

METHODS IN MOLECULAR BIOLOGY™

Volume 262

# Genetic Recombination

*Reviews and Protocols*

*Edited by*

**Alan S. Waldman**



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# Genetic Recombination Reviews And Protocols Methods In Molecular Biology

**Phouthone Keohavong, Stephen G.  
Grant**



## **Genetic Recombination Reviews And Protocols Methods In Molecular Biology:**

**Genetic Recombination** Alan S. Waldman, 2008-02-03 Genetic recombination in the broadest sense can be defined as any process in which DNA sequences interact and undergo a transfer of information producing new recombinant sequences that contain information from each of the original molecules All organisms have the ability to carry out recombination and this striking universality speaks to the essential role recombination plays in a variety of biological processes fundamentally important to the maintenance of life Such processes include DNA repair regulation of gene expression disease etiology meiotic chromosome segregation and evolution One important aspect of recombination is that it typically occurs only between sequences that display a high degree of sequence identity The stringent requirement for homology helps to ensure that under normal circumstances a cell is protected from deleterious rearrangements since a swap of genetic information between two nearly identical sequences is not expected to dramatically alter a genome Recombination between dissimilar sequences which does happen on occasion may have such harmful consequences as chromosomal translocations deletions or inversions For many organisms it is also important that recombination rates are not too high lest the genome become destabilized Curiously certain organisms such as the trypanosome parasite actually use a high rate of recombination at a particular locus in order to switch antigen expression continually and evade the host immune system effectively

**Cytokine Protocols** Marc De Ley, 2008-02-03 A collection of biochemical cellular and molecular techniques for unraveling and quantifying the events occurring between the initial contact of a cytokine at the membrane receptor and the eventual activation of gene transcription The techniques used include the generation of transfectants the immunohistochemical detection of cytokines in tissue sections and optimized staining for cytoplasmic detection Highlights include RT PCR of small amounts of mRNA in situ hybridization biosensor analysis measurement of biological activities and standardization immunohistochemical and single cell detection and receptor isolation characterization and crystallization Enjoy a quick and smooth introduction to the key methods used in cytokine research Use readily reproducible techniques that ensure successful experimental results Employ antisense RNA RT PCR of small amounts of mRNA and in situ hybridization

**Germ Cell Protocols** Heide Schatten, 2008-02-03 All sexually reproducing organisms produce primordial germ cells a small population of cells that differentiate into gametes of either sex and carry to potency an ability to develop into an entire new organism The study of germ cells has undergone enormous advances in recent years and has entered into an explosive phase of new discoveries with the introduction of transgenic technologies and nuclear cloning Basic knowledge and techniques developed for lower vertebrate and invertebrate systems have facilitated the study of higher vertebrates including humans Many experiments that have first been performed on lower vertebrates provided the tools and strategies that could later be applied to other less readily available mammalian systems The discovery of centrosomes in ascidians and sea urchin eggs now benefits studies of fertility and infertility in mammals External in vitro fertilization now a common

technique in assisted fertilization has only been possible as a result of numerous studies in lower systems in which external fertilization is natural Egg activation first explored in sea urchin and ascidian eggs now benefits techniques designed to increase cloning efficiency in farm and domestic animals Gene manipulations and molecular methods have added to the possibilities of producing live offspring with enormous biomedical ecological and economic implications The two volumes on germ cells combine techniques in a variety of different systems we have selected those systems that have provided landmarks in advancing our knowledge on germ cells

**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 premalignant detection of altered oncogenes and of individual variation in biotransformation and DNA repair capacity

Apoptosis Methods and Protocols Hugh J. M. Brady, 2008-02-05 The most fundamental question facing each and every cell within an organism is to survive or to die Cell death is required for normal function some estimates suggest that as many as one million cells undergo cell death every second in the adult human body Almost all cells undergoing physiological or programmed cell death independent of cell type manifest a stereotypic pattern of morphological changes termed apoptosis Typically apoptotic cells display shrinkage membrane blebbing chromatin condensation and nuclear fragmentation The integrity of the cell membrane is not lost during apoptosis and so avoids eliciting the inflammatory response that would have been caused by the spillage of the cell's contents This is quite in contrast to the loss of cell contents typical of necrosis The caspases the family of intracellular cysteine proteases associated with apoptosis are responsible for the stereotypical morphological changes Caspases cleave various substrate proteins that act on DNA fragmentation nuclear envelope integrity the cytoskeleton and cell volume regulation Apoptotic cells are cleared in vivo by the process of phagocytosis in which specific phagocytes move to the site of apoptosis engulf the dying cells and digest them Apoptosis has a central role in many physiological processes for example in the immune system Autoreactive cells are deleted via apoptosis to prevent autoimmunity At the end of an immune response activated lymphocytes are removed to maintain homeostasis within the immune system

Neuroprotection Methods and Protocols Tiziana Borsello, 2007-08-08 This book examines current research into the role of neuronal death in cell signaling pathways and its role in neurodegenerative diseases such as Alzheimer's and Parkinson's After introducing neurodegenerative traumatic and ischemic disorders the authors cover in vitro and animal systems and cellular and molecular

mechanisms      **MAP Kinase Signaling Protocols** Rony Seger,2008-02-03 Mitogen activated protein kinase MAPK signaling cascades are a group of protein kinases that play a central role in the intracellular transmission of extracellular signals These cascades operate as major lines of communication within a complicated signaling network that regulates many cellular processes including proliferation differentiation development stress response and apoptosis More than 15 000 papers on MAPKs have been published over the past few years with the number of publications increasing each year More and more laboratories embark on the study of MAPK cascades in many distinct cellular systems and in particular their role in disease Future challenges in the study of MAPK cascades remain in understanding the role of the various components and isoforms of the cascades in the multiple critical functions that they regulate in the whole organism as well as the diseases caused by their malfunction Data from gene disrupted mice suggest that inhibition of the MAPK cascades may have serious consequences on the development and growth of the animals For example targeted deletion of MEK1 is lethal owing to developmental problems of placental vasculature and abnormal fibroblast migration This lethality occurs in spite of the normal expression of MEK2 indicating that although the two MEK isoforms are apparently similar they do have distinct functions at least during embryogenesis The ERK cascade was also shown to play a central role in brain function and in learning and memory      **B Cell Protocols** Hua Gu,Klaus Rajewsky,2008-02-04 B lymphocyte development and function remains an exciting area of research for those interested in the physiology and pathology of the immune system in higher animals While recent advances in genetics and cellular and molecular biology have provided a large spectrum of powerful new experimental tools in this field it is both time consuming and often very difficult for a student or just any bench side worker to identify a reliable experimental protocol in the ocean of the literature The aim of B Cell Protocols is to provide a collection of diverse protocols ranging from the latest inventions and applications to some classic but still frequently used methods in B cell biology The authors of the various chapters are all highly qualified scientists who are either the inventors or expert users of these methods Their extensive experience in mastering a particular method provides not only the step by step details of a reproducible protocol but also useful troubleshooting tips that readers will appreciate in their daily work We hope that this book will be helpful for both beginning and experienced researchers in the field in designing or modifying an experimental approach and exploring a biological question from multiple angles      **Parasite Genomics Protocols** Sara E. Melville,2008-02-02 Parasitic diseases remain a major health problem throughout the world for both humans and animals For many of us our technologically advanced lifestyle has decreased the prevalence and transmission of parasitic diseases but for the majority of the world's population they are ever present in homes domestic animals food or the environment The study of parasites and parasitic disease has a long and distinguished history In some cases it has been driven by the great importance of the presence of the parasite to the community for example those that affect our livestock In other cases it is clear that applied research has suffered for lack of funding because the parasite affects people with few resources such as the rural poor in resource poor

countries These instances include the so called neglected diseases as defined by the World Health Organization WHO Parasites have complicated life cycles and a thorough understanding of the unique characteristics of a particular parasite species is vital in attempts to avoid prevent or cure infection or to alleviate symptoms Of course the biological characteristics that each parasite has developed to aid survival and transmission to avoid destruction by the immune system and to adapt to a changing environment are of lasting fascination to basic biologists as well The elegance of these biological systems has ensured that the study of protozoan and metazoan parasites also remains an active field of research in countries where the diseases are not a threat to the population

*Receptor Signal Transduction Protocols* Gary B. Willars, R. A. John Challiss, 2008-02-03 This second edition of *Receptor Signal Transduction Protocols* not only has a new editor but also a greater focus on G protein coupled receptors their properties per se and their coupling to immediate downstream binding partners principally although not exclusively the heterotrimeric G proteins The new edition combines updates of key chapters from the first edition as well as a large number of new contributions covering key methodologies that have emerged or been extended to receptor G protein research in the past 5 6 years In common with many fields the range of methods used to assess the first steps in signal transduction are continually expanding and methods that might have been considered too specialized five years ago are now sufficiently routine to be included here Unlike many research areas where off the shelf kits have made research basically foolproof signal transduction research still requires considerable expertise and the methods included here are provided by internationally recognized experts in their fields who have many years of experience using the methods they describe This not only allows each chapter to impart a clear description of the method but also to furnish invaluable troubleshooting advice for when things do not go entirely according to plan Once again we would like to thank the Series Editor John Walker for the invitation to compile this second edition and to express our gratitude to all of the authors who have enthusiastically agreed to provide the uniformly excellent contributions

**Gene Expression Profiling** Richard A. Shinkets, 2008-02-03 Leading scientists in gene expression methodology and bioinformatics data analysis describe readily reproducible methods for measuring RNA levels in cells and tissues The techniques presented include new methods for applying the Affymetrix GeneChip SAR SAGE StaRT PCR SSH the Invader Assay and ADGEM The authors also provide critical bioinformatics insight and resources for data analysis and management By distilling the basic underlying principles of many methods to a few straightforward concepts investigators can easily choose the method most appropriate to their application

Cell Cycle Checkpoint Control Protocols Howard B. Lieberman, 2008-02-02 The field of cell cycle regulation is based on the observation that the life cycle of a cell progresses through several distinct phases G1 M S and G2 occurring in a well defined temporal order Details of the mechanisms involved are rapidly emerging and appear extraordinarily complex Furthermore not only is the order of the phases important but in normal eukaryotic cells one phase will not begin unless the prior phase is completed successfully The point control mechanisms are essentially surveillance systems that monitor the

events in each phase and assure that the cell does not progress prematurely to the next phase. If conditions are such that the cell is not ready to progress for example because of incomplete DNA replication in S or DNA damage that may interfere with chromosome segregation in M a transient delay in cell cycle progression will occur. Once the inducing event is properly handled for example DNA replication is no longer blocked or damaged DNA is repaired cell cycle progression continues. Checkpoint controls have recently been the focus of intense study by investigators interested in mechanisms that regulate the cell cycle. Furthermore the relationship between checkpoint control and carcinogenesis has additionally enhanced interest in these cell cycle regulatory pathways. It is clear that cancer cells often lack these checkpoints and exhibit genomic instability as a result. Moreover several tumor suppressor genes participate in checkpoint control and alterations in these genes are associated with genomic instability as well as the development of cancer.

**Protein-Protein Interactions** Haian Fu, 2008-02-03 As the mysteries stored in our DNA have been more completely revealed scientists have begun to face the extraordinary challenge of unraveling the intricate network of protein-protein interactions established by that DNA framework. It is increasingly clear that proteins continuously interact with one another in a highly regulated fashion to determine cell fate such as proliferation differentiation or death. These protein-protein interactions enable and exert stringent control over DNA replication RNA transcription protein translation macromolecular assembly and degradation and signal transduction. Essentially all cellular functions involve protein-protein interactions. Thus protein-protein interactions are fundamental for normal physiology in all organisms. Alteration of critical protein-protein interactions is thought to be involved in the development of many diseases such as neurodegenerative disorders cancers and infectious diseases. Therefore examination of when and how protein-protein interactions occur and how they are controlled is essential for understanding diverse biological processes as well as for elucidating the molecular basis of diseases and identifying potential targets for therapeutic interventions. Over the years many innovative biochemical biophysical genetic and computational approaches have been developed to detect and analyze protein-protein interactions. This multitude of techniques is mandated by the diversity of physical and chemical properties of proteins and the sensitivity of protein-protein interactions to cellular conditions.

**Signal Transduction Protocols** Robert C. Dickson, Michael D. Mendenhall, 2008-02-02 In 1995 Signal Transduction Protocols edited by David A Kendall and Stephen J Hill was published in the Methods in Molecular Biology series. This second edition represents an update to that previous work with an emphasis on new methodologies that have developed in the last few years. The goal then and now is to provide procedures written by experts with first-hand experience in a detail that goes far beyond what is generally encountered in the methods section of most journals and thus actually permits a particular procedure to be replicated. In addition we have had as a secondary goal the identification of protocols for the assay of general classes of signal transduction components that ideally can be adapted to the assay of any member of that class. The ability to do this has resulted in large part from the use of affinity-based assays the ease with which specific proteins can be specifically tagged and

an explosion in the availability of highly specific antibodies from commercial sources especially antibodies raised against signaling proteins of human origin The number of available approaches is fortunately for those working in signaling research far too great to fit within the confines of this volume so hard choices as to what to include had to be made

*Epigenetics Protocols* Trygve O. Tollefsbol, 2008-02-02 The field of epigenetics has grown exponentially in the past decade and a steady flow of exciting discoveries in this area has served to move it to the forefront of molecular biology Although epigenetics may previously have been considered a peripheral science recent advances have shown considerable progress in unraveling the many mysteries of nontraditional genetic processes Given the fast pace of epigenetic discoveries and the groundbreaking nature of these developments a thorough treatment of the methods in the area seems timely and appropriate and is the goal of *Epigenetics Protocols* The scope of epigenetics is vast and an exhaustive analysis of all of the techniques employed by investigators would be unrealistic However this TM volume of *Methods in Molecular Biology* covers three main areas that should be of greatest interest to epigenetics investigators 1 techniques related to analysis of chromatin remodeling such as histone acetylation and methylation 2 methods in newly developed and especially promising areas of epigenetics such as telomere position effects quantitative epigenetics and ADP ribosylation and 3 an updated analysis of techniques involving DNA methylation and its role in the modification as well as the maintenance of chromatin structure

*RNA Interference, Editing, and Modification* Jonatha M. Gott, 2008-02-05 This volume presents a comprehensive collection of cuttingedge methods for elucidating the function of new genes and altering gene expression These readily reproducible techniques can be used either in transient and stable gene splicing applied to worms flies trypanosomes mammals and plants or in studying RNA editing mechanisms in a wide range of organisms including systems that involve the conversion of one base to another and insertion deletion editing Topics of interest include stable and transient RNA interference gene silencing RNA editing bioinformatics small noncoding RNAs and RNomics Special attention is given to methods for the identification and characterization of small RNAs involved in RNA interference or modification Readily reproducible protocols for discovering new genes or altering gene expression

**Bacterial Artificial Chromosomes** Shaying Zhao, Marvin Stodolsky, 2008-02-03 For both volumes Expert investigators describe not only the classic methods but also the many novel techniques they have perfected for the transfer of large DNAs into the cells of both microbes and animals via large insert recombinant DNAs Volume 1 presents readily reproducible techniques for library construction physical mapping and sequencing An accompanying volume Volume 2 *Functional Studies* provides a wide variety of methods and applications for functional analysis of the DNA transformed organisms Besides protocols each chapter includes scientific reviews software tools database resources genome sequencing strategies and illustrative case studies

*Molecular Biology* Jordanka Zlatanova, 2023-04-21 *Molecular Biology Structure and Dynamics of Genomes and Proteomes* second edition illustrates the essential principles behind the transmission and expression of genetic information at the level of DNA RNA and proteins



Emphasis is on the experimental basis of discovery and the most recent advances in the field while presenting a rigorous yet still concise summary of the structural mechanisms of molecular biology Topics new to this edition include the CRISPR Cas gene editing system Coronaviruses structure genome vaccine and drug development and newly recognized mechanisms for transcription termination The text is written for advanced undergraduate or graduate level courses in molecular biology Key Features Highlights the experimental basis of important discoveries in molecular biology Thoroughly updated with new information on gene editing tools viruses and transcription mechanisms termination and antisense Provides learning objectives for each chapter Includes a list of relevant videos from the Internet about the topics covered in the chapter

Chemoinformatics Jürgen Bajorath, 2008-02-04 In the literature several terms are used synonymously to name the topic of this book chem chemi or chemo informatics A widely recognized definition of this discipline is the one by Frank Brown from 1998 1 who defined chemoinformatics as the combination of all the information resources that a scientist needs to optimize the properties of a ligand to become a drug In Brown's definition two aspects play a fundamentally important role design support by computational means and drug discovery which distinguishes it from the term chemical informatics that was introduced at least ten years earlier and described as the application of information technology to chemistry not with a specific focus on drug discovery In addition there is of course chemometrics which is generally understood as the application of statistical methods to chemical data and the derivation of relevant statistical models and descriptors 2 The pharmaceutical focus of many developments and efforts in this area and the current popularity of gene to drug or similar paradigms is further reflected by the recent introduction of such terms as discovery informatics 3 which takes into account that gaining knowledge from chemical data alone is not sufficient to be ultimately successful in drug discovery Such insights are well in accord with other views that the boundaries between bio and chemoinformatics are fluid and that these disciplines should be closely combined or merged to significantly impact biotechnology or pharmaceutical research 4

**mRNA Processing and Metabolism** Daniel R. Schoenberg, 2008-02-05 Cells possess a wealth of posttranscriptional control mechanisms that impact on every conceivable aspect of the life of an mRNA These processes are intimately intertwined in an almost baroque manner where promoter context influences the recruitment of splicing factors where the majority of pre mRNAs undergo alternative splicing and where proteins deposited during nuclear processing impact distal cytoplasmic processing translation and decay If there is a unifying theme to mRNA Processing and Metabolism Methods and Protocols it is that mRNA processing and metabolism are integrated processes Many of the techniques used to study mRNA have been described in a previous volume of this series RNA Protein Interaction Protocols Susan Haynes ed and specialized methods journals In selecting topics for mRNA Processing and Metabolism Methods and Protocols I sought input on new and novel techniques and approaches that build on this foundation using technological advances in microscopy whole genome sequencing microarrays mass spectrometry fluorescent detection methodologies and RNA interference I have tried not to bias this book toward any single

model organism and approaches described in the various chapters use yeast *Drosophila* *Xenopus* mice plants and cultured mammalian cells

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