

# METHODS IN MOLECULAR BIOLOGY

*Series Editor*

**John M. Walker**

**School of Life and Medical Sciences**

**University of Hertfordshire**

**Hatfield, Hertfordshire, AL10 9AB, UK**

For further volumes:

<http://www.springer.com/series/7651>



# PCR Primer Design

**Second Edition**

Edited by

**Chhandak Basu**

*Department of Biology, California State University, Northridge, Los Angeles, CA, USA*

 **Humana Press**

*Editor*

Chhandak Basu  
Department of Biology  
California State University  
Northridge  
Los Angeles, CA, USA

ISSN 1064-3745                      ISSN 1940-6029 (electronic)  
Methods in Molecular Biology  
ISBN 978-1-4939-2364-9              ISBN 978-1-4939-2365-6 (eBook)  
DOI 10.1007/978-1-4939-2365-6

Library of Congress Control Number: 2015931213

Springer New York Heidelberg Dordrecht London  
© Springer Science+Business Media New York 2015

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

Humana Press is a brand of Springer  
Springer Science+Business Media LLC New York is part of Springer Science+Business Media ([www.springer.com](http://www.springer.com))

*In memory of my brother Professor Saugata Basu*



---

## Preface

The field of molecular biology and biotechnology has revolutionized since the Nobel Prize winning (1993) work of Dr. Kary Banks Mullis. Dr. Mullis first discovered how to synthesize large amount of DNA from infinitesimal small amount of DNA, and the process is known as Polymerase Chain Reaction (PCR). It is now possible for us to make millions of copies of DNA from miniscule amount of starting DNA within a period of few hours. PCR has been widely used in agriculture, medicine, forensics, paternity testing, molecular ecology, biotechnology etc. and the list will continue to grow. The use of PCR in modern day science is impressive, and it is evident with more than half a million hits in the PubMed Central database with a simple keyword search: “PCR.” The tiny PCR tube contains a variety of ingredients including magnesium chloride, dNTPs, the interesting thermostable enzyme *Taq* polymerase (originally isolated from thermophilic bacterium *Thermus aquaticus*), and last but not least the oligonucleotides. The oligonucleotides, also known as primers, play a very important role in successful amplification of a segment of DNA. In other words, a poor primer design may result in less than desirable PCR product. This book specifically focuses on how to design PCR primers for successful DNA amplification. There are 15 chapters in this book, and the chapters cover wide ranges of topics in PCR primer design including primer design strategies for quantitative PCR, for use in forensic biology, for genotyping, for degenerate PCR, for multiplex PCR etc. Besides these, there are also chapters that focus on in silico PCR primer design and primer design using software available over the Internet. This book was a true international effort and scientists from USA, India, Estonia, Spain, Japan, China, Czech Republic, and Brazil contributed to this book. We hope this book will be useful to molecular biology students, researchers, professors, and PCR enthusiasts.

*Los Angeles, CA, USA*

*Chhandak Basu*





---

## Contents

<i>Preface</i> . . . . .	<i>vii</i>
<i>Contributors</i> . . . . .	<i>xi</i>
1 Fast Masking of Repeated Primer Binding Sites in Eukaryotic Genomes. . . . . <i>Reidar Andreson, Lauris Kaplinski, and Maido Remm</i>	1
2 Primer Design for PCR Reactions in Forensic Biology. . . . . <i>Kelly M. Elkins</i>	17
3 Design of Primers and Probes for Quantitative Real-Time PCR Methods . . . . . <i>Alicia Rodríguez, Mar Rodríguez, Juan J. Córdoba, and María J. Andrade</i>	31
4 Large-Scale Nucleotide Sequence Alignment and Sequence Variability Assessment to Identify the Evolutionarily Highly Conserved Regions for Universal Screening PCR Assay Design: An Example of Influenza A Virus . . . . . <i>Alexander Nagy, Tomáš Jiřinec, Lenka Černíková, Helena Jiřincová, and Martina Havlíčková</i>	57
5 Low-Concentration Initiator Primers Improve the Amplification of Gene Targets with High Sequence Variability. . . . . <i>Kenneth E. Pierce and Lawrence J. Wangh</i>	73
6 Multiplex PCR Primer Design for Simultaneous Detection of Multiple Pathogens. . . . . <i>Wenchao Yan</i>	91
7 Degenerate Primer Design for Highly Variable Genomes. . . . . <i>Kelvin Li, Susmita Shrivastava, and Timothy B. Stockwell</i>	103
8 Allele-Specific Real-Time Polymerase Chain Reaction as a Tool for Urate Transporter 1 Mutation Detection. . . . . <i>Juliet O. Makanga, Antonius Christianto, and Tetsuya Inazu</i>	117
9 MultiPLX: Automatic Grouping and Evaluation of PCR Primers. . . . . <i>Lauris Kaplinski and Maido Remm</i>	127
10 In Silico PCR Primer Designing and Validation. . . . . <i>Anil Kumar and Nikita Chordia</i>	143
11 Primer Design Using Primer Express® for SYBR Green-Based Quantitative PCR. . . . . <i>Amarjeet Singh and Girdhar K. Pandey</i>	153
12 Designing Primers for SNaPshot Technique. . . . . <i>Greiciane Gaburro Paneto and Francisco de Paula Careta</i>	165

13	Rapid and Simple Method of qPCR Primer Design . . . . .	173
	<i>Brenda Thornton and Chhandak Basu</i>	
14	PRIMEGENSw3: A Web-Based Tool for High-Throughput Primer and Probe Design. . . . .	181
	<i>Garima Kushwaha, Gyan Prakash Srivastava, and Dong Xu</i>	
15	Selecting Specific PCR Primers with MFEprimer . . . . .	201
	<i>Wubin Qu and Chenggang Zhang</i>	
	<i>Index</i> . . . . .	215

---

## Contributors

- MARÍA J. ANDRADE • *Food Hygiene and Safety, Meat and Meat Products Research Institute, Faculty of Veterinary Science, University of Extremadura, Cáceres, Spain*
- REIDAR ANDRESON • *Department of Bioinformatics, University of Tartu, Tartu, Estonia; Estonian Biocentre, Tartu, Estonia*
- CHHANDAK BASU • *Department of Biology, California State University, Northridge, Los Angeles, CA, USA*
- FRANCISCO DE PAULA CARETA • *Department of Pharmacy and Nutrition, CCA, Federal University of Espirito Santo, Alegre, ES, Brazil*
- LENKA ČERNÍKOVÁ • *Laboratory of Molecular Methods, State Veterinary Institute Prague, Prague, Czech Republic*
- NIKITA CHORDIA • *School of Biotechnology, Devi Ahilya University, Indore, India*
- ANTONIUS CHRISTIANTO • *Laboratory of Functional Genomics, Department of Pharmacy, College of Pharmaceutical Sciences, Ritsumeikan University, Shiga, Japan*
- JUAN J. CÓRDOBA • *Food Hygiene and Safety, Meat and Meat Products Research Institute, Faculty of Veterinary Science, University of Extremadura, Cáceres, Spain*
- KELLY M. ELKINS • *Chemistry Department, Towson University, Towson, MD, USA*
- MARTINA HAVLÍČKOVÁ • *National Institute of Public Health, Centre for Epidemiology and Microbiology, National Reference Laboratory for Influenza, Prague, Czech Republic*
- TETSUYA INAZU • *Laboratory of Functional Genomics, Department of Pharmacy, College of Pharmaceutical Sciences, Ritsumeikan University, Shiga, Japan*
- HELENA JIŘINCOVÁ • *National Institute of Public Health, Centre for Epidemiology and Microbiology, National Reference Laboratory for Influenza, Prague, Czech Republic*
- TOMÁŠ JIŘINEC • *National Institute of Public Health, Centre for Epidemiology and Microbiology, National Reference Laboratory for Influenza, Prague, Czech Republic*
- LAURIS KAPLINSKI • *Department of Bioinformatics, University of Tartu, Tartu, Estonia; Estonian Biocentre, Tartu, Estonia*
- ANIL KUMAR • *School of Biotechnology, Devi Ahilya University, Indore, India*
- GARIMA KUSHWAHA • *Informatics Institute and Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO, USA*
- KELVIN LI • *The J. Craig Venter Institute, Rockville, MD, USA*
- JULIET O. MAKANGA • *Laboratory of Functional Genomics, Department of Pharmacy, College of Pharmaceutical Sciences, Ritsumeikan University, Shiga, Japan*
- ALEXANDER NAGY • *Laboratory of Molecular Methods, State Veterinary Institute Prague, Prague, Czech Republic; National Institute of Public Health, Centre for Epidemiology and Microbiology, National Reference Laboratory for Influenza, Prague, Czech Republic*
- GIRDHAR K. PANDEY • *Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi, India*

- GREICIANE GABURRO PANETO • *Department of Pharmacy, Federal University of Espirito Santo, Vitória, ES, Brazil; Department of Pharmacy, Nutrition Alto Universitario s/n CCA, Alegre, ES, Brazil*
- KENNETH E. PIERCE • *Department of Biology, Brandeis University, Waltham, MA, USA*
- WUBIN QU • *Beijing Institute of Radiation Medicine, State Key Laboratory of Proteomics, Cognitive and Mental Health Research Center of PLA, Beijing, China*
- MAIDO REMM • *Department of Bioinformatics, University of Tartu, Tartu, Estonia; Estonian Biocentre, Tartu, Estonia*
- ALICIA RODRÍGUEZ • *Food Hygiene and Safety, Meat and Meat Products Research Institute, Faculty of Veterinary Science, University of Extremadura, Cáceres, Spain*
- MAR RODRÍGUEZ • *Food Hygiene and Safety, Meat and Meat Products Research Institute, Faculty of Veterinary Science, University of Extremadura, Cáceres, Spain*
- SUSMITA SHRIVASTAVA • *The J. Craig Venter Institute, Rockville, MD, USA*
- AMARJEET SINGH • *Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi, India*
- GYAN PRAKASH SRIVASTAVA • *Department of Neurology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA*
- TIMOTHY B. STOCKWELL • *The J. Craig Venter Institute, Rockville, MD, USA*
- BRENDA THORNTON • *Treasure Coast High School, Delray Beach, FL, USA*
- LAWRENCE J. WANGH • *Department of Biology, Brandeis University, Waltham, MA, USA*
- DONG XU • *Computer Science Department, Informatics Institute, and Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO, USA*
- WENCHAO YAN • *Animal Quarantine Laboratory, College of Animal Science and Technology, Henan University of Science and Technology, Luoyang, Henan Province, China*
- CHENGGANG ZHANG • *Beijing Institute of Radiation Medicine, State Key Laboratory of Proteomics, Cognitive and Mental Health Research Center of PLA, Beijing, China*