

PRINS and *In Situ* PCR Protocols

SECOND EDITION

Edited by

Franck Pellestor

 **HUMANA PRESS**

Prins And In Situ Pcr Protocols

Thomas Liehr



Prins And In Situ Pcr Protocols:

PRINS and In Situ PCR Protocols Franck Pellestor, 2008-02-03 The in situ hybridization and PCR technologies are now well established molecular techniques for studying chromosomal aneuploidy and rearrangements gene localization and expression and genomic organization Over the last decade we have seen increasing applications in these fields By combining the high sensitivity of the PCR reaction and the cytological localization of target sequences both PRINS and in situ PCR techniques have provided highly powerful complements to FISH for in situ cellular and molecular investigations Both these approaches have several advantages in terms of sensitivity and specificity owing to the use of primers and to the fast kinetics of annealing and elongation reactions in situ In the first edition of PRINS and In Situ PCR Protocols edited by John R Gosden experts in the field presented in detail a variety of applications of PRINS and in situ PCR techniques in a wide range of clinical conditions Since the publication of this successful reference book there have been significant improvements in in situ detection techniques This completely revised and updated second edition presents a comprehensive selection of new procedures developed in the field of PRINS and in situ PCR technologies The book has two sections Part I Basic Methodology contains chapters that provide useful protocols for many variations of PRINS and in situ PCR including a new fast multicolor PRINS method and protocols for PRINS detection of unique sequences in situ *PCR Protocols* John M. S. Bartlett, David Stirling, 2008-02-03 In this new edition the editors have thoroughly updated and dramatically expanded the number of protocols to take advantage of the newest technologies used in all branches of research and clinical medicine today These proven methods include real time PCR SNP analysis nested PCR direct PCR and long range PCR Among the highlights are chapters on genome profiling by SAGE differential display and chip technologies the amplification of whole genome DNA by random degenerate oligonucleotide PCR and the refinement of PCR methods for the analysis of fragmented DNA from fixed tissues Each fully tested protocol is described in step by step detail by an established expert in the field and includes a background introduction outlining the principle behind the technique equipment and reagent lists tips on trouble shooting and avoiding known pitfalls and where needed a discussion of the interpretation and use of results **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 technologies has increased the need to temporally and spatially localize the distribution of mRNA expression Our intention in In Situ Hybridization Protocols is to provide ample information 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 reputation as one of the more difficult and capricious

molecular biological techniques 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 biological science The step by step and detailed protocols provided in *In Situ Hybridization Protocols* by researchers active in the field should make it possible for both the molecular biologist with little experience of histology and the histologist with little experience of molecular biology to use the techniques successfully in their laboratories

PRINS and In Situ PCR Protocols. Methods in Molecular Biology Franck Pellestor, 2006 This completely revised and updated edition takes advantage of the many new developments and applications that have occurred in PRINS and in situ PCR technology including a new fast multicolor PRINS method for identifying human chromosomes PRINS protocols for in situ detection of unique sequences and point mutation analysis a new dideoxy PRINS procedure and in situ PCR and PRINS protocols for plants The authors describe in detail various applications of PRINS in human detection of gene deletions in cancer detection of fetal cells in maternal blood and assessment of aneuploidy in brain tissues and embryos as well as plant cells Readily reproducible in situ PCR techniques are also presented for the detection of cytomegalovirus and for use in combination with microdissection In situ RT PCR techniques are also given for use in plant or cancer investigation

PRINS and PNA Technologies in Chromosomal Investigations Franck Pellestor, 2007 Book CD Advances in molecular biotechnology have greatly improved the sensitivity and the efficiency of methods utilised for genetic investigations and diagnosis In the domain of chromosome analysis the introduction of molecular techniques has led to the development of a new approach called Molecular Cytogenetics which has surpassed previously available techniques to become a foremost biological method The fluorescence in situ hybridisation FISH is quickly became the standard technique for in situ chromosomal investigations as illustrated by its large variety of applications in research and diagnosis However during the last decade alternative methods to FISH have been introduced and have shown to be valuable in detecting chromosomes and quantifying chromosomal abnormalities These alternative procedures are the Primed IN Situ PRINS labelling and the Peptide Nucleic Acid PNA probes The two procedures present several advantages for the in situ detection of nucleic acid sequences such as the small size of PNA probes and PRINS primers or the fast kinetics of PRINS and PNA labelling reactions that make them very attractive for a number of cytogenetic purposes This book provides a valuable introduction and overview of the principles and the applications of alternative approaches in the field of molecular cytogenetics

Methods in Molecular Biology: PRINS and in situ PCR protocols John M. Walker, 1984

In Situ Molecular Pathology and Co-expression Analyses Gerard J. Nuovo, 2020-09-03 Major advancements in the field of in situ molecular pathology have occurred since publication of the first edition *In Situ Molecular Pathology and Co expression Analyses* Second Edition continues to teach both the molecular basis for the improvements and the actual protocols This is the unique feature that separates it from the pack of other cook book type approaches The fields of in situ hybridization and immunohistochemistry have expanded rapidly where

computer based analyses systems have greatly expanded the power of these methods Further knowledge of the marked improvements in the reagents themselves since the first edition can make the difference of excellent versus misleading data The automated platforms require that researchers and diagnostic biomedical investigators have a good understanding of the basics of in situ based tests protocols and biochemistry for troubleshooting in order to maximize the use of these platforms This second edition focuses attention on straightforward protocols used to simultaneously detect two or more proteins nucleic acids within intact tissue by doing co expression analyses Practicing molecular pathologists diagnostic pathologists laboratory directors and toxicologists as well as clinicians and researchers in training will benefit from this clear presentation of protocols and theoretical framework Data derived from in situ hybridization and immunohistochemistry Explains the theory and foundation of immunohistochemistry and in situ hybridization and presents easy to follow experimental protocols with tricks of the trade Includes two new chapters Recent improvements in immunohistochemistry and in situ hybridization Quality control for immunohistochemistry and in situ hybridization How to know if the color change is signal or background The second edition also includes a detailed test to help one learn the basics of histologic interpretation of tissues and a separate detailed test in how to differentiate signal from background Includes chapter ending summaries of Key Points to Remember bringing beginners up to speed with any seasoned veteran in the field Thoughtfully tackles the molecular basis of IHC and ISH along with application of that knowledge to improving the techniques is significant Endocrine Pathology: Ricardo V. Lloyd, 2010-01-24 Endocrine Pathology Differential Diagnosis and Molecular Advances Second Edition provides detailed coverage of endocrine pathology with extensive discussion of the differential diagnosis as well as presentation of molecular pathobiology of the major endocrine organs Revised and expanded from the first edition each chapter written by leaders in their respective field has been updated with the latest advances that are transforming the field of endocrine pathology Richly illustrated with color photomicrographs useful diagrams and line drawings each chapter includes differential diagnosis of common and uncommon lesions as well as material on molecular developments with emphasis on the molecular findings that are most helpful in the diagnosis of specific disorders Endocrine Pathology Differential Diagnosis and Molecular Advances Second Edition provides a useful and well organized resource designed not only for the endocrine pathologist and the general surgical pathologist but also for the clinical endocrinologist and the endocrine surgeon

Principles and Technical Aspects of PCR Amplification Elizabeth van Pelt-Verkuil, Alex van Belkum, John P. Hays, 2008-03-14 Kary Mullis was awarded a Nobel Prize for inventing the PCR technique more than a decade ago in 1993 Since its discovery multiple adaptations and variations of the standard PCR technique have been described This publication aims to provide the reader with a guide to the standard PCR technique and its many available variants with particular emphasis being placed on the role of these PCR techniques in the clinical diagnostic laboratory the central theme of this book

Fluorescence In Situ Hybridization (FISH) - Application Guide Thomas Liehr, 2008-11-26 This book is a unique

source of information on the present state of the exciting field of molecular cytogenetics and how it can be applied in research and diagnostics The basic techniques of fluorescence in situ hybridization and primed in situ hybridization PRINS are outlined the multiple approaches and probe sets that are now available for these techniques are described and applications of them are presented in 36 chapters by authors from ten different countries around the world The book not only provides the reader with basic and background knowledge on the topic but also gives detailed protocols that show how molecular cytogenetics is currently performed by specialists in this field The FISH Application Guide initially provides an overview of the historical development of molecular cytogenetics its basic procedures the equipment required and probe generation The book then describes tips and tricks for making different tissues available for molecular cytogenetic studies These are followed by chapters on various multicolor FISH probe sets their availability and their potential for use in combination with other approaches The possible applications that are shown encompass the characterization of marker chromosomes cryptic cytogenetic aberrations and epigenetic changes in humans by interphase and metaphase cytogenetics studies of nuclear architecture as well as the application of molecular cytogenetics to zoology botany and microbiology

Chromosome Painting Arun Kumar Sharma, Archana Sharma, 2011-06-27 Chromosome Painting is the most modern and novel technique for directly identifying several gene sequences simultaneously in the chromosome with the aid of specific probes in molecular hybridization Its resolution ranges from single copy to entire genome sequences It is now applied in plant animal and human systems in gene mapping identification of genetic disorders evolutionary studies and gene transfer experiments This treatise is the first of its kind to cover the technique with all its modifications and applications It is designed for regular use by postgraduate students and research workers in cell and molecular genetics plant and animal sciences agriculture medicine and phylogenetic studies

Plant Cytogenetics Hank Bass, James A. Birchler, 2011-12-02 This reference book provides information on plant cytogenetics for students instructors and researchers Topics covered by international experts include classical cytogenetics of plant genomes plant chromosome structure functional molecular cytology and genome dynamics In addition chapters are included on several methods in plant cytogenetics informatics and even laboratory exercises for aspiring or practiced instructors The book provides a unique combination of historical and modern subject matter revealing the central role of plant cytogenetics in plant genetics and genomics as currently practiced This breadth of coverage together with the inclusion of methods and instruction is intended to convey a deep and useful appreciation for plant cytogenetics We hope it will inform and inspire students researchers and teachers to continue to employ plant cytogenetics to address fundamental questions about the cytology of plant chromosomes and genomes for years to come Hank W Bass is a Professor in the Department of Biological Science at Florida State University James A Birchler is a Professor in the Division of Biological Sciences at the University of Missouri

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

Cell Imaging Techniques

Douglas J. Taatjes, Brooke T. Mossman, 2008-02-04 A diverse collection of state of the art methods for the microscopic imaging of cells and molecules The authors cover a wide spectrum of complimentary techniques including such methods as fluorescence microscopy electron microscopy atomic force microscopy and laser scanning cytometry Additional readily reproducible protocols on confocal scanning laser microscopy quantitative computer assisted image analysis laser capture microdissection microarray image scanning near field scanning optical microscopy and reflection contrast microscopy round out this eclectic collection of cutting edge imaging techniques now available The authors also discuss preparative methods for particles and cells by transmission electron microscopy

Protein Design

Raphael Guerois, Manuela López de la Paz, 2008-02-04 Proteins have evolved through selective pressure to accomplish specific functions The functional properties of proteins depend upon their three dimensional structures which result from particular amino acid sequences folding into tightly packed domains Thus to understand and modulate protein function rationally one definitely needs methods and algorithms to predict and decipher how amino acid sequences shape three dimensional structures Protein design aims precisely at providing the tools to achieve this goal The predictive power of rational protein design methods has dramatically increased over the past five years A broad range of studies now illustrate how the sequence of proteins and peptides can be tuned to engineer biological tools with intended properties 1 3 The extensive characterization of peptides and protein mutants has enormously benefited the understanding of protein sequence to structure relationships Synergies between computational and experimental approaches have also added momentum to the advancing limits of design methods The potential applications in fundamental biochemistry and in biotechnology justify the considerable excitement that this progress has generated within the research community The field is probably mature enough so that expert knowledge can assist researchers of diverse disciplines to rationally create or modify their favorite protein Thus the aim of Protein Design Methods and Protocols is to account for the most up to date protein design and engineering strategies so that readers can undertake their own projects with maximum confidence in a successful return The basic concepts underlying rational design of proteins are intimately related to their three dimensional structures

New and Emerging Proteomic Techniques

Dobrin Nedelkov, Randall W. Nelson, 2008-02-04 Leading researchers and innovators describe in step by step detail the latest

techniques that promise to significantly impact the practice of proteomics as well as its success in developing novel clinical agents. The methods span the entire spectrum of top down and bottom up approaches including microarrays, gels, chromatography and affinity separations and address every aspect of the human proteome both quantitatively and qualitatively. The techniques of protein detection utilized are diverse and range from fluorescence and resonance light scattering to surface plasmon resonance and mass spectrometry. The protocols follow the successful *Methods in Molecular Biology*TM series format, each offering step by step laboratory instructions, an introduction outlining the principles behind the technique, lists of the necessary equipment and reagents, and tips on troubleshooting and avoiding known pitfalls. **Ovarian**

Cancer John M. S. Bartlett, 2008-02-02 If there is one aspect of current cancer research that represents a major challenge in both novice and experienced researchers, it is the rapid advance in our understanding of the disease. Researchers can be required to switch from analysis of gene expression to kinetics of protein activation, from genetic studies to the analysis of protein function. Cancers are highly complex disease systems and researchers aiming to understand the functioning of cancer systems require access to a wide range of laboratory techniques from a broad range of research disciplines. Increasingly, however, published methods are incomplete or refer back to a series of previous publications, each containing only a small part of the complete protocol. The aim of *Ovarian Cancer Methods and Protocols* is to provide for ovarian cancer researchers in the first instance a laboratory handbook that will facilitate research into cancer systems by providing a series of expert protocols with proven efficacy across a broad range of technical expertise. Thus, there are sections on tumor genetics and cellular signal transduction as well as sections on apoptosis and RNA analysis. The value of *Ovarian Cancer Methods and Protocols* to the ovarian cancer researcher will, I trust, be considerably enhanced by 1) the provision of a series of overviews relating to the biology, diagnosis and treatment of this important neoplasm, and 2) the provision of a series of technical overviews introducing each part that provides an expert review of the applications and pitfalls of the various techniques included. *PCR Applications* Michael A. Innis, David H. Gelfand, John J. Sninsky, 1999-05-11 PCR is the most powerful

technique currently used in molecular biology. It enables the scientist to quickly replicate DNA and RNA on the benchtop. From its discovery in the early 80s, PCR has blossomed into a method that enables everything from ready mutation of DNA/RNA to speedy analysis of tens of thousands of nucleotide sequences. *PCR Applications* examines the latest developments in this field. It is the third book in the series, building on the previous publications *PCR Protocols* and *PCR Strategies*. The manual discusses techniques that focus on gene discovery, genomics and DNA array technology, which are contributing factors to the now occurring bioinformatics boom. **Key Features:** Focuses on gene discovery, genomics and DNA array technology. Covers quantitative PCR techniques, including the use of standards and kinetic analysis. Includes statistical refinement of primer design parameters. Illustrates techniques used in microscopic tissue samples, such as single cell PCR, whole cell PCR, laser capture microdissection, and in situ PCR. Entries provide information on Nomenclature, Expression

Sequence analysis Structure and function Electrophysiology Pharmacology Information retrieval *Practical Manual on Plant Cytogenetics* Ram J. Singh, 2017-11-27 Earlier books on the handling of plant chromosomes have not included many of the innovations in cytological techniques for many important crops that have become available in recent years including information on associating genes with chromosomes The aim of this book is to compile all the plant cytogenetic techniques previously published in earlier books into a laboratory manual The first part of the book describes standard cytological techniques that are routinely used by students The second part covers methods used for specific crops for which common cytological methods do not work satisfactorily The third part discusses cytogenetic techniques cytology and genetics for physically locating genes on specific chromosomes This novel book will be highly useful to students teachers and researchers as it is a convenient and comprehensive reference for all plant cytogenetic techniques and protocols The Nucleic Acid Protocols Handbook Ralph Rapley, 2000-03-24 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*TM 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

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