly not restricted to the genomics field indeed a prerequisite for many procedures in this discipline is that they require an extremely high throughput beyond the scope of the average investigator However what we have endeavored here to achieve is both to compile a collection of procedures concerned with geno scale investigations and to incorporate the key components of bottom up and top down approaches to gene finding The technologies described extend from those traditionally recognized as coming under the genomics umbrella touch on proteomics the study of the expressed protein complement of the genome through to early therapeutic approaches utilizing the potential of genome programs via gene therapy Chapters 27 30 Genomic Imprinting Andrew Ward, 2008-02-02

Genomic imprinting is the process by which gene activity is regulated according to parent of origin Usually this means that either the maternally inherited or the paternally inherited allele of a gene is expressed while the opposite allele is repressed The phenomenon is largely restricted to mammals and flowering plants and was first recognized at the level of whole genomes Nuclear transplantation experiments carried out in mice in the late 1970s established the non equivalence of the maternal and paternal genomes in mammals and a similar conclusion was drawn from studies of interploidy crosses of flowering plants that extend back to at least the 1930s Further mouse genetic studies involving animals carrying balanced translocations reviewed in Chapter 3 indicated that imprinted genes were likely to be widely scattered and would form a minority within the mammalian genome The first imprinted genes were identified in the early 1990s over forty are now known in mammals and the list continues steadily to expand *Methods in Plant Biochemistry: Amino acids, proteins and nucleic acids* L. J. Rogers, 1991

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