Genomics Protocols

# METHODS IN MOLECULAR BIOLOGY<sup>TM</sup>

#### John Walker, SERIES EDITOR

- 439. Genomics Protocols: Second Edition, edited by Mike Starkey and Ramnath Elaswarapu, 2008
- 438. Neural Stem Cells: Methods and Protocols, Second Edition, edited by Leslie P. Weiner, 2008
- 437. Drug Delivery Systems, edited by *Kewal K. Jain, 2008*
- 436. Avian Influenza Virus, edited by Erica Spackman, 2008
- 435. Chromosomal Mutagenesis, edited by Greg Davis and Kevin J. Kayser, 2008
- 434. Gene Therapy Protocols: Volume 2: Design and Characterization of Gene Transfer Vectors, edited by Joseph M. LeDoux, 2008
- 433. Gene Therapy Protocols: Volume 1: Production and In Vivo Applications of Gene Transfer Vectors, edited by Joseph M. LeDoux, 2008
- 432. **Organelle Proteomics**, edited by *Delphine Pflieger and Jean Rossier*, 2008
- 431. Bacterial Pathogenesis: Methods and Protocols, edited by Frank DeLeo and Michael Otto, 2008
- 430. Hematopoietic Stem Cell Protocols, edited by *Kevin D. Bunting*, 2008
- 429. Molecular Beacons: Signalling Nucleic Acid Probes, Methods and Protocols, edited by Andreas Marx and Oliver Seitz, 2008
- 428. Clinical Proteomics: Methods and Protocols, edited by Antonio Vlahou, 2008
- 427. Plant Embryogenesis, edited by Maria Fernanda Suarez and Peter Bozhkov, 2008
- 426. Structural Proteomics: High-Throughput Methods, edited by Bostjan Kobe, Mitchell Guss, and Huber Thomas, 2008

- 425. **2D PAGE:** Sample Preparation and Fractionation, Volume 2, edited by *Anton Posch, 2008*
- 424. **2D PAGE:** Sample Preparation and Fractionation, Volume 1, edited by *Anton Posch, 2008*
- 423. Electroporation Protocols: Preclinical and Clinical Gene Medicine, edited by *Shulin Li, 2008*
- 422. **Phylogenomics**, edited by *William J. Murphy*, 2008
- 421. Affinity Chromatography: Methods and Protocols, Second Edition, edited by *Michael Zachariou*, 2008
- 420. Drosophila: Methods and Protocols, edited by Christian Dahmann, 2008
- 419. Post-Transcriptional Gene Regulation, edited by *Jeffrey Wilusz*, 2008
- 418. Avidin–Biotin Interactions: Methods and Applications, edited by *Robert J. McMahon*, 2008
- 417. **Tissue Engineering**, Second Edition, edited by *Hannsjörg Hauser and Martin Fussenegger*, 2007
- 416. Gene Essentiality: Protocols and Bioinformatics, edited by Svetlana Gerdes and Andrei L. Osterman, 2008
- 415. **Innate Immunity**, edited by Jonathan Ewbank and Eric Vivier, 2007
- 414. Apoptosis in Cancer: Methods and Protocols, edited by *Gil Mor and Ayesha Alvero, 2008*
- 413. Protein Structure Prediction, Second Edition, edited by *Mohammed Zaki* and Chris Bystroff, 2008
- 412. Neutrophil Methods and Protocols, edited by Mark T. Quinn, Frank R. DeLeo, and Gary M. Bokoch, 2007
- 411. **Reporter Genes: A Practical Guide**, edited by *Don Anson, 2007*

# **Genomics Protocols**

Second Edition

Edited by

Mike Starkey Animal Health Trust, Lanwades Park, Newmarket, Suffolk, United Kingdom

Ramnath Elaswarapu Oxford Gene Technology Ltd., Oxford, United Kingdom



*Editors* Mike Starkey Animal Health Trust Lanwades Park, Newmarket Suffolk, United Kingdom

Series Editor John M. Walker University of Hertfordshire Hatfield, Herts United Kingdom Ramnath Elaswarapu Oxford Gene Technology Ltd. Oxford, United Kingdom

ISBN: 978-1-58829-871-3

e-ISBN: 978-1-59745-188-8

Library of Congress Control Number: 2007938729

© 2008 Humana Press, a part of Springer Science+Business Media, LLC

All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (Humana Press, 999 Riverview Drive, Suite 208, Totowa, NJ 07512 USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden.

The use in this publication of trade names, trademarks, service marks, and similar terms, even if they are not identified as such, is not to be taken as an expression of opinion as to whether or not they are subject to proprietary rights.

Printed on acid-free paper

9 8 7 6 5 4 3 2 1

springer.com

#### Preface

The genomics research world has moved on since the first volume of *Genomics Protocols* in the Methods in Molecular Biology series was published in 2001. This progression is reflected in the themes of the chapters that adorn the current volume. It is true that there is the same apparently eclectic mixture of subjects as in the former volume. However, undeniably, the emphasis has switched from gene identification to functional genomics and the characterization of genes and gene products.

Although essentially a volume of "wet lab" techniques, in silico approaches also are represented through three chapters addressing the detection of genome sequence variation and the prediction of gene function, respectively.

We make no apologies for including texts that may appear in volumes devoted to other of the "omics." Rather, we feel that the majority of these relative newcomers are derivatives of genomics, and from the perspective of being able to see "the bigger picture," a great value is to be gained from considering these research disciplines collectively.

For some of the specialist techniques, it is difficult to avoid the use of a specific/ particular commercial platform or piece of equipment. For these procedures, the authors have focused on the generic issues (e.g., data analysis), which are all too often given inadequate coverage in user manuals. While not wishing to advocate particular systems, by including these topics we acknowledge the importance, and possibly the uniqueness, of the technologies involved.

Through the choice of some of the topics, we tried to remember that not everyone is engaged in investigation of organisms for which an annotated sequenced genome or a high-density genetic map is available. Similarly, lest we forget that not all researchers are affiliated to well-equipped laboratories, Chapter 5 describes a relatively inexpensive and low-tech approach to genetic mapping using microsatellites.

To our minds, the jewel in the crown of the Methods in Molecular Biology series remains the "Notes" section of each chapter. In spite of the complexity of some of the subject matter, the authors have endeavored to ensure that as little technical detail as possible is left to either the reader's imagination or to the extensivity of their organization's library. The first chapter addresses an issue that all too often is a prerequisite for comprehensive genome analysis: high fidelity whole genome amplification. Chapters 2–4 focus on a spectrum of procedures that are a prelude to genetic linkage analysis (Chapter 5).

The molecular profiling of genomic DNA in a variety of guises is encompassed in Chapters 6–9. Chapters 10–12 are devoted to transcriptional profiling, and focusing on small samples and the use of formalin-fixed archival tissue, deal with two of the thornier issues confronting researchers. The profiling of microRNAs (Chapter 12) is an exciting area of interest in the study of the regulation of gene expression in disease.

The line between proteomics and functional genomics can be a little fuzzy, but Chapters 12–17 describe alternative strategies for protein profiling, while Chapters 18–26 arguably constitute approaches for gene characterization and the identification of protein interaction networks. The volume concludes with a detailed tutorial (Chapter 27) about an exciting genomics technology useful in the elucidation of gene function and with huge potential therapeutic applications.

So there you have it. All that remains is for us to thank a group of redoubtable authors, the series editor, John Walker, and all those involved at Humana Press. We truly hope that you find the book informative and a valuable addition to your laboratory bookshelf.

Mike Starkey Ramnath Elaswarapu

## Contents

Preface		V
Contributo	rs	xi
Chapter 1	Whole Genome Amplification with Phi29 DNA Polymerase to Enable Genetic or Genomic Analysis of Samples of Low DNA Yield Kaisa Silander and Janna Saarela	1
Chapter 2	Scanning for DNA Variants by Denaturant Capillary Electrophoresis Per O. Ekstrøm	19
Chapter 3	Identification of SNPs, or Mutations in Sequence Chromatograms Nicole Draper	35
Chapter 4	BeadArray-Based Genotyping	53
Chapter 5	Microsatellite-Based Candidate Gene Linkage Analysis Studies Cathryn Mellersh	75
Chapter 6	Full Complexity Genomic Hybridization on 60-merOligonucleotide Microarrays for Array ComparativeGenomic Hybridization (aCGH)Alexei Protopopov, Bin Feng, and Lynda Chin	87
Chapter 7	<b>Detection of Copy Number Changes at Multiple Loci in DNA</b> <b>Prepared from Formalin-Fixed, Paraffin-Embedded Tissue</b> <b>by Multiplex Ligation-Dependent Probe Amplification</b> Minoru Takata	101

Chapter 8	Application of Microarrays for DNAMethylation ProfilingAxel Schumacher, Andreas Weinhäusl, and Arturas Petronis	109
Chapter 9	Genomewide Identification of Protein Binding Locations Using Chromatin Immunoprecipitation Coupled with Microarray Byung-Kwan Cho, Eric M. Knight, and Bernhard Ø. Palsson	131
Chapter 10	Transcriptional Profiling of Small Samples in the Central Nervous System Stephen D. Ginsberg	147
Chapter 11	Quantitative Expression Profiling of RNA from Formalin-Fixed, Paraffin-Embedded Tissues Using Randomly Assembled Bead Arrays Marina Bibikova, Joanne M. Yeakley, Jessica Wang-Rodriguez, and Jian-Bing Fan	159
Chapter 12	Expression Profiling of microRNAs in Cancer Cells: Technical Considerations Mouldy Sioud and Lina Cekaite	179
Chapter 13	Identification of Disease Biomarkers by Profiling of Serum Proteins Using SELDI-TOF Mass Spectrometry Sigrun Langbein	191
Chapter 14	The Applicability of a Cluster of Differentiation Monoclonal Antibody Microarray to the Diagnosis of Human Disease Peter Ellmark, Adrian Woolfson, Larissa Belov, and Richard I. Christopherson	199
Chapter 15	Protein Profiling Based on Two-Dimensional Difference Gel Electrophoresis Gert Van den Bergh and Lutgarde Arckens	211
Chapter 16	Quantitative Protein Profiling by Mass Spectrometry Using Isotope-Coded Affinity Tags Arsalan S. Haqqani, John F. Kelly, and Danica B. Stanimirovic	225
Chapter 17	Quantitative Protein Profiling by Mass Spectrometry Using Label-Free Proteomics Arsalan S. Haqqani, John F. Kelly, and Danica B. Stanimirovic	241

Contents
----------

Chapter 18	Peptides in Extracts from an Infected Mouse	
	John F. Kelly and Wen Ding	25
Chapter 19	Baculovirus Expression Vector System:An Emerging Host for High-Throughput EukaryoticProtein ExpressionBinesh Shrestha, Carol Smee, and Opher Gileadi	26
Chapter 20	Coimmunoprecipitation and Proteomic Analyses S. Fabio Falsone, Bernd Gesslbauer, and Andreas J. Kungl	29
Chapter 21	Tandem Affinity Purification Combined with Mass Spectrometry to Identify Components of Protein ComplexesOf Protein ComplexesPeter Kaiser, David Meierhofer, Xiaorong Wang, and Lan Huang	309
Chapter 22	Mammalian Two-Hybrid Assay for Detecting Protein-Protein Interactions in vivo Runtao He and Xuguang Li	327
Chapter 23	<b>Detection of Protein-Protein Interactions in Live</b> <b>Cells and Animals with Split Firefly Luciferase</b> <b>Protein Fragment Complementation</b> Victor Villalobos, Snehal Naik, and David Piwnica-Worms	33
Chapter 24	Subcellular Localization of Intracellular Human Proteins by Construction of Tagged Fusion Proteins and Transient Expression in COS-7 Cells John E. Collins	353
Chapter 25	GeneFAS: A Tool for the Prediction of Gene Function Using Multiple Sources of Data Trupti Joshi, Chao Zhang, Guan Ning Lin, Zhao Song, and Dong Xu	369
Chapter 26	Comparative Genomics-Based Prediction of Protein Function Toni Gabaldón	38
Chapter 27	Design, Manufacture, and Assay of the Efficacy of siRNAs for Gene Silencing Louise A. Dawson and Badar A. Usmani	40.
Index		42

### Contributors

Lutgarde Arckens Laboratory of Neuroplasticity and Neuroproteomics, Katholieke Universiteit Leuven, Leuven, Belgium

Larissa Belov Medsaic Pty Ltd, National Innovation Centre, Eveleigh, Australia

Marina Bibikova Illumina Inc., San Diego, CA

Helen Butler Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom

Lina Cekaite Department of Tumor Biology, Institute for Cancer Research, Rikshopitalet– Radiumhospitalet Medical Center, Montebello, Oslo, Norway

Lynda Chin Center for Applied Cancer Science, Belfer Institute for Innovative Cancer Science, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

Byung-Kwan Cho Department of Bioengineering, University of California–San Diego, La Jolla, CA

Richard I. Christopherson School of Molecular and Microbial Biosciences, University of Sydney, Sydney, Australia

John E. Collins Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Louise A. Dawson Institute of Cellular and Molecular Biology, University of Leeds, Leeds, United Kingdom

Wen Ding Institute for Biological Sciences, National Research Council, Ottawa, Ontario, Canada Nicole Draper Oxford Gene Technology Ltd, Yarnton, Oxford, United Kingdom

Per O. Ekstrøm The Norwegian Radium Hospital, Oslo, Norway

Peter Ellmark Department of Immunotechnology, Lund University, Lund, Sweden

S. Fabio Falsone Institute of Pharmaceutical Sciences, University of Graz, Graz, Austria

Jian-Bing Fan Illumina Inc., San Diego, CA

Bin Feng Center for Applied Cancer Science, Belfer Institute for Innovative Cancer Science, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

Toni Gabaldón Bioinformatics Department, CIPF, Valencia, Spain

Bernd Gesslbauer Institute of Pharmaceutical Sciences, University of Graz, Graz, Austria

Opher Gileadi Structural Genomics Consortium, Botnar Research Centre, University of Oxford, Oxford, United Kingdom

Stephen D. Ginsberg Center for Dementia Research, Nathan Kline Institute, and Departments of Psychiatry and Physiology and Neuroscience, New York University School of Medicine, Orangeburg, NY

Arsalan S. Haqqani Institute for Biological Sciences, National Research Council, Ottawa, Ontario, Canada

Runtao He National Microbiology Laboratory, Public Health Agency of Canada Manitoba, Manitoba, Canada

Lan Huang Department of Physiology & Biophysics and Department of Developmental and Cell Biology, University of California Irvine, Irvine, CA

Trupti Joshi Digital Biology Laboratory, Computer Science Department and Christopher S. Bond Life Sciences Center, University of Missouri–Columbia, Columbia, MO

Peter Kaiser Department of Biological Chemistry, College of Medicine, University of California Irvine, Irvine, CA John F. Kelly Institute for Biological Sciences, National Research Council, Ottawa, Ontario, Canada

Eric M. Knight Department of Bioengineering, University of California–San Diego, La Jolla, CA

Andreas J. Kungl Institute of Pharmaceutical Sciences, University of Graz, Graz, Austria

Sigrun Langbein Department of Urology, Uro-Oncology, Academic Medic Centrum (AMC), University of Amsterdam, Amsterdam, The Netherlands

Xuguang Li Centre for Biologics Research, Biological and Genetic Therapies Directorate, Health Canada, Ottawa, Canada

Guan Ning Lin Digital Biology Laboratory, Computer Science Department, Christopher S. Bond Life Sciences Center, University of Missouri–Columbia, Columbia, MO

David Meierhofer Department of Biological Chemistry, College of Medicine, University of California Irvine, Irvine, CA

Cathryn Mellersh Animal Health Trust, Suffolk, United Kingdom

Snehal Naik Molecular Imaging Center, Mallinckrodt Institute of Radiology, and Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO

Bernhard Ø. Palsson Department of Bioengineering, University of California–San Diego, La Jolla, CA

Arturas Petronis The Krembil Family Epigenetics Laboratory, Centre for Addiction and Mental Health, Toronto, Ontario, Canada

David Piwnica-Worms Molecular Imaging Center, Mallinckrodt Institute of Radiology, and Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO

Alexei Protopopov Center for Applied Cancer Science, Belfer Institute for Innovative Cancer Science, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA **Jiannis Ragoussis** Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom Janna Saarela National Public Health Institute, Helsinki, Finland Axel Schumacher Epigenetics Lab, Department of Medicine II, Klinikum rechts der Isar, Technical University Munich, Munich, Germany **Binesh Shrestha** Structural Genomics Consortium, Botnar Research Centre, University of Oxford, Oxford, United Kingdom Kaisa Silander National Public Health Institute, Helsinki, Finland Mouldy Sioud Department of Immunology, Institute for Cancer Research, Rikshopitalet -Radiumhospitalet Medical Center, Montebello, Oslo, Norway Carol Smee Structural Genomics Consortium, Botnar Research Centre, University of Oxford, Oxford, United Kingdom Zhao Song Digital Biology Laboratory, Computer Science Department, Christopher S. Bond Life Sciences Center, University of Missouri-Columbia, Columbia, MO Danica B. Stanimirovic Institute for Biological Sciences, National Research Council, Ottawa, Ontario, Canada Minoru Takata Shinshu University School of Medicine, Matsumoto, Japan Badar A. Usmani Institute of Cellular and Molecular Biology, University of Leeds, Leeds, United Kingdom Gert Van den Bergh Laboratory of Neuroplasticity and Neuroproteomics, Katholieke Universiteit Leuven, Leuven, Belgium Victor Villalobos Molecular Imaging Center, Mallinckrodt Institute of Radiology, and Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO

#### Contributors

Xiaorong Wang Department of Physiology and Biophysics and Department of Developmental and Cell Biology, University of California Irvine, Irvine, CA

Jessica Wang-Rodriguez University of California, San Diego, Department of Pathology and the Veterans Administration San Diego Healthcare System, San Diego, CA

Andreas Weinhäusl ARCS, Austrian Research Center, Seibersdorf, Austria

Adrian Woolfson University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Cambridge, United Kingdom

Dong Xu Digital Biology Laboratory, Computer Science Department, Christopher S. Bond Life Sciences Center, University of Missouri–Columbia, Columbia, MO

Joanne M. Yeakley Illumina, Inc., San Diego, CA

Chao Zhang Digital Biology Laboratory, Computer Science Department, Christopher S. Bond Life Sciences Center, University of Missouri–Columbia, Columbia, MO