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Nora Engel *Editor*

Genomic Imprinting

Methods and Protocols

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Genomic Imprinting Methods And Protocols Methods In Molecular Biology

Alan R. Clarke



Genomic Imprinting Methods And Protocols Methods In Molecular Biology:

Genomic Imprinting Nora Engel, 2012-08-21 Genomic imprinting the process by which the non equivalence of the paternal and maternal genomes is established has been fascinating us for over three decades and has provided many emerging scientists with the chance to hit their stride in a frontier posing many unexpected questions and even more surprising answers In *Genomic Imprinting Methods and Protocols* experts in the field provide a survey of the technologies that are being applied to advance the study of imprinting This detailed volume features new technologies that are accelerating the pace of discovery of imprinted genes and characterization of their epigenetic profile bioinformatic procedures for prediction and comparative analyses of imprinted genes as well as methods in embryology and basic molecular biology that have been employed for many years some appearing in new versions for small cell numbers Written in the highly successful *Methods in Molecular Biology*TM 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 easy to use *Genomic Imprinting Methods and Protocols* will aid scientists in unveiling both much awaited answers and all new questions to keep this vital field busy for many exciting years to come

Methods in Molecular Biology: Genomic imprinting: methods and protocols John M. Walker, 1984 *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

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.

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.

GTPase Protocols Ed Manser, Thomas Leung, 2008-02-03 In the last 10 years, researchers have firmly established key roles for Ras related GTPases in almost every aspect of cell biology. In the 1980s, the proto-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.

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 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 **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 **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

Genomic Imprinting Andrew Ward, 2001-10-10 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.

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.

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.

RT-PCR Protocols Nicola King, Joe O'Connell, 2008-02-04. Until the mid 1980s, the detection and quantification of a specific mRNA was a difficult task, usually only undertaken by a skilled molecular biologist. With the advent of PCR, it became possible to amplify specific mRNA after first converting the mRNA to cDNA via reverse transcriptase. The arrival of this technique, termed reverse transcription PCR (RT-PCR), meant that mRNA suddenly became amenable to rapid and sensitive analysis without the need for advanced training in molecular biology. This new accessibility of mRNA, which has been facilitated by the rapid accumulation of sequence data for human mRNAs, means that every biomedical researcher can now include measurement of specific mRNA expression as a routine component of his/her research plans. In view of the ubiquity of the use of standard RT-PCR, the main objective of RT-PCR Protocols is essentially to provide novel, useful applications of RT-PCR. These include some useful adaptations and applications that could be relevant to the wider research community who are already familiar with the basic RT-PCR protocol. For example, a variety of different adaptations are described that have been employed to obtain quantitative data from RT-PCR. Quantitative RT-PCR provides the ability to accurately measure changes in specific mRNA expression between normal and diseased tissues.

DNA Methylation Protocols Ken I. Mills, Bernie H. Ramsahoye, 2008-02-05. DNA Methylation Protocols offer a set of readily reproducible protocols for 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.

Proteoglycan Protocols Renato V. Iozzo, 2008-02-02. Proteoglycans are some of the most elaborate macromolecules of mammalian and lower organisms. The covalent attachment of at least five types of glycosaminoglycan side chains to more than forty individual protein cores makes these molecules quite complex and endows them with a multitude of biological functions. Proteoglycan Protocols offers a comprehensive and up-to-date collection of preparative and analytical methods for the in-depth analysis of proteoglycans. Featuring step-by-step detailed protocols, this book will enable both novice and experienced researchers to isolate intact proteoglycans from tissues and cultured cells, to establish the composition of their carbohydrate moieties, to generate strategies for prokaryotic and eukaryotic expression, to utilize methods for the suppression of specific proteoglycan gene expression, and for the detection of

mutant cells and degradation products and to study specific interactions between proteoglycans and extracellular matrix proteins as well as growth factors and their receptors The readers will find concise yet comprehensive techniques carefully drafted by leading experts in the field Each chapter commences with a general Introduction followed by a detailed Materials section and an easy to follow Methods section An asset of each chapter is the extensive notation that includes troubleshooting tips and practical considerations that are often lacking in formal methodology papers The reader will find this section most valuable because it is clearly provided by experienced scientists who have first hand knowledge of the techniques they outline In addition most of the chapters are well illustrated with examples of typical data generated with each method

Steroid Receptor Methods Benjamin A. Lieberman, 2001-08-10 A distinguished team of principal investigators and their associates describe in step by step detail a cross section of the latest research techniques available for studying the endocrine system As a basis for sophisticated biochemical analysis of receptor properties the contributors provide methods for the production and purification of a variety of receptors including progesterone glucocorticoid and androgen Other protocols allow the reader to experiment with DNA binding characteristics hormone binding assays and the use of combinatorial chemistry for drug discovery A series of novel methods utilizing the latest advances in immunochemistry yeast two hybrid screening and fluorescence are included for the detection and analysis of a variety of cellular proteins that influence steroid receptor effectiveness

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

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

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

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