

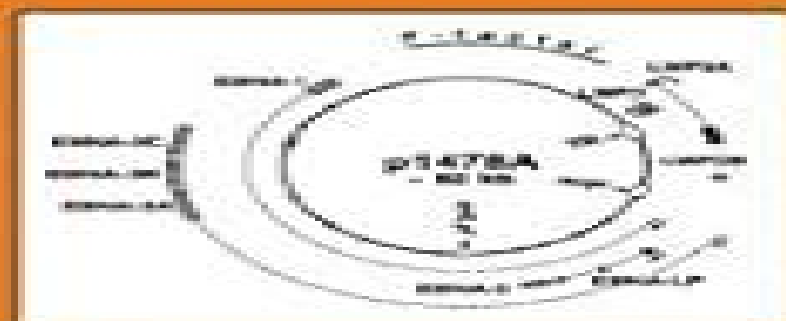
Methods in Molecular Biology

VOLUME 174

# Epstein-Barr Virus Protocols

*Edited by*

Joanna B. Wilson  
Gerhard H. W. May



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# Epstein Barr Virus Protocols Methods In Molecular Biology

**Martin J. Tymms, Ismail Kola**



## **Epstein Barr Virus Protocols Methods In Molecular Biology:**

**Methods in Molecular Biology: Epstein-Barr virus protocols** John M. Walker, 1984     *Epstein-Barr Virus Protocols* Joanna B. Wilson, Gerhard H. W. May, 2010-11-10 The discovery of Epstein Barr virus EBV by Epstein Achong and Barr reported in 1964 Lancet 1 702 703 was stimulated by Denis Burkitt's recognition of a novel African childhood lymphoma and his postulation that an infectious agent was involved in the tumor's etiology Nature 194 232 234 1962 Since then molecular and cellular biological and computational technologies have progressed by leaps and bounds The advent of recombinant DNA technology opened the possibilities of genetic research more than most would have realized Not only have the molecular tools permitted the analyses of viral mechanisms but importantly they have formed the basis for discerning viral presence and subsequently viral involvement in an increasing number of diseases Though in every field of science the search for further knowledge is likely to be a limitless phenomenon the distinct goal in EBV research namely to gain sufficient insight into the viral host interaction to be able to intercept the pathogenic process is beginning to be realized Epstein Barr virus research has effectively entered the postgenomic era that began with the sequencing of the first strains cloned in the mid to late 1980s     Epstein-Barr Virus Protocols. Methods in Molecular Biology Joanna B. Wilson, Gerhard H. W. May, 2001 The application of recombinant DNA technology to the analysis of Epstein Barr virus EBV is rapidly developing sufficient insight into the virus host interaction so that its role in disease pathology is now often discernible and can increasingly be interdicted In Epstein Barr Virus Protocols Joanna Wilson and Gerhard May have assembled a collection of the key molecular biology protocols used in the analysis of Epstein Barr virus along with a series of valuable immunology cell biology and transgenic mouse protocols Described in step by step detail by experts who use them regularly these readily reproducible techniques include methods for gene expression with mini EBV plasmids for expression analysis by FISH for EBV detection and quantitation and for cell proliferation and death assays In addition the authors provide information on EBV based vectors an up to date map of EBV a comprehensive table of available latent protein antisera and assays from in vitro to cell to organ to organism levels Timely and highly practical Epstein Barr Virus Protocols provides powerful tools for elucidating the life cycle of EBV and its host interactions work that promises the emergence of major new treatments and cures for EBV associated diseases including several forms of human cancer     **Epstein Barr Virus** Janos Minarovits, Hans Helmut Niller, 2017 This volume explores data from the applications of molecular biological methods and the applications of recent immunological and cytogenetic methods in Epstein Barr Virus EBV that will offer readers possible new solutions to the unresolved problems in the EBV field Chapters in this book cover topics such as viral life cycle latency EBV associated diseases and EBV diagnostics in vitro methods including organotypic cultures for the analysis of EBV epithelial cell interactions identification of the interacting viral and cellular proteins using affinity purification mass spectrometry methods 3D telomere FISH transcription analysis using high throughput RNA sequencing qPCR and nuclear run on assay analysis of

viral and cellular microRNAs isolation and characterization of exosomes and the assessment of their function  
characterization of the viral genome by terminal repeat analysis and sequencing the use of chromatin immunoprecipitation coupled sequencing ChIP Seq for the analysis of Zta DNA interactions epigenetic analysis by bisulfite sequencing and ChIP novel in vivo models for the study of EBV infection and how immunological virological tissue culture and molecular methods can be combined to yield Good Manufacturing Practice compliant EBV specific T cells for the immunotherapy of EBV associated post transplant lymphoproliferative diseases PTLT Written in the highly successful Methods in Molecular Biology 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 Cutting edge and comprehensive Epstein Barr Virus Methods and Protocols is a valuable resource for anyone who is interested in this fascinating and evolving field

**Immunotoxin Methods and Protocols** Walter A. Hall, 2008-02-02 Immunotoxins represent a new class of human therapeutics that have widespread applications and a potential that has not yet been fully recognized since they were first conceived of by Paul Ehrlich in 1906 The majority of advances in the development and implementation of immunotoxins has occurred over the last 20 years The reasons for this use of immunotoxins in basic science and clinical research are the powerful concurrent advances in genetic engineering and receptor physiology Recombinant technology has allowed investigators to produce sufficient quantities of a homogeneous compound that allows clinical trials to be performed The identification of specific receptors on malignant cell types has enabled scientists to generate immunotoxins that have had positive results in clinical trials As more cellular targets are identified in coming years additional trials will be conducted in different disease states affecting still larger patient populations Modulation of the immune system to decrease the humoral response to immunotoxins may improve their overall efficacy As increasingly more effective compounds are generated it will be necessary to decrease the local and systemic toxicity associated with these agents and methods for doing so are presently being developed The work presented in Immunotoxin Methods and Protocols focuses on three specific areas of immunotoxin investigation that are being conducted by experts throughout the world The first section describes the construction and development of a variety of immunotoxins

**Connexin Methods and Protocols** Roberto Bruzzone, Christian Giaume, 2008-02-05 Direct cell cell communication is a common property of multicellular organisms that is achieved through membrane channels which are organized in gap junctions The protein subunits of these intercellular channels the connexins form a multigene family that has been investigated in great detail in recent years It has now become clear that in different tissues connexins speak several languages that control specific cellular functions This progress has been made possible by the availability of new molecular tools and the improvement of basic techniques for the study of membrane channels as well as by the use of genetic approaches to study protein function in vivo More important connexins have gained visibility because mutations in some connexin genes have been found to be linked to human genetic disorders

Connexin Methods and Protocols presents in detail a collection of techniques currently used to study the cellular and molecular biology of connexins and their physiological properties. The field of gap junctions and connexin research has always been characterized by a multidisciplinary approach combining morphology, biochemistry, biophysics and cellular and molecular biology. This book provides a series of cutting edge protocols and includes a large spectrum of practical methods that are available to investigate the function of connexin channels. Connexin Methods and Protocols is divided into three main parts.

**Cytoskeleton Methods and Protocols** Ray H. Gavin, 2008-02-03 Over the past two decades experimental studies have solidified the interpretation of the cytoskeleton as a highly dynamic network of microtubules, actin microfilaments, intermediate filaments and myosin filaments. Rather than a network of disparate fibers, these polymers are often interconnected and display synergy which is the combined action of two or more cytoskeletal polymers to achieve a specific cellular structure or function. Cross communication among cytoskeletal polymers is thought to be achieved through cytoskeletal polymer accessory proteins and molecular motors that bind two or more cytoskeletal polymers. Development of the modern concept of the cytoskeleton is a direct result of advances in experimental tools and reagents that are available to cell and molecular biologists. Technological advances and refinements in cell imaging have made it possible to selectively image a single cytoskeletal polymer and monitor its dynamics through the use of fluorescence probes in vitro and in vivo. Two decades ago cytoskeletal research was limited to a few perturbation reagents that included colchicine and cytochalasin. Today the perturbation arsenal has expanded to a highly selective group of reagents that includes Taxol, nocodazole, benomyl, latrunculin, jasplakinolide and such endogenous proteins as gelsolin. These reagents enable the investigator to selectively perturb or destroy a cytoskeletal polymer while leaving other cytoskeletal polymers intact. Site specific monoclonal antibodies that target a specific cytoskeletal polymer have proven to be highly selective affinity tools for cytoskeletal research. **Amino**

**Acid Analysis Protocols** Catherine Cooper, 2008-02-05 A collection of classic and cutting edge techniques of high utility in answering specific biological questions about amino acids. Common methods include those based on HPLC or gas chromatography separation and analysis after precolumn derivatization. New techniques based on capillary electrophoresis separation, high performance anion exchange chromatography and mass spectrometry are also presented. Each method is described in step by step detail to ensure successful experimental results and emphasizes sample preparation, particularly the collection and storage of bodily fluids. Up to date and highly practical Amino Acid Analysis Protocols offers analytical and clinical chemists as well as a broad range of biological and biomedical investigators a rich compendium of laboratory tools for the productive analysis of both common and uncommon amino acids. **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      **Neurotrophin Protocols** Robert A. Rush, 2008-02-03 The past decade has seen an extraordinary growth in research interest in neurotrophic factors and the study of the neurotrophin family has led this activity Nevertheless this area of research has often struggled as a result of techniques that were either inadequate or just emerging from other research fields and disciplines Neurotrophin Protocols has brought together many leaders in the neurotrophin field who detail their special expertise in a wide variety of techniques Though most procedures are valid across many different fields of research some of those described here have been developed to address particular issues within the neurotrophic factor field The protocols cover a broad range of biochemical histological and biological techniques that are often required by the modern laboratory However all have been written with sufficient detail to allow any laboratory to achieve proficiency without need of reference to other texts Neurotrophin Protocols is divided into four sections dealing with protein RNA recombinant and in vivo techniques Protein techniques have in general been less successfully employed than those dealing with RNA or DNA However procedures that achieve localization and quantification of the neurotrophins are now being used more extensively Their inclusion here should assist further studies at the protein level Transgenic cell lines and animals are commonplace in the scientific research literature but their inclusion in several chapters in this book provide some novel uses that are not readily available elsewhere      **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 attachment of liposomes to cell surfaces permeation of liposomes through the plasma membranes and 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      **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

***Mycotoxin Protocols*** Mary W. Trucksess, Albert E. Pohland, 2008-02-05 Mycotoxins produced by molds are common contaminants of many important crops including wheat corn rice and peanuts Some mycotoxins are found in fruits and vegetables These contaminants have a broad range of toxic effects including carcinogenicity neurotoxicity and reproductive and developmental toxicity The occurrence of mycotoxins in foods is an unavoidable worldwide problem About 80 countries have imposed regulatory limits to minimize human and animal exposure to mycotoxins Regulatory limits including international standards have tremendous economic impact and must be developed using science based risk assessments The purpose of Mycotoxin Protocols is to provide the scientific and technological basis for analytical methods for use in obtaining the exposure data needed for risk assessments Mycotoxin Protocols is divided into four sections which are interconnected The first section Chapters 1 5 describe the general techniques for mycotoxin analysis with emphasis on the importance of method validation based on statistical parameters sampling procedures for collecting a sample as representative as possible of a bulk lot the isolation of mycotoxins for use as analytical standards or for toxicological studies the evaluation of purity and preparation of standards and the detection and identification of impurities in isolated mycotoxins Sections 2 4 Chapters 6 19 describe the most current chromatographic and immunochemical methods for studies on the major mycotoxins

***Gene Knockout Protocols*** Martin J. Tymms, Ismail Kola, 2008-02-03 As the major task of sequencing the human genome is near completion and full complement of human genes are catalogued attention will be focused on the ultimate goal to understand the normal biological functions of these genes and how alterations lead to disease states In this task there is a severe limitation in working with human material but the mouse has been adopted as the favored animal model because of the available genetic resources and the highly conserved gene conservation linkage organization In just of ten years since the first gene targeting experiments were performed in

embryonic stem ES cells and mutations transmitted through the mouse germline more than a thousand mouse strains have been created These achievements have been made possible by pioneering work that showed that ES cells derived from preimplantation mouse embryos could be cultured for prolonged periods without differentiation in culture and that homologous recombination between targeting constructs and endogenous DNA occurred at a frequency sufficient for recombinants to be isolated In the next few years the mouse genome will be systematically altered and the techniques for achieving manipulations are constantly being streamlined and improved

### **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

Matrix Metalloproteinase Protocols Ian M. Clark, 2008-02-05 Research in the matrix metalloproteinase field began with the demonstration by Gross and Lapiere in 1962 that resorbing tadpole tail expressed an enzyme that could degrade collagen gels These humble beginnings have led us to the elucidation of around twenty distinct vertebrate MMPs along with a variety of homologs from such diverse organisms as sea urchin plants nematode worm and bacteria This coupled with four known specific inhibitors of MMPs the TIMPs gives a complex picture Part I of Matrix Metalloproteinase Protocols provides the reader with a selective overview of the MMP arena and a chance to come to grips with where the field has been where it is and where it is going I hope that this complements all of the methodology that comes later Part II presents the reader with a diverse set of methods for the expression and purification of MMPs and TIMPs bringing together the long and often hard earned experience of a number of researchers Part III allows the reader to detect MMPs and TIMPs at both the protein and mRNA level whereas Part IV gives the ability to assay MMP and TIMP activities in a wide variety of circumstances

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 pr



ently widely used and that will continue to be the standard for researchers worldwide Each chapter is aimed at supplying detailed methodologies to allow reproduction in any laboratory while also providing the general principles on which the methods are based Some of the techniques grouped in the first section apply broadly to small GTPases whereas others in Part II are more applicable within each GTPase subfamily **PCR Cloning Protocols** Bing-Yuan Chen, Harry W.

Janes, 2008-02-05 PCR Cloning Protocols Second Edition updates and expands Bruce White's best selling PCR Cloning Protocols 1997 with the newest procedures for DNA cloning and mutagenesis Here the researcher will find readily reproducible methods for all the major aspects of PCR use including PCR optimization computer programs for PCR primer design and analysis and novel variations for cloning genes of special characteristics or origin with emphasis on long distance PCR and GC rich template amplification Also included are both conventional and novel enzyme free and restriction site free procedures to clone PCR products into a range of vectors as well as state of the art protocols to facilitate DNA mutagenesis and recombination and to clone the challenging uncharacterized DNA flanking a known DNA fragment *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

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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