

METHODS IN MOLECULAR BIOLOGY

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Reverse Genetics of RNA Viruses

Methods and Protocols

Edited by

Daniel R. Perez

*Department of Population Health, Poultry Diagnostic and Research Center,
College of Veterinary Medicine, University of Georgia, Athens, GA, USA*

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Editor

Daniel R. Perez
Department of Population Health,
Poultry Diagnostic and Research Center,
College of Veterinary Medicine
University of Georgia
Athens, GA, USA

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Preface

The International Committee on Taxonomy of Viruses (ICTV) classifies RNA viruses as those that belong to Group III, Group IV, or Group V of the Baltimore classification system and contain ribonucleic acid (RNA) as genetic material throughout their entire life cycle. Group III includes double-stranded RNA viruses (dsRNAs), whereas Groups IV and V contain single-stranded RNA viruses (ssRNAs) of positive and negative polarity, respectively. Positive sense RNA viruses (+ssRNAs) are those in which the RNA itself is translated by the host cell translation machinery and initiates an infectious cycle *de novo*. In contrast, negative sense RNA viruses (–ssRNAs) cannot be translated directly and require copying of the negative sense RNA into a positive sense RNA strand before the infection can proceed.

In biology, the term “forward genetics” is used to define an approach that seeks to find the genetic basis of a phenotype or trait. Forward genetics of RNA viruses implies imposing them to various stress conditions and then defining the genetic changes that occurred in the process. The term “reverse genetics” is an approach to unravel the function of a gene by establishing and analyzing the phenotypic effects of (artificially) engineered gene sequences. In case of RNA viruses, reverse genetics invariably requires the *de novo* reconstitution of the virus from a cDNA copy. Using molecular biology, cDNA copies of RNA viruses are cloned into a variety of vectors, most typically and in order of preference, plasmids, bacterial artificial chromosomes or bacmids, or recombinant viral vectors. The ability to further manipulate DNA elements encoding portions or entire cDNA copies of RNA viruses has revolutionized the manner in which these viruses can be studied and understood. Thanks to reverse genetics, it is possible to better define the molecular mechanisms that modulate pathogenesis, transmission, and host range of RNA viruses, to study virus evolution, receptor binding characteristics, virus entry, replication, assembly, and budding. Reverse genetics allows the development of novel vaccine strategies and to better test and/or develop alternative intervention strategies such as novel antivirals. Perhaps the initial perception is to think that reverse genetics of dsRNAs and +ssRNAs is easier than –ssRNAs; however, genome size, secondary RNA structures, genome segmentation, cryptic signal sequences, among other issues, make reverse genetics of all kinds of RNA viruses equally challenging.

This book *Reverse Genetics of RNA Viruses: Methods and Protocols* is a compilation of 16 chapters summarizing reverse genetics breakthroughs and detailed reverse genetics protocols. The book does not cover every reverse genetics protocol for every RNA virus. Instead, it does provide comprehensive protocols for those RNA viruses that were initially the most challenging to obtain and/or that were developed most recently. This book, of course, would not have been possible without the outstanding and most generous contributions of our authors who are leaders in their respective fields and that have shared their insights and step-by-step protocols to help you, our colleagues, with your own research endeavors. I hope you find this book helpful.

Athens, GA, USA

Daniel R. Perez

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Contributors

- CLAUDIO L. AFONSO • *Southeast Poultry Research Laboratory, United States Department of Agriculture, Athens, GA, USA*
- MATTHEW ANGEL • *Department of Population Health, Poultry Diagnostic and Research Center, University of Georgia, Athens, GA, USA*
- SHRINGKHALA BAJIMAYA • *Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA*
- UDENI B.R. BALASURIYA • *Maxwell H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY, USA*
- RALPH BARIC • *Department of Epidemiology, University of North Carolina-Chapel Hill, Chapel Hill, NC, USA; Departments of Microbiology and Immunology, University of North Carolina-Chapel Hill, Chapel Hill, NC, USA*
- ANNE BEALL • *Department of Microbiology and Immunology, University of North Carolina-Chapel Hill, Chapel Hill, NC, USA*
- ERICA BICKERTON • *The Pirbright Institute, Pirbright, UK*
- KARL W. BOEHME • *Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR, USA*
- PAUL BRITTON • *The Pirbright Institute, Pirbright, UK*
- STIVALIS CARDENAS-GARCIA • *United States Department of Agriculture, Southeast Poultry Research Laboratory, Athens, GA, USA; Department of Population Health, Poultry Diagnostic and Research Center, College of Veterinary Medicine, The University of Georgia, Athens, GA, USA*
- ADAM S. COCKRELL • *Department of Epidemiology, University of North Carolina-Chapel Hill, Chapel Hill, NC, USA*
- YIJUN DU • *Department of Pathobiology University of Illinois at Urbana-Champaign, Urbana, IL, USA; Shandong Key Laboratory of Animal Disease Control and Breeding, Institute of Animal Science and Veterinary Medicine, Shandong Academy of Agricultural, Sciences, Jinan, China*
- HEINZ FELDMANN • *Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA*
- COURTNEY FINCH • *Division of Viral Products, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, US Food and Drug Administration, Silver Spring, MD, USA*
- STEFAN FINKE • *Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut, Greifswald, Insel Riems, Germany*
- R.A.M. FOUCHIER • *Department of Viroscience, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands*
- ANA SILVIA GONZALEZ-REICHE • *Department of Population Health, Poultry Diagnostic and Research Center, University of Georgia, Athens, GA, USA*

- MINGYUAN HAN • *Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA; Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, MI, USA*
- TSUYOSHI HAYASHI • *Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA*
- THOMAS HOENEN • *Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA; Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut, Greifswald, Insel Riems, Germany*
- ANNE L. HOTARD • *Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, USA; Children's Healthcare of Atlanta, Atlanta, GA, USA*
- HANZHONG KE • *Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA*
- SARAH M. KEEP • *The Pirbright Institute, Pirbright, UK*
- LUIS MARTÍNEZ-SOBRIDO • *Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA*
- JIA MENG • *Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, USA; Children's Healthcare of Atlanta, Atlanta, GA, USA*
- MARTIN L. MOORE • *Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, USA; Children's Healthcare of Atlanta, Atlanta, GA, USA*
- MARCELO CORTEZ-SAN MARTÍN • *Laboratory of Molecular Virology, Faculty of Chemistry and Biology, University of Santiago of Chile, Santiago, Chile*
- AITOR NOGALES • *Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA*
- TOBIAS NOLDEN • *Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut, Greifswald, Insel Riems, Germany; ViraTherapeutics, Innsbruck, Austria*
- ADEBIMPE OBADAN • *Department of Population Health, Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia, Athens, GA, USA*
- SLOBODAN PAESSLER • *University of Texas Medical Branch, Galveston, TX, USA*
- DANIEL R. PEREZ • *Department of Population Health, Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia, Athens, GA, USA*
- MATTHEW B. PHILLIPS • *Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR, USA*
- JEFFERSON SANTOS • *Department of Population Health, Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia, Athens, GA, USA*
- CHAO SHAN • *Departments of Biochemistry & Molecular Biology, Pharmacology & Toxicology, and Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch, Galveston, TX, USA*
- PEI-YONG SHI • *Departments of Biochemistry & Molecular Biology and Pharmacology & Toxicology, and Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch, Galveston, TX, USA*
- CHRISTOPHER C. STOBART • *Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, USA; Children's Healthcare of Atlanta, Atlanta, GA, USA*
- JOHNASHA D. STUART • *Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR, USA*
- TORU TAKIMOTO • *Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA*

JUAN CARLOS DE LA TORRE • *Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, CA, USA*

DANIELA TORO-ASCUY • *Laboratory of Molecular Virology, Faculty of Chemistry and Biology, University of Santiago of Chile, Santiago, Chile*

B.G. VAN DEN HOOGEN • *Department of Viroscience, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands*

XUPING XIE • *Departments of Biochemistry & Molecular Biology and Pharmacology & Toxicology, and Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch, Galveston, TX, USA*

DONGWAN YOO • *Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

BOYD YOUNT • *Department of Epidemiology, University of North Carolina-Chapel Hill, Chapel Hill, NC, USA*

JIANQIANG ZHANG • *Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA*

QINGZHAN ZHANG • *Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA*