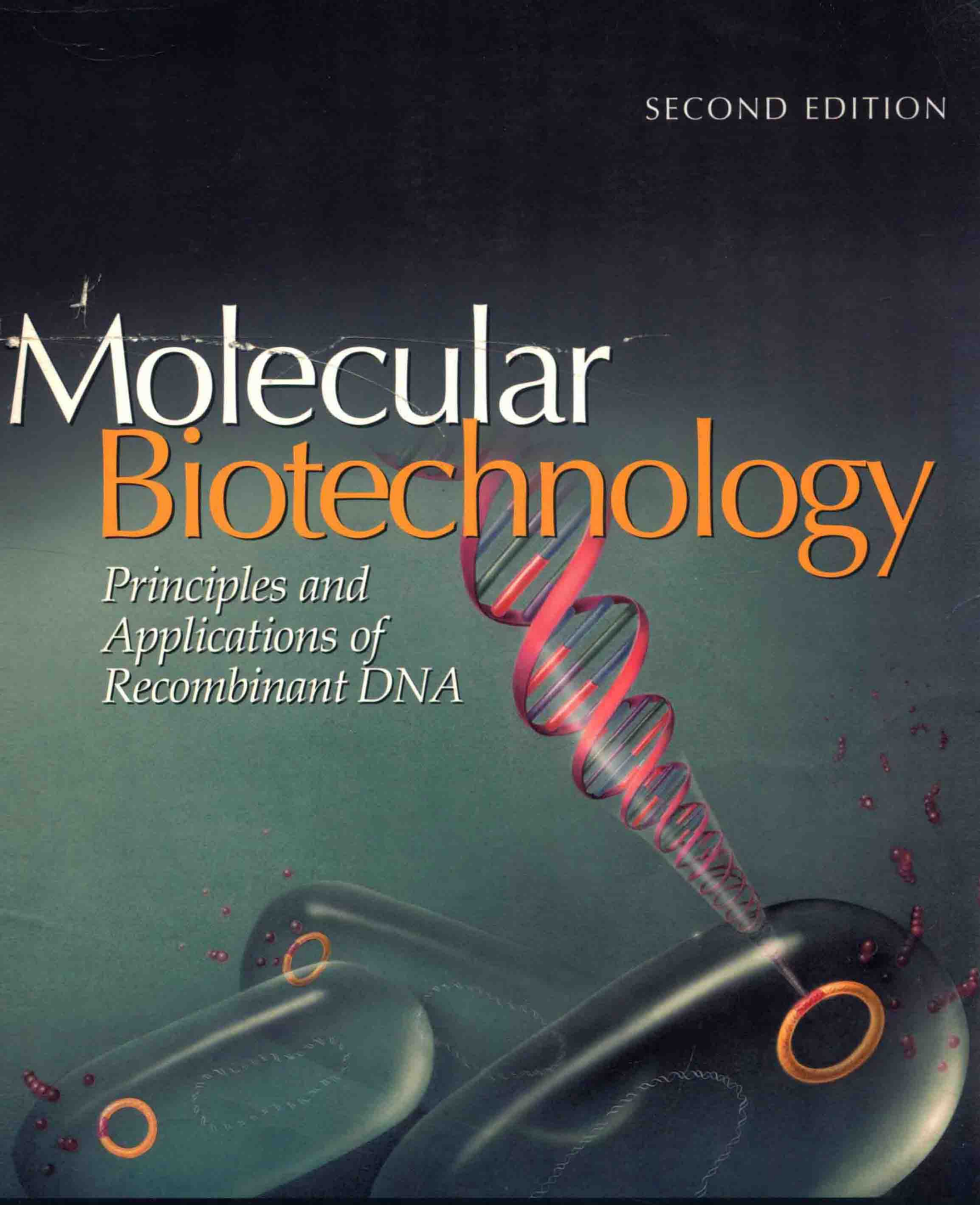
SECOND EDITION

Molecular Biotechnology

Principles and Applications of Recombinant DNA



Bernard R. Glick
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Principles and Applications of Recombinant DNA

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To Marcia, for being Marcia
BRG

To Lili, for everything
JJP

Preface

In the past 4 years, since the publication of the first edition of *Molecular Biotechnology: Principles and Applications of Recombinant DNA*, there has been an outpouring of new discoveries from biotechnology laboratories, and many gene-based innovations have been introduced into the marketplace. For example, human pharmaceuticals that are the products of cloned genes have been commercialized, while many other promising biotechnology drugs are being tested; immunological and PCR-based diagnostic assays for several disease-causing organisms and human disorders have become the standard feature of clinical testing procedures; novel recombinant vaccines that are based on gene products and cloned genes have been developed; many human disease genes have been identified and characterized; the number of human gene therapy clinical trials has increased 10-fold; detailed genetic and physical maps of human chromosomes have been constructed; the first mammal has been cloned from a differentiated somatic cell; and the first commercial transgenic plants have been used on a large scale.

In the first edition, we presented the principles and applications of molecular biotechnology, in a broad biological context, in a way that would be interesting and informative. Since then, we have received many helpful comments from colleagues, graduate students, and undergraduates from many different institutions around the world. With our original objectives in mind and taking into consideration the advice of a number of readers, we have updated, expanded, and reorganized *Molecular Biotechnology: Principles and Applications of Recombinant DNA*. We hope that the second edition conveys the excitement of molecular biotechnology, the scientific underpinnings of this area of study, the significance of recent discoveries, and how this information will be used to create "goods and services." Specifically, a new chapter that discusses the organisms that are commonly used by molecular biotechnologists has been added. As well, there is a complete chapter that describes the fundamental principles of molecular biology. The chapters dealing with human molecular genetics, human gene therapy, and

plant biotechnology have been extensively expanded to account for the recent advances in these areas. The chapters pertaining to diagnostic assays and vaccines have been thoroughly reorganized. The coverage of material has been updated throughout the book. In addition to these extensive changes, the numbers of figures and tables have been increased by approximately 50% to facilitate the understanding of both experimental strategies and important theoretical concepts. The glossary has also been updated and expanded. Notwithstanding all of the revising and reorganization, we have tried to preserve the same jargon-free and "reader-friendly" style that made the first edition popular with students.

We thank the crew at ASM Press, including Ken April, Susan Birch, Greg Payne, and Jeff Holtmeier for their support and encouragement. Susan Schmidler, as usual, did an excellent job with design of the book. Special thanks to Ken April, who handled the manuscript with diligence and thoroughness and us with patience.

Bernard R. Glick
Jack J. Pasternak

Preface to the First Edition

Molecular biotechnology emerged as a new research field that arose as a result of the fusion in the late 1970s of recombinant DNA technology and traditional industrial microbiology. Whether one goes to the movies to see *Jurassic Park* with its ingenious but scientifically untenable plot of cloning dinosaurs, reads in the newspaper about the commercialization of a new "biotech" tomato that has an extended shelf life, or hears one of the critics of molecular biotechnology talking about the possibility of dire consequences from genetic engineering, there is a significant public awareness about recombinant DNA technology. In this book, we introduce and explain what molecular biotechnology actually is as a scientific discipline, how the research in the area is conducted, and how this technology may realistically impact on our lives in the future.

We have written *Molecular Biotechnology: Principles and Applications of Recombinant DNA* to serve as a text for courses in biotechnology, recombinant DNA technology, and genetic engineering or for any course introducing both the principles and the applications of contemporary molecular biology methods. The book is based on the biotechnology course we have offered for the past 12 years to advanced undergraduate and graduate studies from the biological and engineering sciences at the University of Waterloo. We have written this text for students who have an understanding of basic ideas from biochemistry, molecular genetics, and microbiology. We are aware that it is unlikely that students will have had all of these courses before taking a course on biotechnology. Thus, we have tried to develop the topics in this text by explaining their broader biological context before delving into molecular details.

This text emphasizes how recombinant DNA technology can be used to create various useful products. We have, wherever possible, used experimental results and actual methodological strategies to illustrate basic concepts, and we have tried to capture the flavor and feel of how molecular biotechnology operates as a scientific venture. The examples that we have selected—from a vast and rapidly growing literature—were chosen as case studies that not only illustrate particular points but also provide the reader with a solid basis for understanding current research in specialized areas of

molecular biotechnology. Nevertheless, we expect that some of our examples will be out of date by the time the book is published, because molecular biotechnology is such a rapidly changing discipline.

For the ease of the day-to-day practitioners, scientific disciplines often develop specialized terms and nomenclature. We have tried to minimize the use of technical jargon and, in many instances, have deliberately used a simple phrase to describe a phenomenon or process that might otherwise have been expressed more succinctly with technical jargon. In any field of study, synonymous terms that describe the same phenomenon exist. In molecular biotechnology, for example, recombinant DNA technology, gene cloning, and genetic engineering, in a broad sense, have the same meaning. When an important term or concept appears for the first time in this text, it is followed in parentheses with a synonym or equivalent expression. An extensive glossary can be found at the end of the book to help the reader with the terminology of molecular biotechnology.

Each chapter opens with an outline of topics and concludes with a detailed summary and list of review questions to sharpen students' critical thinking skills. All of the key ideas in the book are carefully illustrated by the more than 200 full-color diagrams in the pedagogical belief that a picture is indeed worth a thousand words. After introducing molecular biotechnology as a scientific and economic venture in Chapter 1, the next five chapters (2 to 6) deal with the methodologies of molecular biotechnology. The chapters of Part I act as a stepping-stone for the remainder of the book. Chapters 7 to 12 in Part II present examples of microbial molecular biotechnology covering such topics as the production of metabolites, vaccines, therapeutics, diagnostics, bioremediation, biomass utilization, bacterial fertilizers, and microbial pesticides. Chapter 13 describes some of the key components of large-scale fermentation processes using genetically engineered (recombinant) microorganisms. In Part III, we deal with the molecular biotechnology of plants and animals (Chapters 14 and 15). The isolation of human disease-causing genes by using recombinant DNA technology and how, although it is in its early stages, genetic manipulation is being currently contemplated for the treatment of human diseases are presented in Chapters 16 and 17. The book concludes with coverage of the regulation of molecular biotechnology and patents in Part IV.

A brief mention should be made about the reference sections that follow each chapter. Within many of the chapters we have relied upon the published work of various researchers. In all cases, although not cited directly in the body of a chapter, the original published articles are noted in the reference section of the appropriate chapter. In some cases, we have taken "pedagogic license" and either extracted or reformulated data from the original publications. Clearly, we are responsible for any distortions or misrepresentations from these simplifications, although we hope that none has occurred. The reference sections also contain other sources that we used in a general way, which might, if consulted, bring the readers closer to a particular subject.

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