

# **PCR Mutation Detection Protocols**

Second Edition

**Edited by**  
**Bimal D. M. Theophilus**  
**Ralph Rapley**

# Pcr Mutation Detection Protocols

**Subhash C. Basu, Manju Basu**



## **Pcr Mutation Detection Protocols:**

**PCR Mutation Detection Protocols** Bimal D. M. Theophilus, Ralph Rapley, 2008-02-02 1 Bimal D Theophilus and Ralph Rapley provide biological and clinical investigators with a comprehensive collection of new recent and updated PCR based screening methods suitable for detecting the presence of both known and novel mutations The methods cover point mutations e g ASO PCR SSCP DGGE chemical cleavage deletions multiplex PCR FISH blotting non sense mutations PTT and more The new and exciting techniques of DNA array analysis along with such recently developed experimental methods as conformation sensitive gel electrophoresis are also included Each chapter explains the basic theory behind the technique and provides valuable notes essential for its successful execution *PCR Mutation Detection Protocols* Bimal D. M. Theophilus, Ralph Rapley, 2002

*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 *Superantigen Protocols* Teresa Krakauer, 2008-02-05

Leading researchers in the biological chemical and physical investigation of superantigens describe in step by step detail their best experimental techniques to assess the physical characteristics and biological effects of superantigens Their protocols range from those for investigating the interactions of superantigens with cellular receptors to those for the analysis of their immunological and biological effects including methods for using BIOcore to determine binding kinetics and establishing various lymphocyte cell culture systems There are also accounts of such methods as the RNase protection assay cytokine ELISA FACS analysis and cytokine production at the single cell level *Molecular Toxicology Protocols* Phouthone Keohavong, Stephen G. Grant, 2008-02-03

A collection of cutting edge techniques for analyzing genotoxic exposure and detecting the resulting biological effects including endogenous metabolites up to and including the development of cancer The authors emphasize analytical methods that can be specifically applied to human populations and patients Among the applications detailed are the analysis of interactions between such cellular macromolecules as DNA and proteins and chemical and physical agents the assessment of medically relevant toxicity and the characterization of genetic alterations induced in transgenic animals by in vivo systems There are also methods for the analysis of genotoxic exposure during gene expression of cytotoxicity caused by the induction of apoptosis of genetic alterations in reporter genes and oncogenes early

pre-malignant detection of altered oncogenes and of individual variation in biotransformation and DNA repair capacity      **In Vitro Mutagenesis Protocols** Jeff Braman, 2008-02-05 Hands on researchers with proven track records describe in stepwise fashion their advanced mutagenesis techniques The contributors focus on improvements to conventional site directed mutagenesis including a chapter on chemical site directed mutagenesis PCR based mutagenesis and the modifications that allow high throughput mutagenesis experiments and mutagenesis based on gene disruption both in vitro and in situ based Additional methods are provided for in vitro gene evolution for gene disruption based on recombination transposon and cassette mutagenesis and for facilitating the introduction of multiple mutations Time tested and highly practical the protocols in *In Vitro Mutagenesis Protocols* 2nd Edition offer today's molecular biologists reliable and powerful techniques with which to illuminate the proteome      **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      *Epithelial Cell Culture Protocols* Clare Wise, 2008-02-05 There have been significant advances in research involving the isolation and culture of epithelial cells in the past decade and many new techniques have

been developed Monolayer cultures can be used to evaluate the nature and behavior of cells while the use of epithelial cells in model systems has allowed a deeper understanding of cellular and molecular mechanisms and interactions The aim of this book is to provide a comprehensive step by step guide to many techniques for epithelial cell culture combining in one volume the more commonly used protocols along with many that are more specialized Epithelial Cell Culture Protocols should help those who are new to this field and want to learn the basic culture techniques as well as those needing to use more wide ranging and specific protocols It should be a useful resource on its own and also complement the other volumes that have been written about cell culture in the Methods in Molecular Biology series Epithelial Cell Culture Protocols covers a wide variety of protocols mostly aimed at the researcher but also a few aimed at clinicians The establishment and maintenance of primary cultures derived from many different tissues and different species is covered Particular emphasis has been placed on protocols needed to further analyze and assess epithelial cells for example by looking at apoptosis and integrins and by measuring membrane capacitance and confluence Using different coculture techniques it is possible also to develop models to investigate many different systems in vitro

**Transgenic Mouse Methods and Protocols** Marten H. Hofker, Jan van Deursen, 2008-02-04 Marten Hofker and Jan van Deursen have assembled a multidisciplinary collection of readily reproducible methods for working with mice and particularly for generating mouse models that will enable us to better understand gene function Described in step by step detail by highly experienced investigators these proven techniques include new methods for conditional induced knockout and transgenic mice as well as for working with mice in such important research areas as immunology cancer and atherosclerosis Such alternative strategies as random mutagenesis and viral gene transduction for studying gene function in the mouse are also presented

**MHC Protocols** Stephen H. Powis, Robert W. Vaughan, 2008-02-05 The aim of MHC Protocols is to document protocols that can be used for the analysis of genetic variation within the human major histocompatibility complex MHC HLA region The human MHC encompasses approximately 4 million base pairs on the short arm of chromosome 6 at cytogenetic location 6p21.3 The region is divided into three subregions The telomeric class I region contains the genes that encode the HLA class I molecules HLA A B and C The centromeric class II region contains the genes encoding the HLA class II molecules HLA DR DQ and DP In between is the class III region originally identified because it contains genes encoding components of the complement pathway The entire human MHC has recently been sequenced and each subregion is now known to contain many other genes a number of which have immunological functions The study of polymorphism within the MHC is well established because the region contains the highly polymorphic HLA genes HLA polymorphism has been used extensively in solid organ and bone marrow transplantation to match donors and recipients As a result large numbers of HLA alleles have been identified a process that has been further driven by recent interest in HLA gene diversity in ethnic populations The extreme genetic variation in HLA genes is believed to have been driven by the evolutionary response to infectious agents but relatively few studies have

analyzed associations between HLA genetic variation and infectious disease which has been difficult to demonstrate *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 **Hemostasis and Thrombosis**

**Protocols** David J. Perry, K. John Pasi, 2008-02-02 Laboratory studies in hemostasis have traditionally focused on abnormalities of platelet function or the quantitative and qualitative disorders that affect the proteins involved in blood coagulation However over the last 10 years there has been an explosion in our understanding of the molecular bases that underlie many of the inherited and acquired disorders of hemostasis Many of these disorders are now routinely diagnosed and assessed by methods that involve genotypic analysis Indeed in the late 1990s the distinction between molecular methods for research and for routine diagnosis is becoming increasingly blurred The techniques and approaches that are used in hemostasis are manifold and published in isolation in a variety of publications The aim therefore of this volume *Hemostasis and Thrombosis Protocols* is to pull together into a single volume the variety of techniques that are frequently used in the field of hemostasis We have targeted this volume at laboratories who wish to move into the field of molecular hemostasis or who may already have some experience in this area but wish to develop new areas of research and diagnosis The chapters are wide ranging and hopefully provide a broad overview of the differing applications in which these standard techniques can be used Though the articles may appear relatively specific the techniques contained within them are applicable to the study of many different disorders and we hope that they provide a series of ideas and concepts well suited to problem solving **Transgenesis**

**Techniques** Alan R. Clarke, 2008-02-05 The past decade has witnessed a spectacular explosion in both the development and use of transgenic technologies Not only have these been used to aid our fundamental understanding of biologic mechanisms but they have also facilitated the development of a range of disease models that are now truly beginning to impact upon our approach to human disease Some of the most exciting model systems relate to neurodegenerative disease and cancer where the availability of appropriate models is at last allowing radically new therapies to be developed and tested This latter point is of particular significance given the current concerns of the wider public over both the use of animal models and the merits of using genetically modified organisms Arguably advances of the greatest significance have been made using mammalian systems driven by the advent of embryonic stem cell based strategies and more recently by cloning through nuclear transfer For this reason this new edition of *Transgenesis Techniques* focuses much more heavily on manipulation of the mammalian genome both in the general discussions and in the provision of specific protocols Protein Sequencing Protocols Bryan

John Smith, 2008-02-02 Determination of the protein sequence is as important today as it was a half century ago even though the techniques and purposes have changed over time Mass spectrometry has continued its recent rapid development to find notable application in the characterization of small amounts of protein for example in the field of proteomics The traditional chemical N terminal sequencing is still of great value in quality assurance of the increasing number of biopharmaceuticals that are to be found in the clinic checking processing events of recombinant proteins and so on It is joined in the armory of methods of protein analysis by such techniques as C terminal sequencing and amino acid analysis These methods are continually developing The first edition of Protein Sequencing Protocols was a snapshot of methods in use in protein biochemistry laboratories at the time and this the second edition is likewise Methods have evolved in the intervening period and the content of this book has similarly changed the content of some chapters having been superseded and replaced by other approaches Thus in this edition there is inclusion of approaches to validation of methods for quality assurance work reflecting the current importance of biopharmaceuticals and also a guide to further analysis of protein sequence information acknowledging the importance of bioinformatics

**Molecular Cytogenetics** Yao-Shan Fan, 2008-02-05 The new techniques of molecular cytogenetics mainly fluorescence in situ hybridization FISH of DNA probes to metaphase chromosomes or interphase nuclei have been developed in the past two decades Many FISH techniques have been implemented for diagnostic services whereas some others are mainly used for investigational purposes Several hundreds of FISH probes and hybridization kits are now commercially available and the list is growing rapidly FISH has been widely used as a powerful diagnostic tool in many areas of medicine including pediatrics medical genetics maternal fetal medicine reproductive medicine pathology hematology and oncology Frequently a physician may be puzzled by the variety of FISH techniques and wonder what test to order It is not uncommon that a sample is referred to a laboratory for FISH without indicating a specific test On the other hand a cytogeneticist or a technologist in a laboratory needs from case to case to determine which procedure to perform and which probe to use for an informative result To obtain the best results one must use the right DNA probes and have reliable protocols and measures of quality assurance in place Also one must have sufficient knowledge in both traditional and molecular cytogenetics as well as the particular areas of medicine for which the test is used in order to appropriately interpret the FISH results and to correlate them with clinical diagnosis treatment and prognosis

**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 **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 **Inflammation**

**Protocols** Paul G. Winyard, Derek A. Willoughby, 2008-02-03 Inflammation has been described as the basis of many pathologies of human disease When one considers the updated signs of inflammation they would be vasodilation cell migration and in the case of chronic inflammation cell proliferation often with an underlying autoimmune basis Generally inflammation may be divided into acute chronic and autoimmune though the editors believe that most if not all chronic states are often the result of an autoimmune response to an endogenous antigen Thus a proper understanding of the inflammatory basis may provide clues to new therapeutic targets not only in classical inflammatory diseases but atherosclerosis cancer and ischemic heart disease as well The lack of advances in classical inflammatory diseases such as rheumatoid arthritis may in part arise from a failure to classify the disease into different forms That different forms exist is exemplified in patients with differing responses to existing antiinflammatory drugs ranging from nonresponders to very positive responders for a particular nonsteroidal antiinflammatory drug NSAID Though researchers have progressively unraveled the mechanisms the story is far from complete It should also be noted that the inflammatory response is part of the innate immune response or to use John Hunter's words in 1795 inflammation is a salutary response That may be applied in particular to the defensive response to invading microorganisms **Capillary Electrophoresis of Carbohydrates** Pierre Thibault, Susumu Honda, 2008-02-03 A

collection of cutting edge techniques for using capillary electrophoresis CE to analyze complex carbohydrates These readily reproducible protocols provide methods for sample preparation analysis of mono and oligosaccharides glycoproteins and glycoconjugates A useful appendix describes the structures of the most commonly encountered carbohydrate residues and oligosaccharides from mammalian and bacterial origins Each protocol contains detailed information on reagents apparatus notes comments and tips on procedures



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