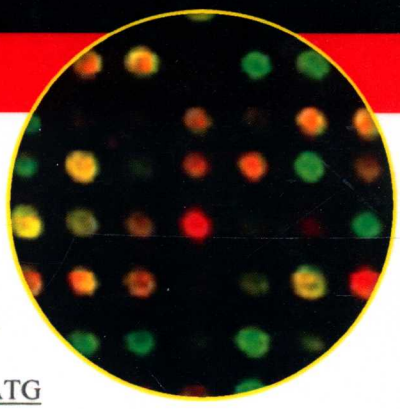
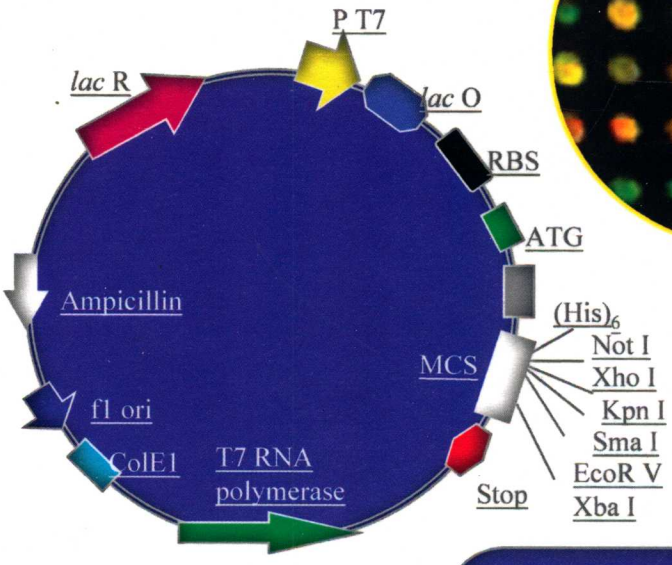
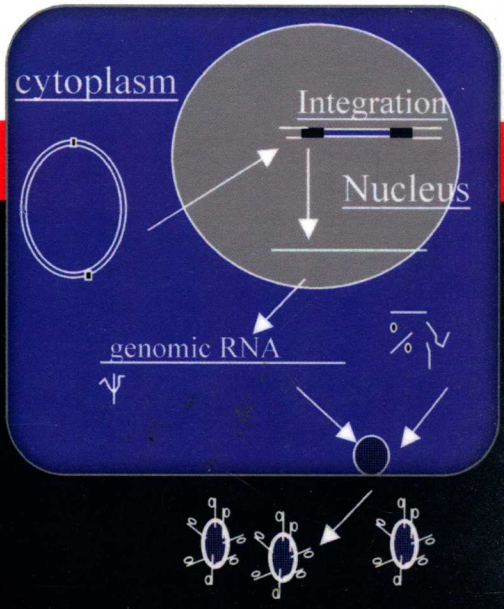
# Gene Biotechnology

## Third Edition






William Wu
Helen H. Zhang
Michael J. Welsh
Peter B. Kaufman

CRC Press
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# Preface

We are living in an era of biotechnology revolution. To many molecular and cellular biologists, the flood of information on human genome and new methodologies is particularly overwhelming because biotechnology is forging ahead and bringing rapid changes every day. Because every single organism depends on molecular actions for survival, molecular biology research has become more and more dominant in multiple disciplines. In fact, there is a general trend for the U.S. National Institutes of Health (NIH) and other funding agencies to award grants with high priority to those research proposals using molecular biology approaches. How can one catch up with new biotechnologies, and use the most recent, proven techniques for novel research? One of the aims of this book is to provide investigators with the tools needed for modern molecular and cellular biology research.

Another aim of this book is to guide graduate students in their thesis research. In our experience, good graduate training mandates independent performance with minimal advice from one's mentor. How do you select a novel research project for your thesis? What are your hypotheses, objectives, and experimental designs? How can you grasp technical problems and master current techniques? Where do you begin and what are the predicted results? How do you write your thesis and research manuscripts for publications? It is apparent that a graduate student needs some help with these questions. This book will provide the clues.

This book covers a wide range of current biotechnologies and methods that have been developed and are widely used in molecular biology, biochemistry, cell biology, immunology, recombinant protein biochemistry, biopharmaceutics, etc. The methods and protocols described in the appropriate chapters include the following: approaches to novel research, rapid isolation of specific cDNAs or genes by PCR; construction and screening of cDNA and genomic DNA libraries; preparation of DNA constructs; nonisotopic and isotopic DNA or RNA sequencing; information superhighway and computer databases of nucleic acids and proteins; characterization of DNA, RNA, or proteins by Southern, northern, or western blot hybridization; fluorescent techniques; gene overexpression; gene underexpression; and gene knockout in mammalian systems; analysis of cellular DNA or abundance of mRNA by radioactivity *in situ* hybridization; localization of DNA or abundance mRNA by fluorescence *in situ* hybridization; *in situ* PCR hybridization of low copy genes and *in situ* RT-PCR detection of low abundance mRNAs; strategies for gene double knockout, large-scale expression, and purification of recombinant proteins in cultured cells; high-throughput analysis of gene expression by real-time RT-PCR; gene expression profiling via DNA microarray, phage display, and siRNA technology; protein–protein binding and proteomic interactions; conditional gene knockout; and determination of gene copy numbers. Each chapter covers the principle(s) underlying methods and techniques, detailed step-by-step descriptions of each protocol, notes, tips, and a troubleshooting guide. We have observed that many of the currently available books in molecular biology contain only protocol recipes. Unfortunately, many

fail to explain the principles and concepts behind the methods outlined or to inform the reader about the possible pitfalls in the methods described. We intend to fill these gaps.

More importantly, this technology book provides unique features: (1) how to write a research paper for publication in English journals, (2) how to protect research discoveries and inventions via patents, and (3) discussion on practical biocalculation. The authors believe that research paper publication, intellectual property (IP) protection via patent(s), and biocalculations are very important topics to graduate students, researchers, and university faculty members.

For the information of the reader, Chapter 1 was written by William Wu, Peter B. Kaufman, and Michael J. Welsh. Chapters 2, 3, 6, 7, 9, 10, 13–17, 19–22, 23, 25, and 26 were written by William Wu. Chapter 8 was written by William Wu and Michael J. Welsh. Chapter 5 was written by William Wu and Peter B. Kaufman. Chapters 4, 11, 12, 18, 24, and 27 were written by William Wu and Helen Zhang. Nonetheless, all four authors have worked as a team.

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# Authors

**William Wu** has extensive experience in both academic and industrial settings. Throughout the years, Dr. Wu has held various positions in several organizations, including investigator and research assistant professor at the University of Michigan Medical School, Ann Arbor, Michigan; professor at the Hunan Normal University, Hunan, China; and senior scientist, team leader, and chief technology officer in several biomedical and pharmaceutical companies. He received his master's in biology from the Hunan Normal University, Hunan, China, in 1984. In 1992, he received his PhD in molecular and cellular biology at The Ohio University, Athens, Ohio, USA. This was followed by 3 years of postdoctoral training in molecular biology at the University of Michigan.

Dr. Wu is an internationally recognized expert in molecular biology, cell biology, protein biochemistry, immunology, and biotechnology. He has presented and published more than 35 research papers and abstracts at scientific meetings and in national and international journals. As a senior author, Dr. Wu has contributed 39 chapters on molecular and cellular biology methodologies in four books. He has an extensive knowledge and profound understanding of molecular biology, biotechnology, protein biochemistry, cellular biology, and molecular genetics. He is highly experienced and has extensive hands-on expertise in a variety of current molecular biology technologies in both academic and bioindustrial settings. As inventor and coinventor, Dr. Wu has five biomedical patents granted or in pending status.

**Helen H. Zhang** is a senior research associate at the University of Michigan Medical School, Ann Arbor, Michigan. In 1996, she received her MS in molecular and cellular biology at the Eastern Michigan University, Ypsilanti, Michigan.

Zhang has a strong background in molecular biology, microbiology, biochemistry, and immunology. She is highly experienced in DNA recombination, polymerase chain reaction (PCR) applications, cell transfection, and gene expression in bacterial and animal systems. Additionally, she has extensive experience in protein–peptide purification, enzymatic assays, and analysis of drug–protein binding as well as cell culture techniques. As a coauthor, she has published many research papers in internationally recognized journals.

**Michael J. Welsh** is a professor of cell biology in the Department of Cell and Developmental Biology at the University of Michigan Medical School, Ann Arbor, Michigan. He is also a professor of toxicology in the School of Public Health at the University of Michigan. Dr. Welsh received his PhD in 1977 from the University of Western Ontario, London, Ontario, Canada.

Dr. Welsh is an internationally recognized top scientist in molecular and cellular biology. He has published more than 120 research papers and has contributed chapters in several reference books. Dr. Welsh is a leading authority on mammalian heat shock protein (HSP27) and is a recipient of several major grants from the U.S.

National Institutes of Health; some of these projects utilized his expertise to examine the function and mechanisms of the HSP27 gene. He is also a fellow of the American Association for the Advancement of Science.

**Peter B. Kaufman** is a professor of biology in the Plant Cellular and Molecular Biology Program in the Department of Biology and a member of the faculty of the Bioengineering Program at the University of Michigan, Ann Arbor, Michigan. Dr. Kaufman received his PhD in 1954 in plant biology at the University of California, Davis, California.

Dr. Kaufman is an internationally recognized scholar in the field of molecular biology and physiology. He is a fellow of the American Association for the Advancement of Science and Secretary–Treasurer of the American Society for Gravitational and Space Biology. He has served on the editorial board of *Plant Physiology* for 10 years, has published more than 190 research papers and seven professional books. Dr. Kaufman teaches a popular course in plant biotechnology yearly at the University of Michigan. He has been awarded research grants from the National Science Foundation, the National Aeronautics and Space Administration, the U.S. Department of Agriculture, and Parke-Davis Pharmaceutical Research Laboratories in Ann Arbor, Michigan.

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