DNA Viruses
M E T H O D S  I N  M O L E C U L A R  B I O L O G Y™

John M. Walker, Series Editor

304 Human Retrovirus Protocols: Virology and Molecular Biology, edited by Twofu Zha, 2005
303 NanoBiotecnology Protocols, edited by Sandra J. Rosenthal and David W. Wright, 2005
302 Handbook of ELISOT, edited by Alexander E. Kalyuzhny, 2005
301 Ubiquitin–Proteasome Protocols, edited by Cam Patterson and Douglas M. Cyr, 2005
300 Protein Nanotechnology: Protocols, Instrumentation, and Applications, edited by Tuan Vo-Dinh, 2005
299 Amyloid Proteins: Methods and Protocols, edited by Einar M. Sigurdsson, 2005
298 Peptide Synthesis and Application, edited by John Howl, 2005
297 Forensic DNA Typing Protocols, edited by Angel Carracedo, 2005
296 Cell Cycle Protocols, edited by Tim Humphrey and Gavin Brooks, 2005
295 Cell Migration: Developmental Methods and Protocols, edited by Jan-Lin Guan, 2005
294 Laser Capture Microdissection: Methods and Protocols, edited by Graeme I. Murray and Stephanie Curran, 2005
293 DNA Viruses: Methods and Protocols, edited by Paul M. Lieberman, 2005
292 Molecular Toxicology Protocols, edited by Phouathone Keohavong and Stephen G. Grant, 2005
289 Epidermal Cells, Methods and Applications, edited by Kursad Turksen, 2005
288 Oligonucleotide Synthesis, Methods and Applications, edited by Piet Heredewijn, 2005
287 Epigenetics Protocols, edited by Trygve O. Tollefsbol, 2004
286 Transgenic Plants: Methods and Protocols, edited by Leandro Peña, 2005
285 Cell Cycle Control and Dysregulation Protocols: Cyclins, Cyclin-Dependent Kinases, and Other Factors, edited by Antonio Giordano and Gaetano Romano, 2004
283 Bioconjugation Protocols, edited by Christof M. Niemeyer, 2004
282 Apoptosis Methods and Protocols, edited by Hugh J. M. Brady, 2004
280 Checkpoint Controls and Cancer, Volume 1: Reviews and Model Systems, edited by Axel H. Schönhthal, 2004
277 Trinucleotide Repeat Protocols, edited by Yoshinori Kohwi, 2004
276 Capillary Electrophoresis of Proteins and Peptides, edited by Mark A. Strege and Avinash L. Lagu, 2004
275 Chemoinformatics, edited by Jürgen Bajorath, 2004
274 Photosynthesis Research Protocols, edited by Robert Carpentier, 2004
273 Platelets and Megakaryocytes, Volume 2: Perspectives and Techniques, edited by Jonathan M. Gibbins and Martyn P. Mahaut-Smith, 2004
272 Platelets and Megakaryocytes, Volume 1: Functional Assays, edited by Jonathan M. Gibbins and Martyn P. Mahaut-Smith, 2004
271 B Cell Protocols, edited by Hua Gu and Klaus Rajewsky, 2004
270 Parasite Genomics Protocols, edited by Sara E. Melville, 2004
269 Vaccina Virus and Poxvirology: Methods and Protocols, edited by Stuart N. Isaacs, 2004
266 Genomics, Proteomics, and Clinical Bacteriology: Methods and Reviews, edited by Neil Woodford and Alan Johnson, 2004
265 RNA Interference, Editing, and Modification: Methods and Protocols, edited by Jonatha M. Gott, 2004
264 Protein Arrays: Methods and Protocols, edited by Eric Fung, 2004
262 Genetic Recombination Protocols, edited by Alan S. Waldman, 2004
261 Protein–Protein Interactions: Methods and Applications, edited by Haian Fu, 2004
Preface

The application of modern methods in molecular biology and biotechnology to the study of human, animal, and plant viruses continues to revitalize the age-old discipline of virology. Modern virology remains at the vanguard of contemporary biomedical research largely owing to the impact of viruses in human disease and pathogenesis, but also because of the utility of viruses as model systems for investigation of basic biological processes. DNA Viruses: Methods and Protocols describes innovative approaches to solving important problems in modern virology and also provides methodologies that can equally be applied to numerous other biological systems. Since virology, like cell biology, covers a vast expanse of methodological approaches, it is virtually impossible to cover all aspects of this dynamic field. The scope of the book is limited to DNA viruses, and it includes only a small sample of the many exciting methodological innovations of the last few years. This book does not include any specific applications to RNA viruses, but some of the methods describe techniques that have general applications to RNA viruses, as well as to cell biology.

In DNA Viruses: Methods and Protocols I have tried to include a sample of exciting advances in what I see as the major areas of DNA virology today. The methods presented here are representative of, but do not exhaust, the many important contributions to this field. I have divided the book into nine parts that include: viral detection, structure, entry, gene expression, replication, pathogenesis, complex cellular models, and recombinant genetics, with the addition of computational/systems approaches toward virology. Some of these divisions are arbitrary and have obvious overlaps. Nevertheless, I thought it useful to divide this volume into sections to emphasize the various methodological approaches as they are applied to important questions in virology.

Although DNA Viruses: Methods and Protocols attempts to cover numerous aspects of modern virology, it is apparent that many significant methodological advances have not been included. I ask those readers who would have preferred either a more focused or a more comprehensive volume to understand the book’s constraints, and those authors who should have been asked to contribute to accept my apology for the oversight. Regardless of these obvious limits, I hope you find this book of interest and value in your experimental molecular biology pursuits.

Paul M. Liebermann
## Contents

Preface ................................................................................................................................. v
Contributors ......................................................................................................................... xi

### PART I. VIRAL DETECTION

1 Viral Detection

*Feng Wang-Johanning and Gary L. Johanning* ................................................................. 3

2 Quantitative Detection of Epstein-Barr Virus DNA in Clinical Specimens by Rapid Real-Time PCR Targeting a Highly Conserved Region of EBNA-1

*Servi J. C. Stevens, Sandra A. W. M. Verkuijlen, and Jaap M. Middeldorp* ................................. 15

3 Profiling of Epstein-Barr Virus Latent RNA Expression in Clinical Specimens by Gene-Specific Multiprimed cDNA Synthesis and PCR

*Servi J. C. Stevens, Antoinette A. T. P. Brink, and Jaap M. Middeldorp* ................................. 27

4 Quantitative Detection of Viral Gene Expression in Populations of Epstein-Barr Virus-Infected Cells In Vivo

*Donna R. Hochberg and David A. Thorley-Lawson* ............................................................... 39

5 Detection and Quantification of the Rare Latently Infected Cell Undergoing Herpes Simplex Virus Transcriptional Activation in the Nervous System In Vivo

*Nancy M. Sawtell* ............................................................................................................. 57

6 Reporter Cell Lines for the Detection of Herpes Simplex Viruses

*Szu-Hao Kung* ................................................................................................................ 73

### PART II. VIRUS STRUCTURE AND IMAGING

7 Unraveling the Architecture of Viruses by High-Resolution Atomic Force Microscopy

*Alexander J. Malkin, Marco Plomp, and Alexander McPherson* ................................. 85

8 Studying the Structure of Large Viruses With Multiresolution Imaging

*Carmen San Martín* ............................................................................................................ 109

9 Herpes Simplex Virus–Cell Interactions Studied by Low-Fading Contrasted Immunofluorescence

*Helle Lone Jensen and Bodil Norrild* .............................................................................. 129
Contents

10 Herpes Simplex Virus–Cell Interactions Studied by Immunogold Cryosection Electron Microscopy
   Helle Lone Jensen and Bodil Norrild ................................................ 143

11 FTIR Microscopy Detection of Cells Infected With Viruses
   Vitaly Erukhimovitch, Marina Talyshinsky, Yelena Souprun, and Mahmoud Huleihel ................................................ 161

PART III. VIRUS ENTRY

12 The JC Virus-Like Particle Overlay Assay
   Hirofumi Sawa and Rika Komagome ................................................ 175

13 Analysis of Fusion Using a Virus-Free Cell Fusion Assay
   Marisa P. McShane and Richard Longnecker ................................... 187

14 Pseudovirions as Specific Tools for Investigation of Virus Interactions With Cells
   Martin Sapp and Hans-Christoph Selinka ......................................... 197

PART IV. GENE EXPRESSION

15 Simultaneous In Situ Detection of RNA, DNA, and Protein Using Tyramide-Coupled Immunofluorescence
   Brian A. Van Tine, Thomas R. Broker, and Louise T. Chow ............. 215

16 Identification and Characterization of Herpesviral Immediate-Early Genes
   Yan Yuan ........................................................................................... 231

PART V. REPLICATION AND GENOME MAINTENANCE

17 Methods for Measuring the Replication and Segregation of Epstein-Barr Virus-Based Plasmids
   Priya Kapoor and Lori Frappier ....................................................... 247

18 DNA Affinity Purification of Epstein-Barr Virus OriP-Binding Proteins
   Constandache Atanasiu, Larissa Lezina, and Paul M. Lieberman .... 267

PART VI. PATHOGENESIS

19 Pre-B-Cell Colony Formation Assay
   Masato Ikeda and Richard Longnecker .......................................... 279

20 Luciferase Real-Time Bioluminescence Imaging for the Study of Viral Pathogenesis
   Gary D. Luker and David A. Leib ..................................................... 285

PART VII. COMPLEX CELL SYSTEMS

21 Culturing Primary and Transformed Neuronal Cells for Studying Pseudorabies Virus Infection
   Toh Hean Ch’ng, E. Alexander Flood, and Lynn William Enquist ....... 299
Contents ix

22 Human Papillomavirus Type 31 Life Cycle:
Methods for Study Using Tissue Culture Models
Frauke Fehrmann and Laimonis A. Laimins ............................... 317

PART VIII. RECOMBINANT GENETICS

23 Molecular Genetics of Herpesviruses:
A Recombinant Technology Approach
Jason S. Knight, Subhash C. Verma, Ke Lan, and Erle S. Robertson .... 333

24 Molecular Genetics of DNA Viruses:
Recombinant Virus Technology
Bernhard Neuhierl and Henri-Jacques Delecluse ......................... 353

25 Genetic Analysis of Cytomegalovirus by Shuttle Mutagenesis
Manfred Lee and Fenyong Liu ............................................... 371

26 Construction of a Gene Inactivation Library for Bovine
herpesvirus 1 Using Infectious Clone Technology
Timothy J. Mahony, Fiona M. McCarthy, Jennifer L. Gravel,
and Peter L. Young .................................................................. 387

27 Selective Silencing of Viral Gene E6 and E7 Expression
in HPV-Positive Human Cervical Carcinoma Cells
Using Small Interfering RNAs
Ming Jiang and Jo Milner .......................................................... 401

PART IX. COMPUTATION/SYSTEMS BIOLOGY OF VIRUSES

28 Design of a Herpes Simplex Virus Type 2 Long
Oligonucleotide-Based Microarray: Global Analysis of HSV-2
Transcript Abundance During Productive Infection
J. S. Aguilar, Peter Ghazal, and Edward K. Wagner ...................... 423

29 Real-Time Quantitative PCR Analysis of Viral Transcription
James Papin, Wolfgang Vahrson, Rebecca Hines-Boykin,
and Dirk P. Dittmer ................................................................ 449

30 Rapid Screening of Chemical Inhibitors That Block
Processive DNA Synthesis of Herpesviruses:
Potential Application to High-Throughput Screening
Robert P. Ricciardi, Kai Lin, Xulin Chen, Dorjbal Dorjsuren,
Robert Shoemaker, and Shizuko Sei ........................................ 481

Index ......................................................................................... 493
Contributors

J. S. AGUILAR • Department of Molecular Biology and Biochemistry and Center for Virus Research, University of California, Irvine, Irvine, CA
CONSTANDACHE ATANASIU • The Wistar Institute, Philadelphia, PA
ANTOINETTE A. T. P. BRINK • Department of Pathology, VU Medical Centre, Amsterdam, The Netherlands
THOMAS R. BROKER • Department of Biochemistry & Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL
XULIN CHEN • Department of Microbiology, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA
TOH HEAN CH'NG • Department of Molecular Biology, Princeton University, Princeton, NJ
LOUISE T. CHOW • Department of Biochemistry & Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL
HENRI-JACQUES DELECLUSE • CR-UK Institute for Cancer Studies, Department of Pathology, University of Birmingham, Birmingham, UK
DIRK P. DITTMER • Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK
DORJBAL DORJSUREN • Laboratory of Antiviral Drug Mechanisms, SAIC-Frederick, National Cancer Institute, Frederick, MD
LYNN WILLIAM ENQUIST • Department of Molecular Biology, Princeton University, Princeton, NJ
VITALY ERUKHIMOVITCH • The Institute for Applied Biosciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel
FRAUKE FEHRMANN • Department of Microbiology-Immunology, Feinberg School of Medicine, Northwestern University, Chicago, IL
E. ALEXANDER FLOOD • Department of Molecular Biology, Princeton University, Princeton, NJ
LORI FRAPPIER • Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Ontario Canada
PETER GHAZAL • Scottish Centre for Genomic Technology and Informatics, University of Edinburgh Medical School, The Chancellor’s Building, Little France Crescent, Edinburgh, UK
JENNIFER L. GRAVEL • Department of Primary Industries, Agency for Food and Fibre Sciences, St. Lucia, Queensland, Australia
DONNA R. HOCHEMBERG • Department of Pathology, Tufts University School of Medicine, Boston, MA
REBECCA HINES-BOYKIN • Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK
MAHMOUD HULEIHEL • The Institute for Applied Biosciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel

MASATO IKEDA • Department of Microbiology-Immunology, Northwestern University Feinberg School of Medicine, Chicago, IL

HELLE LONE JENSEN • Department of Pathology, Rigshospitalet, University Hospital of Copenhagen, Copenhagen, Denmark

MING JIANG • Yorkshire Cancer Research P53 Laboratory, Department of Biology, University of York, York, UK

GARY L. JOHANNING • Department of Veterinary Sciences, University of Texas M. D. Anderson Cancer Center, Bastrop, TX

PRIYA KAPOOR • Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Ontario Canada

JASON S. NIGHT • Department of Microbiology and the Abramson Comprehensive Cancer Center, University of Pennsylvania Medical School, Philadelphia, PA

RIKA KOMAGOME • Laboratory of Molecular and Cellular Pathology, Graduate School of Medicine, Hokkaido University; CREST, JST, Sapporo, Japan

Szu-Hao Kung • Faculty of Medical Technology and Institute of Biotechnology in Medicine, National Yang-Ming University, Taipei, Taiwan, Republic of China

LAIMONIS A. LAIMINS • Department of Microbiology-Immunology, Feinberg School of Medicine, Northwestern University, Chicago, IL

KE LAN • Department of Microbiology and the Abramson Comprehensive Cancer Center, University of Pennsylvania Medical School, Philadelphia, PA

MANFRED LEE • Division of Infectious Diseases, School of Public Health, University of California, Berkeley, CA

DAVID A. LEIB • Department of Ophthalmology and Visual Sciences; Department of Molecular Microbiology Washington University School of Medicine, St. Louis, MO

PAUL M. LIEBERMAN • The Wistar Institute, Philadelphia, PA

LARISSA LEZINA • The Wistar Institute, Philadelphia, PA

KAI LIN • Department of Biochemistry and Biophysics, School of Medicine University of Pennsylvania, Philadelphia, PA

FENYONG LIU • Division of Infectious Diseases, School of Public Health, University of California, Berkeley, CA

RICHARD LONGNECKER • Department of Microbiology-Immunology, Northwestern University Feinberg School of Medicine, Chicago, IL

GARY D. LUKER • Department of Radiology, University of Michigan Medical School, Ann Arbor, MI

TIMOTHY J. MAHONY • Department of Primary Industries, Agency for Food and Fibre Sciences, St. Lucia, Queensland, Australia

ALEXANDER J. MALIK • BioSecurity and NanoSciences Laboratory, Department of Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore CA; and Department of Molecular Biology and Biochemistry, University of California, Irvine, CA
Contributors

Fiona M. McCARTHY • Department of Primary Industries, Agency for Food and Fibre Sciences, St. Lucia, Queensland, Australia
Alexander McPherson • Department of Molecular Biology and Biochemistry, University of California, Irvine, CA
Marisa P. McShane • Department of Microbiology and Immunology, Northwestern University Feinberg School of Medicine, Chicago, IL
Jaap M. Middeldorp • Department of Pathology, VU Medical Centre, Amsterdam, The Netherlands
Jo Milner • Yorkshire Cancer Research P53 Laboratory, Department of Biology, University of York, York, UK
Bernhard Neuhiem • GSF-National Research Centre for Environment and Health, Department of Gene Vectors, Munich, Germany
Bodil Norrild • Institute of Molecular Pathology, University of Copenhagen, Copenhagen, Denmark
James Papin • Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK
Marco Plomp • Biosecurity and Nanosciences Laboratory, Department of Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, CA
Robert P. Ricciardi • Departments of Microbiology and Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA
Erle S. Robertson • Department of Microbiology and the Abramson Comprehensive Cancer Center, University of Pennsylvania School of Medicine, Philadelphia, PA
Carmen San Martín • The Wistar Institute, Philadelphia, PA; Biocomputing Department, Centro Nacional de Biotecnologia (CSIC), Madrid, Spain
Martin Sapp • Institute for Medical Microbiology and Hygiene, University of Mainz, Mainz, Germany
Hiroyoshi Sawa • Laboratory of Molecular and Cellular Pathology, Graduate School of Medicine, Hokkaido University; 21st Century COE Program for Zoonosis, CREST, JST, Sapporo, Japan
Nancy M. Sawtell • Division of Infectious Diseases, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH
Shizuko Sei • Laboratory of Antiviral Drug Mechanisms, SAIC-Frederick, National Cancer Institute, Frederick, MD
Hans-Christoph Selinka • Institute for Medical Microbiology and Hygiene, University of Mainz, Mainz, Germany
Robert Shoemaker • Screening Technology Branch, Developmental Therapeutics Program, National Cancer Institute, Frederick, MD
Yelena Souproun • The Institute for Applied Biosciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel
Servi J. C. Stevens • Department of Pathology, VU Medical Centre, Amsterdam, The Netherlands
Marina Talyshinsky • The Institute for Applied Biosciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel
Contributors

DAVID A. THORLEY-LAWSON • Department of Pathology, Tufts University School of Medicine, Boston, MA
BRIAN A. VAN TINE • Department of Pathology, University of Alabama at Birmingham, Birmingham, AL,
WOLFGANG VAHRSON • Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK
SANDRA A. W. M. VERKUIJLEN • Department of Pathology, VU Medical Centre, Amsterdam, The Netherlands
SUBHASH C. VERMA • Department of Microbiology and the Abramson Comprehensive Cancer Center, University of Pennsylvania School of Medicine, Philadelphia, PA
EDWARD K. WAGNER • Department of Molecular Biology and Biochemistry, Institute for Genomics and Bioinformatics, and Center for Virus Research, University of California, Irvine, CA
FENG WANG-JOHANNING • Department of Veterinary Sciences, University of Texas MD Anderson Cancer Center, Houston, TX
PETER L. YOUNG • Department of Primary Industries, Agency for Food and Fibre Sciences, St. Lucia, Queensland, Australia
YAN YUAN • Department of Microbiology, University of Pennsylvania School of Dental Medicine, Philadelphia, PA