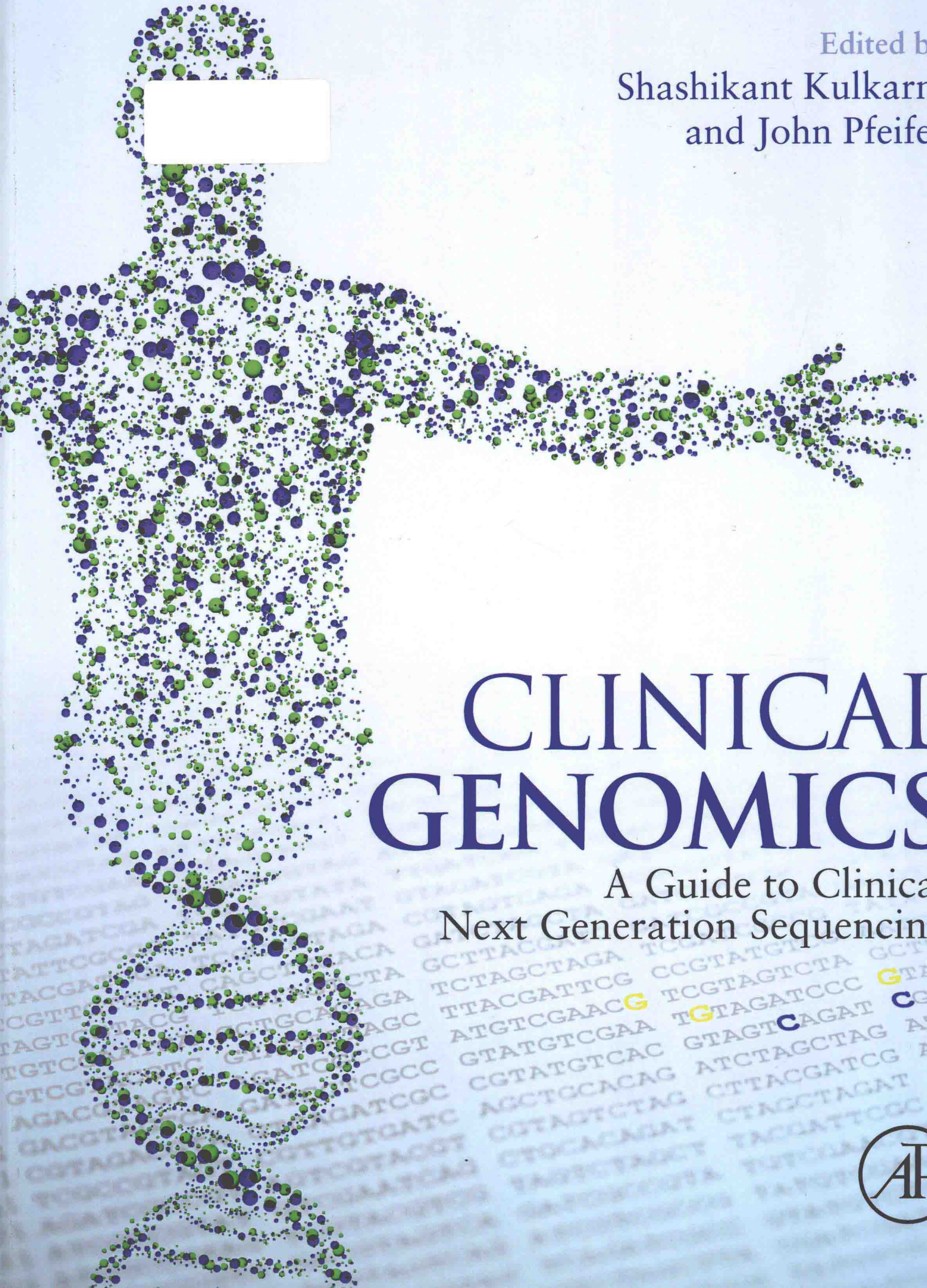

Edited by
Shashikant Kulkarni
and John Pfeifer

CLINICAL GENOMICS

A Guide to Clinical Next Generation Sequencing

CLINICAL GENOMICS

Edited by

SHASHIKANT KULKARNI M.S (MEDICINE)., PH.D, FACMG

Washington University School of Medicine, St. Louis, MO, USA

JOHN PFEIFER M.D, PH.D

Washington University School of Medicine, St. Louis, MO, USA

ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
32 Jamestown Road, London NW1 7BY, UK
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA
225 Wyman Street, Waltham, MA 02451, USA
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK

Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

ISBN: 978-0-12-404748-8

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress.

Typeset by MPS Limited, Chennai, India
www.adi-mps.com

CLINICAL GENOMICS

Dedication

This book is dedicated

With honor to our patients and their loved ones; my family (Shamika and Sonya-BGOW); my teachers and my parents —SK

To Jennifer, who has made it all worthwhile —JDP

List of Contributors

Haley Abel Division of Statistical Genetics, Washington University School of Medicine, St. Louis, MO, USA

Shankar Ajay Illumina Clinical Services Laboratory, San Diego, CA, USA

Hussam Al-Kateb Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA

Sami S. Amr Department of Pathology, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, USA; Laboratory for Molecular Medicine, Partners Healthcare Personalized Medicine, Cambridge, MA, USA

Andrew J. Bredemeyer Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA

Fengqi Chang Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

Elizabeth C. Chastain Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA

Deanna M. Church Personalis, Inc., Menlo Park, CA, USA

Paul Cliften Department of Genetics, Washington University School of Medicine, St. Louis, MO, USA

Catherine E. Cottrell Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA

Andrew Drury Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA

Eric Duncavage Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA

Birgit Funke Department of Pathology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA; Laboratory for Molecular Medicine, Partners Healthcare Personalized Medicine, Cambridge, MA, USA

Amy S. Gargis Division of Preparedness and Emerging Infections, Laboratory Preparedness and Response Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Ian S. Hagemann Departments of Pathology and Immunology and of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO, USA

Tina M. Hambuch Illumina Clinical Services Laboratory, San Diego, CA, USA

Madhuri R. Hegde Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA

Michelle Hogue Illumina Clinical Services Laboratory, San Diego, CA, USA

Vanessa L. Horner Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA

Lisa Kalman Division of Laboratory Programs, Services, and Standards, Centers for Disease Control and Prevention, Atlanta, GA, USA

Shamika Ketkar Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA

Roger D. Klein Department of Molecular Pathology, Robert J. Tomsich Pathology and Laboratory Medicine Institute, Cleveland Clinic, Cleveland, OH, USA

Shashikant Kulkarni Department of Pathology and Immunology, Department of Pediatrics, and Department of Genetics, Washington University School of Medicine, St. Louis, MO, USA

William A. LaFramboise Genomics Division of the Cancer Biomarker Facility, Shadyside Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Marilyn M. Li Dan Duncan Cancer Center, Baylor College of Medicine, Houston, TX, USA; Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

Cindy J. Liu Research and Computing Services, Harvard Business School, Cambridge, MA, USA

Geoffrey L. Liu Department of Human Genetics, University of Chicago, Chicago, IL, USA

Ira M. Lubin Division of Laboratory Programs, Services, and Standards, Centers for Disease Control and Prevention, Atlanta, GA, USA

Elaine Lyon ARUP Laboratories, Salt Lake City, UT, USA; Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, USA

Donna R. Maglott National Center for Biotechnology Information/National Library of Medicine/National Institutes of Health, Bethesda, MD, USA

Rong Mao ARUP Laboratories, Salt Lake City, UT, USA; Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, USA

John Mayfield Illumina Clinical Services Laboratory, San Diego, CA, USA

Carri-Lyn Mead Illumina Clinical Services Laboratory, San Diego, CA, USA

Rakesh Nagarajan Department of Pathology and Immunology, and Department of Genetics, Washington University School of Medicine, St. Louis, MO, USA

Yuri E. Nikiforov Division of Molecular and Genomic Pathology, Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Marina N. Nikiforova Division of Molecular and Genomic Pathology, Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Alex Nord Center for Neuroscience, Departments of Neurobiology, Physiology and Behavior and Psychiatry, University of California at Davis, CA, USA

Brendan D. O'Fallon ARUP Laboratories, Salt Lake City, UT, USA

John Pfeifer Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA

Colin Pritchard Department of Laboratory Medicine, University of Washington, Seattle, WA, USA

Erica Ramos Illumina Clinical Services Laboratory, San Diego, CA, USA

Kris Rickhoff Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA

Kristina A. Roberts ARUP Laboratories, Salt Lake City, UT, USA; Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, USA

Wendy S. Rubinstein National Center for Biotechnology Information/National Library of Medicine/National Institutes of Health, Bethesda, MD, USA

Stephen J. Salipante Department of Laboratory Medicine, University of Washington, Seattle, WA, USA

Marc Salit Biosystems and Biomaterials Division, National Institute of Standards and Technology, Gaithersburg, MD, USA

Jennifer K. Sehn Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA

Benjamin D. Solomon Medical Genetics Branch, National Human Genome Research Institute/National Institutes of Health, Bethesda, MD, USA

Stephanie Solomon Albert Gnaegi Center for Health Care Ethics, Saint Louis University, Salus Center, St. Louis, MO, USA

David H. Spencer Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA

Bin Zhang Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA

Justin Zook Biosystems and Biomaterials Division, National Institute of Standards and Technology, Gaithersburg, MD, USA

Foreword

Genomics is a young scientific discipline, surprisingly so considering the rapid prominence that it has gained across nearly the entire landscape of biological and biomedical research. In fact, the word "genomics" was first described in the scientific literature in 1987 [1]. That year was personally significant for me—I graduated medical and graduate school in 1987 and started my residency in laboratory medicine (clinical pathology). Later that same year, I also made the decision to shift my area of research from cell biology/biochemistry (which had encompassed my undergraduate and graduate research efforts) to this nascent, heavily discussed area involving the comprehensive study of genomes—a.k.a., genomics.

At that time, the raging debate in the biomedical research circles focused on the proposal for a big "genome project." Some argued that it was simply a bad idea; a subset of those also claimed that it would be a "career dead end" for young trainees who foolishly joined in the effort. Fortunately, I ignored both of those views and went "all in"— dedicating the research component of my pathology training to genomics and becoming a front line participant of the Human Genome Project (both at its launch and throughout its 13 years). While admittedly vague, at that time I could envision productive connections between enhanced knowledge of the human "genomic blueprint" and the diagnostic work of pathologists. My earliest training in clinical pathology (combined with a deep appreciation for other areas of pathology) had quickly revealed the need for more robust tools to refine diagnoses and empower clinicians to acquire insights about the "uniqueness" of each patient. In short, too much of medical care was generic, lacking the fine-tuning needed for truly personalized care. I thought that genomics could possibly help to provide that fine-tuning. Let me emphasize—these were very nascent insights; I had no well-formulated ideas about timetable or implementation, and assumed that any sizable infusion of genomics into diagnostic medicine was many decades away. In hindsight, I should have been more brash!

Fast forward to 2014—slightly more than a quarter century later and a blink of an eye in the history of scientific inquiry. Since that time, we have sequenced the human genome—the first time as part of the Human Genome Project and then tens of thousands of times since then, as the cost of sequencing DNA plummeted roughly a million-fold with the development and implementation of powerful new technologies. These new capabilities catalyzed a flurry of key advances in understanding the genomic bases of specific disorders (most notably, rare diseases and cancer) and drug response, yielding convincing and prototypic examples in which genomic information has clinical utility. In aggregate, these early triumphs have helped to clarify the vague notion of "genomic medicine" and to illuminate a path forward for more widespread utilization of genomics in patient care. To make this vision a reality, genome sequencing will need to become a tool fully adapted for use in clinical settings—and that will require bringing together genomic technologies and clinical implementation.

However, bringing the genomic and clinical worlds together is not easy. To help make it happen, scholarly guides are needed. Kulkarni and Pfeifer have created such a resource in producing *Clinical Genomics*. For this book, an assembled group of key experts and opinion leaders wrote 26 chapters that collectively cover a wide swath of territory relevant to the use of genome sequencing for medical diagnostics. Their audience: clinical laboratory professionals and physicians (both established and in-training) seeking an overview of and guidance for adoption of genome sequencing as a clinical tool. The general areas covered include genome sequencing methods, data analysis, interpreting and reporting genomic information, genomic-based diagnostics for specific disorders, and ancillary (but critically important) topics related to regulation, reimbursement, and legal issues. It is particularly impressive that chapters covering ethical and legal challenges associated with clinical genomics were included. In addition to providing key technical details for diagnostic-based genome sequencing, *Clinical Genomics* effectively converts the knowledge of human genomics gained in basic science research settings into factual, practice-based information to facilitate the use of genome sequencing in clinical settings.

In summary, Kulkarni, Pfeifer, and their recruited authors aimed to compile a first-rate book that would benefit interested physicians, pathologists, and other healthcare professionals wishing to learn about the opportunities and challenges of using genome sequencing for the diagnosis, prognosis, and management of inherited and somatic disorders—something key for realizing a future of genomic medicine. As evident from the pages that follow, that goal has been clearly reached. Readers of *Clinical Genomics* will gain important insights about the future

of medicine, a future in which genomic information increasingly becomes a mainstream component of diagnostics and clinical care. I applaud Kulkarni and Pfeifer for helping to make genomic medicine a reality!

Eric Green, MD, PhD
Director, National Human Genome Research Institute (NHGRI)

Reference

[1] McKusick VA, Ruddle FH. Genomics 1987;1:1–2.

Eric Green received his graduate and postgraduate training at Washington University in St. Louis, earning an MD-PhD in 1987 and then pursuing clinical training (in clinical pathology) and postdoctoral research training (in genomics) until 1992. Following 2 years as an assistant professor at Washington University, Dr. Green joined the National Human Genome Research Institute of the National Institutes of Health, where he has been for just over 20 years. During that time, he has assumed multiple leadership positions, being appointed the Institute's Director in 2009.

Preface

In 2011, next generation sequencing (NGS)-based clinical diagnostic testing was implemented for precision medicine at Washington University School of Medicine, St. Louis by Genomics and Pathology Services (GPS). Even before GPS began accepting patient specimens for testing of a set of genes that provided information to direct the clinical care of oncology patients, it was evident that there was a lack of real-world educational resources for healthcare providers who recognized the need to incorporate NGS-based tests into their clinical practice. There was the need for a textbook specifically devoted to the practice-based issues that are unique to NGS on technical, bioinformatic, interpretive, ethical, and regulatory levels. And as the portfolio of tests offered by GPS has grown, as the volume of testing has increased, and as a wider variety of physicians and trainees have incorporated NGS-based tests into their clinical practice, the need for a textbook focused on clinical genomics has become more and more obvious.

This book was produced in order to meet that need. The chapters are authored by nationally recognized experts (with practical experience in that they are associated with clinical laboratories that are actively performing clinical NGS-based testing for either constitutional/hereditary diseases or somatic/acquired diseases such as cancer). The topics covered include the technical details of the platforms and chemistries used to perform NGS; the conceptual underpinnings of assay design for testing gene panels, exomes, or whole genomes; the bioinformatics pipelines required to identify and annotate sequence variants; the clinical settings in which NGS can contribute to patient care; the ethical and regulatory issues surrounding NGS testing; and the reimbursement issues that govern payment for testing. The focus throughout is on the advantages and disadvantages; capabilities and limitations; and clinical settings for which genomic methods can be used clinically to enhance patient care, and the key elements that must be considered in the design, validation, and implementation of NGS-based tests for this purpose.

The book is not a laboratory manual or a compilation of laboratory protocols, for two reasons. First, generic protocols are of little use since many clinical NGS labs develop customized tests based on gene panels, the exome, or the whole genome as required to meet the clinical needs of their patient population. Second, given the rapid pace of change in NGS platforms, bioinformatics pipelines, and relevant genetic loci, any set of laboratory protocols would be hopelessly out of date before it ever appeared in print!

The book should be useful to a broad medical audience, including medical directors, pathologists, and geneticists who are responsible for designing and implementing NGS-based tests; oncologists, pediatricians, geneticists, and other clinicians who order genetic tests as part of the care of their patients whether for diagnosis or prediction of therapeutic response; laboratory personnel who perform the hands-on component of the testing; and trainees (whether medical students, residents, or fellows). The book should also prove useful to basic science and translational researchers who are interested in the clinical application of NGS in order to guide the research and development activities within their laboratories. And, since the book covers the bioethical, legal, and regulatory issues related to NGS, it can also serve as a textbook for undergraduate and graduate level courses focused on the broader topic of clinical genomics.

Acknowledgments

First and foremost, we thank our colleagues who contributed their time to write the chapters that comprise the work; the book would not have been possible without them. They are all extremely busy people, and their willingness to participate in this project is a sign of their commitment to share their expertise regarding the opportunities provided by next generation sequencing techniques to enhance patient care. We are fortunate to count them as not only colleagues but also friends, and have greatly benefited from their expert advice.

In addition, we want to thank the leadership team at Genomics and Pathology Services (GPS) at Washington University School of Medicine in St. Louis. We especially want to acknowledge Dr. Karen Seibert, Director of GPS; Dr. Cathy Cottrell, Medical Director; Dr. Andrew Bredemeyer, Chief Operations Officer, and Dr. John Heusel, Chief Medical Officer, and thank them for their availability and willingness to assist us with this project. They have been tireless in their efforts to ensure that NGS-based methods can become part of the clinical testing performed to improve patient care. And we want to acknowledge our colleagues on the GPS team, including Drs. Eric Duncavage, Haley Abel, David Spencer, Ian Hageman, Hussam Al-Kateb, Ian Hagemann, Tu Nguyen, Robi Mitra, Rakesh Nagarajan, Richard Head, and Paul Clifton, all of whom generously supported the effort.

We want to express our sincere gratitude to our hardworking colleagues and staff at the Genome Technology Access Center (GTAC), Center for Biomedical Informatics (CBMI), GPS and Cytogenomics and Molecular Pathology for providing valuable direct and indirect support of the book as well.

We also want to acknowledge Dr. Herbert "Skip" Virgin, Chairman of the Department of Pathology and Immunology, and Dr. Jeffrey Milbrandt, Chairman of the Department of Genetics, both at Washington University School of Medicine, for their support of the development and operation of GPS. They have provided a model of commitment to patient care, academic productivity, and focus on resident and fellow education that has made it possible for a laboratory focused on clinical genomics to flourish.

We want to thank our assistants, specifically Elease Barnes and Amy Dodson, for their hard work typing and editing the various revisions of the chapters of this book, a task which they performed with endless patience and good humor.

We have also been extremely fortunate to interact with a great group of people at Academic Press and Elsevier. Graham Nisbet, Senior Acquisitions Editor, was receptive to our idea for a book focused on clinical genomics using next generation sequencing technologies, and helped launch the project. Catherine Van Der Laan, Associate Acquisitions Editor, provided constant guidance and support; there is no doubt that her encouragement and strict attention to deadlines were absolutely essential for bringing the book to completion.

Most importantly, we also owe a debt of gratitude to our families for their love, sustained encouragement, support, and patience during the long hours we spent writing and editing the book.

Shashikant Kulkarni and John Pfeifer,
St. Louis, 2014

Contents

II BIOINFORMATICS

III INTERPRETATION