Preface

Thirty years ago, the investigation of gene sequences and, in particular, disease-causing mutations was a tedious, highly skilled operation; however, this field was revolutionized in the mid-1980s with the introduction and subsequent development of a technique which enabled the reliable amplification of minute quantities of starting DNA into readily detectable levels. Nowadays, this technique, otherwise known as reverse-transcription polymerase chain reaction (RT-PCR), has become routine in most laboratories. Indeed, such is the popularity of RT-PCR that a protocol has been published describing how this could be carried out in a kitchen using common household equipment. The aim of this volume is to translate RT-PCR theory into practice. To achieve this, a comprehensive guide to currently available RT-PCR techniques is given in the form of user-friendly protocols. These protocols contain precise information about all the necessary chemical, consumable, and equipment resources and detailed instructions about how to perform each stage of the different methods. Furthermore, each protocol concludes with a comprehensive notes section, where authors provide helpful hints, trouble-shooting tips, and other must-know information in a format which is accessible to the beginner.

RT-PCR protocols were the subject of an earlier edition in the Methods in Molecular Biology™ series, which was published in August 2002. In this second edition, some of the contents of the previous edition are revisited bringing these technologies up to date, for example, competitive RT-PCR, nested RT-PCR, RT-PCR from single cells, and RT-PCR for cloning. In addition, the second volume also describes the newer technologies that have been developed and applied in the last 7 years including multiplex RT-PCR and RT-LATE-PCR. This growth and development is reflected in the wide selection of basic RT-PCR techniques which are presented in the first section entitled “The RT-PCR Detective: Hunting Down the Right Method”. Arguably, however, the greatest advances in RT-PCR have come in the field of real-time quantitative RT-PCR, and this, along with all the other means of quantifying PCR products, is explained in the second section, “The RT-PCR Mathematician: Assessing Gene and RNA Expression”. Finally, since designing RT-PCR experiments requires both the correct recipe and the best ingredients, the last section, “The RT-PCR Master Chef: Finding the Best Ingredients,” is devoted to recent advances in some of the individual elements that go together to make the optimum RT-PCR reaction, e.g. RNA extraction, primer design, and reverse transcription.

This volume is not intended to be a hard-core technical manual that is accessible to a few Nobel Laureate molecular biologists. Rather, the goal is that it should act as a handy companion to anyone who wants to explore the marvels of gene expression. This includes students and their tutors, researchers, laboratory managers, and technologists from many diverse disciplines ranging from biochemistry to zoology and forensics to physiology.

Armidale, NSW

Nicola King
Contents

Preface ........................................................................................................... v
Contributors ................................................................................................ x

PART I THE RT-PCR DETECTIVE: HUNTING DOWN THE BEST METHOD

1 Single Cell RT-PCR on Mouse Embryos: A General Approach
   for Developmental Biology ................................................................. 3
   Wolfgang Mann and Thomas Haaf

2 Poly(A) cDNA Real-Time PCR for Indicator Gene Measurement in Cancer ...... 13
   Melissa Oliveira-Cunha, Ajith K. Siriwardena, and Richard J. Byers

3 Transcriptome Profiling of Host–Microbe Interactions
   by Differential Display RT-PCR ..................................................... 33
   Leong Wai Fook and Vincent T.K. Chow

4 Quantitative RT-PCR Methods for Mature microRNA Expression Analysis ..... 49
   Stephanie D. Fiedler, Martha Z. Carletti, and Lane K. Christenson

5 Detection of Influenza A Virus Neuraminidase and PB2 Gene
   Segments by One Step Reverse Transcription Polymerase Chain Reaction .... 65
   Alejandra Castillo Alvarez, Victoria Boyd, Richard Lai,
   Sandy Pineda, Cheryl Bletchly, Hans G. Heine, and Ross Barnard

6 Detection and Identification of CD46 Splicing Isoforms
   by Nested RT-PCR. ........................................................................ 83
   Anita Szalmás, József Kónya, István Sziklai, and Tamás Karosi

7 Simultaneous Detection of Bluetongue Virus RNA, Internal
   Control GAPDH mRNA, and External Control Synthetic
   RNA by Multiplex Real-Time PCR .............................................. 97
   Frank Vandenbussche, Elise Vandemeulebroucke, and Kris De Clercq

8 Detection of West Nile Viral RNA from Field-Collected Mosquitoes
   in Tropical Regions by Conventional and Real-Time RT-PCR. .......... 109
   Ana Silvia González-Reiche, Maria de Lourdes Monzón-Pineda,
   Barbara W. Johnson, and María Eugenia Morales-Betoulle

9 Detection of Antisense RNA Transcripts by Strand-Specific RT-PCR. ....... 125
   Eric C.H. Ho, Michael E. Donaldson, and Barry J. Saville

10 RT-PCR Amplification and Cloning of Large Viral Sequences ................. 139
    Xiaofeng Fan and Adrian M. Di Bisceglie

PART II THE RT-PCR MATHEMATICIAN: ASSESSING GENE
   AND RNA EXPRESSION

11 One-Step RT-LATE-PCR for mRNA and Viral RNA Detection
    and Quantification ......................................................................... 153
    Cristina Hartshorn and Lawrence J. Wangh
Contents

12 Changes in Gene Expression of Caveolin-1 After Inflammatory Pain Using Quantitative Real-Time PCR ............................... 187
   Fiza Rashid-Doubell

13 Real-Time Quantitative Reverse Transcriptase Polymerase Chain Reaction ...... 199
   Hongxin Fan and Ryan S. Robetorye

14 The Use of Comparative Quantitative RT-PCR to Investigate the Effect of Cysteine Incubation on GPx1 Expression in Freshly Isolated Cardiomyocytes ........................................ 215
   Nicola King

15 The Renaissance of Competitive PCR as an Accurate Tool for Precise Nucleic Acid Quantification ........................................ 233
   Lorena Zentilin and Mauro Giacca

PART III THE RT-PCR MASTER CHEF: FINDING THE RIGHT INGREDIENTS

16 Skeletal Muscle RNA Extraction in Preparation for RT-PCR ...................... 251
   Janelle P. Mollica

17 Reverse Transcription of the Ribonucleic Acid: The First Step in RT-PCR Assay ........................................ 261
   Fadia Haddad and Kenneth M. Baldwin

18 Primer Design for RT-PCR ................................................ 271
   Kelvin Li and Anushka Brownley

19 Hot Start PCR ................................................................. 301
   Natasha Paul, Jonathan Shum, and Tony Le

20 Real-time RT-PCR for Automated Detection of HIV-1 RNA During Blood Donor Screening ........................................ 319
   Jens Müller

Index .................................................................................. 337
Contributors

ALEJANDRA CASTILLO ALVAREZ • Biochip Innovations Pty Ltd., 8 Mile Plains, Brisbane, QLD, Australia
KENNETH M. BALDWIN • Physiology and Biophysics Department, University of California Irvine, Irvine, CA, USA
ROSS BARNARD • School of Chemistry & Molecular Biosciences, The University of Queensland, St. Lucia, QLD, Australia
VICTORIA BOYD • CSIRO Livestock Industries, Australian Animal Health Laboratory (AAHL), Geelong, VIC, Australia
ANUSHKA BROWNLEY • Complete Genomics, Inc., Mountain View, CA, USA
RICHARD J. BYERS • Histopathology Department, Manchester Royal Infirmary, Manchester, UK
MARTHA Z. CARLETTI • Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, USA
VINCENT T.K. CHOW • Human Genome Laboratory, Department of Microbiology, Infectious Diseases Program, Yong Loo Lin School of Medicine, National University Health System, National University of Singapore, Kent Ridge, Singapore
LANE K. CHRISTENSON • Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, USA
KRIS DE CLERCOQ • Department of Virology, Veterinary Agrochemical Research Center, Ukkel, Belgium
ADRIAN M. DI BISCEGLIE • Division of Gastroenterology and Hepatology, Department of Internal Medicine, Saint Louis University Liver Center, Saint Louis University School of Medicine, St. Louis, MO, USA
MICHAEL E. DONALDSON • Environmental & Life Sciences Graduate Program, Trent University, Peterborough, ON, Canada
HONGXIN FAN • Molecular Diagnostics Laboratory, Department of Pathology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA
XIAOFENG FAN • Division of Gastroenterology and Hepatology, Department of Internal Medicine, Saint Louis University Liver Center, Saint Louis University School of Medicine, St. Louis, MO, USA
STEPHANIE D. FIEDLER • Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, USA
MAURO GIACCA • Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy
ANA SILVIA GONZÁLEZ-REICHE • Centro de Estudios en Salud, Centers for Disease Control and Prevention, Universidad del Valle de Guatemala, Regional Office for Central America and Panama, Guatemala City, Guatemala
Thomas Haaf • Institute of Human Genetics, Johannes Gutenberg University, Mainz, Germany
Fadia Haddad • Physiology and Biophysics Department, University of California Irvine, Irvine, CA, USA
Cristina Hartshorn • Department of Biology, Brandeis University, Waltham, MA, USA
Hans G. Heine • CSIRO Livestock Industries, Australian Animal Health Laboratory (AAHL), Geelong, VIC, Australia
Eric C.H. Ho • Department of Medical Biophysics, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, ON, Canada
Barbara W. Johnson • Diagnostic & Reference Laboratory, Arbovirus Diseases Branch, Division of Vector-Borne Infectious Diseases (DVBID), Centers for Disease Control and Prevention (CDC), Fort Collins, CO, USA
Tamás Karosi • Department of Otolaryngology Head and Neck Surgery, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary
Nicola King • School of Science and Technology, University of New England, Armidale, NSW, Australia
József Kónya • Department of Medical Microbiology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary
Richard Lai • Biochip Innovations Pty Ltd., 8 Mile Plains, Brisbane, QLD, Australia; School of Chemistry & Molecular Biosciences, The University of Queensland, St. Lucia, QLD, Australia
Tony Le • Department of Research and Development, TriLink BioTechnologies, Inc., San Diego, CA, USA
Leong Wai Fook • Institute of Molecular and Cell Biology, Proteos, Singapore
Kelvin Li • J. Craig Venter Institute, Rockville, MD, USA
Wolfgang Mann • Olympus Life Science Research Europe, München, Germany
Janelle P. Mollica • Department of Zoology, LaTrobe University, Bundoora, VIC, Australia
María de Lourdes Monzón-Pineda • Centro de Estudios en Salud, Centers for Disease Control and Prevention, Universidad del Valle de Guatemala, Regional Office for Central America and Panama, Guatemala City, Guatemala
María Eugenia Morales-Betoulle • Centro de Estudios en Salud, Centers for Disease Control and Prevention, Universidad del Valle de Guatemala, Regional Office for Central America and Panama, Guatemala City, Guatemala
Jens Müller • Institute for Experimental Haematology and Transfusion Medicine, University of Bonn, Bonn, Germany
Melissa Oliveira-Cunha • Hepatobiliary Surgery Unit, Manchester Royal Infirmary, Manchester, UK
Natasha Paul • Department of Research and Development, TriLink BioTechnologies, Inc., San Diego, CA, USA
Sandy Pineda • Biochip Innovations Pty Ltd., 8 Mile Plains, Brisbane, QLD, Australia
Fiza Rashid-Doubell • School of Applied Sciences, University of Northumbria, Newcastle upon Tyne, UK
RYAN S. ROBETORYE • Molecular Diagnostics Laboratory, Department of Pathology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA
BARRY J. SAVILLE • Forensic Science Program and Environmental & Life Sciences Graduate Program, Trent University, Peterborough, ON, Canada
JONATHAN SHUM • Department of Research and Development, TriLink BioTechnologies, Inc., San Diego, CA, USA
AJITH K. SIRIWARDENA • Hepatobiliary Surgery Unit, Manchester Royal Infirmary, Manchester, UK
ANITA SZALMÁS • Department of Medical Microbiology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary
ISTVÁN SZIKLÁI • Department of Otolaryngology Head and Neck Surgery, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary
ELISE VANDEMEULEBROUCKE • Department of Virology, Veterinary Agrochemical Research Center, Ukkel, Belgium
FRANK VANDENBUSSCHE • Department of Virology, Veterinary Agrochemical Research Center, Ukkel, Belgium
LAWRENCE J. WANGH • Departments of Biology and Molecular Diagnostics, Brandeis University, Waltham, MA, USA
LORENA ZENTILIN • Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy