国外优秀生命科学教学用书

Gene Cloning and DNA Analysis

An Introduction

基因克隆和DNA分析

影印版

Fourth Edition

• T. A. Brown

高等教育出版社
Higher Education Press

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出 版 前 言

随着克隆羊的问世和人类基因组计划的完成，生命科学成为21世纪名副其实的领头学科，生物高新技术产业逐步成为高科技产业的核心。生物科技和生物产业的发展对世界科技、经济、政治和社会发展等方面产生着深刻的影响，这也是我国赶超世界发达国家生产力水平最有前途和希望的领域。生命科学与技术全方位的发展呼唤高等教育培养更多高水平的复合型科技人才。

为此，教育部在《关于加强高等学校本科教学工作　提高教学质量的若干意见》[教高（2001）4号文件]中提出，高等学校要大力提倡编写、引进和使用先进教材，其中信息科学、生命科学等发展迅速、国际通用性强、可比性强的学科和专业可以直接引进先进的、能反映学科发展前沿的原版教材。教育部高等教育司还于2001年11月向全国主要大学和出版社下发了“关于开展‘国外生命科学类优秀教学用书’推荐工作的通知”，有力推动了生命科学类教材的引进工作。

高等教育出版社对国外生命科学教材进行了充分的调研，并委托教育部高等学校生物科学与工程教学指导委员会的专家教授开展了“引进国外优秀生命科学教材及其教学辅助材料专项研究”，并就国内外同类教材进行了比较，提出了具体的引进教材书目。经过版权谈判，目前我社已经购买了Pearson Education, McGraw-Hill, John Wiley & Sons, Blackwell Science, Thomson Learning, Cambridge University Press, Lippincott Williams & Wilkins等出版的13种教材的影印权，学科领域涉及生物化学、细胞生物学、遗传学、微生物学、生态学、免疫学、神经科学、发育生物学、解剖学与生理学、分子生物学、普通生物学等。这些教材具有以下特点：（1）所选教材基本是近2年出版的，及时反映了学科发展的最新进展，在国际上使用广泛，具有权威性和时代感；（2）内容简明，篇幅适中，结构合理，兼具一定的深度和广度，适用范围广；（3）插图精美、丰富，既有很强的艺术性，又不失严谨的科学性，图文并茂，与正文相辅相成；（4）语言简练、流畅，十分适合非英语国家的学生阅读。其中9种已入选教育部高等教育司推荐“国外优秀生命科学教学用书”。

考虑到中国国情，为了让学生买得起，同时又能让学生看到原版书彩色精美的插图，我们在引进学生用原版教材时，一方面采用黑白影印，最大限度地降低定价，另一方面随书附赠含有原书彩色插图的光盘，以充分体现原教材的风格、特色，为读者提供方便。

引进国外优秀生命科学教学用书是我社一项长期的重点工作，因此，我们衷心希望广大专家教授和同学提出宝贵的意见和建议，如有更好的教材值得引进，请与高等教育出版社生命科学分社联系，联系电话：010-68344002，E-mail地址：lifesciences-hep@x263.net。

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2002年11月

国外优秀生命科学教学用书

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Essential Biology	生物学导论

Preface to the Fourth Edition

Fifteen years ago, when the first edition of this book was published, gene cloning was a relatively new technique that provided the basis of virtually all studies of DNA. Now non-cloning approaches, notably the polymerase chain reaction (PCR), are equally important. To reflect these changes, the fourth edition has a new title. This does not mean that the philosophy of the book has changed. It is still an introductory text that begins at the beginning and does not assume the reader has any prior knowledge of the techniques used to study genes and genomes. The new title simply means that the breadth of the book is greater than it needed to be in 1986.

This edition has two entirely new chapters and some organizational changes. The first of the new chapters is about the methods used to sequence genomes and to understand the sequence after it has been obtained. This is, of course, much more than simply sequencing on a grand scale and involves strategies for assembling contiguous sequences, methods for identifying genes in a genome sequence, and techniques for studying the transcriptome and proteome. The second new chapter is the final one, on the applications of gene cloning and DNA analysis in forensic science. This is a popular topic with students and one that provides an excellent illustration of the applications of DNA analysis in the real world.

The organizational changes see PCR moved to an earlier position so that its applications in research and biotechnology can be dealt with adequately in Parts 2 and 3. I have also moved a few other sections around to try to produce a more logical flow of information. As usual, there are updates and the few errors have been corrected.

For some time I was unsure if there would be a fourth edition, and I would therefore like to thank Nigel Balmforth of Blackwell Science for ensuring that the book survived the takeover of the previous publishers. I must also thank my colleagues Lubomira Stateva and Keri Brown for allowing me to make use of their teaching materials as cribs for some of the new information in Part 3. At the end of the Preface I usually say how much hard work the new edition has been, but to be honest doing this one was very enjoyable.

T.A. Brown
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Preface to the Third Edition

The third edition of this book is longer and more detailed than either of its predecessors, but it retains the same basic philosophy. It is still unashamedly introductory and is still aimed at undergraduates and other individuals who have no previous experience of experiments with DNA. The last few years have seen a proliferation of cloning manuals and other 'hands-on' texts for gene cloners, but few of these address the needs of a student encountering the subject for the first time. I hope that this new edition will continue to help these newcomers get started.

I have made revisions and updates at many places throughout the book, but major changes do not occur until the last few chapters. A major weakness of the second edition was the poor coverage that I had given to the polymerase chain reaction, my excuse being that the sudden rise of the technique occurred just as I had completed the manuscript. The third edition attempts to correct this failing with a new chapter devoted entirely to PCR. I appreciate that PCR is not really a component of gene cloning, so the title of the book is no longer appropriate, but *Gene Cloning, the Polymerase Chain Reaction, and Related Techniques: An Introduction* seemed a bit long-winded. The 'gene cloning' of the title is now something of a generic term, and I apologize to any purists among the readership.

The other major rewrites are in Part 3, where again I have extended the coverage, this time by bringing in a new chapter on the applications of gene cloning in agriculture, or to be precise, in plant genetic engineering. Here, and at a few other places in the book, I have attempted in a rather hesitant (and I fear inadequate) fashion to discuss some of the broader issues that arise from our ability to clone genes. These issues are now so prominent in the public perception that they must be addressed by all students of genetic engineering. So the real title of the book is *Gene Cloning, the Polymerase Chain Reaction, Related Techniques and Some of the Implications: An Introduction.*

As with the first and second editions, I would not have got very far with this book if my wife Keri had not been prepared to put up with several months of lonely evenings as I struggled with the word processor. Once again her unwavering encouragement has been the most important factor in completion of the task.

T.A. Brown
Manchester

Preface to the Second Edition

It was only when I started writing the second edition to this book that I fully appreciated how far gene cloning has progressed since 1986. Being caught up in the day-to-day excitement of biological research it is sometimes difficult to stand back and take a considered view of everything that is going on. The pace with which new techniques have been developed and applied to recombinant DNA research is quite remarkable. Procedures which in 1986 were new and innovative are now *de rigueur* for any self-respecting research laboratory and many of the standard techniques have found their way into undergraduate practical classes. Students are now faced with a vast array of different procedures for cloning genes and an even more diverse set of techniques for studying them once they have been cloned.

In revising this book I have tried to keep rigidly to a self-imposed rule that I would not make the second edition any more advanced than the first. There are any number of advanced texts for students or research workers who need detailed information on individual techniques and approaches. In contrast, there is still a surprising paucity of really introductory texts on gene cloning. The first edition was unashamedly introductory and I hope that the second edition will be also.

Nevertheless, changes were needed and on the whole the second edition contains more information. I have resisted the temptation to make many additions to Part 1, where the fundamentals of gene cloning are covered. A few new vectors are described, especially for cloning in eukaryotes, but on the whole the first seven chapters are very much as they were in the first edition. Part 2 has been redefined so it now concentrates more fully on techniques for studying cloned genes, in particular with a description of methods for analysing gene regulation. Recombinant DNA techniques in general have become more numerous since 1986 and an undergraduate is now expected to have a broader appreciation of how cloned genes are studied. In Part 3 the main theme is still biotechnology, but the tremendous advances in this area have required more extensive rewriting. The use of eukaryotes for synthesis of recombinant protein is now standard procedure, and we have seen the first great contributions of gene cloning to the study of human disease. The applications of gene cloning really make up a different book to this one, but nonetheless in Part 3 I have tried to give a flavour of what is going on.

A number of people have been kind enough to comment on the first edition and make suggestions for this revision. Don Grierson and Paul Sims again provided important and sensible advice. I must also thank Stephen Oliver and Richard Walmsley for their comments on specific parts of the book. Once again my wife's patience and encouragement has been a major factor in getting a second edition done at all. Finally I would like to thank all the students who have used the first edition for the mainly nice things they have said about it.

T.A. Brown
Manchester

Preface to the First Edition

This book is intended to introduce gene cloning and recombinant DNA technology to undergraduates who have no previous experience of the subject. As such, it assumes very little background knowledge on the part of the reader – just the fundamental details of DNA and genes that would be expected of an average sixth-former capable of a university entrance grade at A-level biology. I have tried to explain all the important concepts from first principles, to define all unfamiliar terms either in the text or in the glossary, to avoid the less helpful jargon words, and to reinforce the text with as many figures as are commensurate with a book of reasonable price.

Although aimed specifically at first- and second-year undergraduates in biochemistry and related degree courses, I hope that this book will also prove useful to some experienced researchers. I have been struck over the last few years by the number of biologists, expert in other aspects of the science, who have realized that gene cloning may have a role in their own research projects. Possibly this text can act as a painless introduction to the complexities of recombinant DNA technology for those of my colleagues wishing to branch out into this new discipline.

I would like to make it clear that this book is not intended as competition for the two excellent gene cloning texts already on the market. I have considerable regard for the books by Drs Old and Primrose and by Professor Glover, but believe that both texts are aimed primarily at advanced undergraduates who have had some previous exposure to the subject. It is this 'previous exposure' that I aim to provide. My greatest satisfaction will come if this book is accepted as a primer for Old and Primrose or for Glover.

I underestimated the effort needed to produce such a book and must thank several people for their help. The publishers provided the initial push to get the project under way. I am indebted to Don Grierson at Nottingham University and Paul Sims at UMIST for reading the text and suggesting improvements; all errors and naïveties are, however, mine. Finally, my wife Keri typed most of the manuscript and came to my rescue on several occasions with the right word or turn of phrase. This would never have been finished without her encouragement.

T.A. Brown
Manchester

Contents

PART 1
THE BASIC PRINCIPLES OF GENE CLONING AND DNA ANALYSIS

Chapter 1 Why Gene Cloning and DNA Analysis are Important

In the middle of the nineteenth century, Gregor Mendel formulated a set of rules to explain the inheritance of biological characteristics. The basic assumption of these rules is that each heritable property of an organism is controlled by a factor, called a **gene**, that is a physical particle present somewhere in the cell. The rediscovery of Mendel's laws in 1900 marks the birth of **genetics**, the science aimed at understanding what these genes are and exactly how they work.

1.1 The early development of genetics

For the first 30 years of its life this new science grew at an astonishing rate. The idea that genes reside on **chromosomes** was proposed by W. Sutton in 1903, and received experimental backing from T.H. Morgan in 1910. Morgan and his colleagues then developed the techniques for **gene mapping**, and by 1922 had produced a comprehensive analysis of the relative positions of over 2000 genes on the four chromosomes of the fruit fly, *Drosophila melanogaster*.

Despite the brilliance of these classical genetic studies, there was no real understanding of the molecular nature of the gene until the 1940s. Indeed, it was not until the experiments of Avery, MacLeod and McCarty in 1944, and of Hershey and Chase in 1952, that anyone believed deoxyribonucleic acid (DNA) to be the genetic material: up until then it was widely thought that genes were made of protein. The discovery of the role of DNA was a tremendous stimulus to genetic research, and many famous biologists (Delbrück, Chargaff, Crick and Monod were among the most influential) contributed to the second great age of genetics. In the 14 years between 1952 and 1966 the structure of DNA was elucidated, the genetic code cracked, and the processes of transcription and translation described.

1.2 The advent of gene cloning and the polymerase chain reaction

These years of activity and discovery were followed by a lull, a period of anticlimax when it seemed to some molecular biologists (as the new generation of geneticists styled themselves) that there was little of fundamental importance that was not understood. In truth there was a frustration that the experimental techniques of the late 1960s were not sophisticated enough to allow the gene to be studied in any greater detail.

Then in the years 1971–1973 genetic research was thrown back into gear by what at the time was described as a revolution in experimental biology. A whole new methodology was developed, enabling previously impossible experiments to be planned and carried out, if not with ease, then at least with success. These methods, referred to as **recombinant DNA technology** or **genetic engineering**, and having at their core the process of **gene cloning**, sparked another great age of genetics. They led to rapid and efficient DNA sequencing techniques that enabled the structures of individual genes to be determined, reaching a culmination in the 1990s with the massive genome sequencing projects, including the human project which was completed in 2000. They led to procedures for studying the regulation of individual genes, which have allowed molecular biologists to understand how aberrations in gene regulation can result in human diseases such as cancer. The techniques spawned modern **biotechnology**, which puts genes to work in production of proteins and other compounds needed in medicine and industrial processes.

During the 1980s, when the excitement engendered by the gene cloning revolution was at its height, it hardly seemed possible that another, equally novel and equally revolutionary process was just around the corner. According to DNA folklore, Kary Mullis invented the **polymerase chain reaction (PCR)** during a drive along the coast of California one evening in 1985. His brainwave was an exquisitely simple technique that acts as a perfect complement to gene cloning. PCR has made easier many of the techniques that were possible but difficult to carry out when gene cloning was used on its own. It has extended the range of DNA analysis and led to molecular biology finding new applications in areas of endeavour outside of its traditional range of medicine, agriculture and biotechnology. Molecular ecology, biomolecular archaeology and DNA forensics are just three of the new disciplines that have become possible as a direct consequence of the invention of PCR, and molecular biologists are devising new ways of using DNA to ask questions about human evolution and the impact of environmental change on the biosphere. Thirty years after the gene cloning revolution we are still riding the rollercoaster and there is no end to the excitement in sight.

1.3 What is gene cloning?

The basic steps in a gene cloning experiment are as follows (Figure 1.1).

(1) A fragment of DNA, containing the gene to be cloned, is inserted into a circular DNA molecule called a **vector**, to produce a **chimera** or **recombinant DNA molecule**.

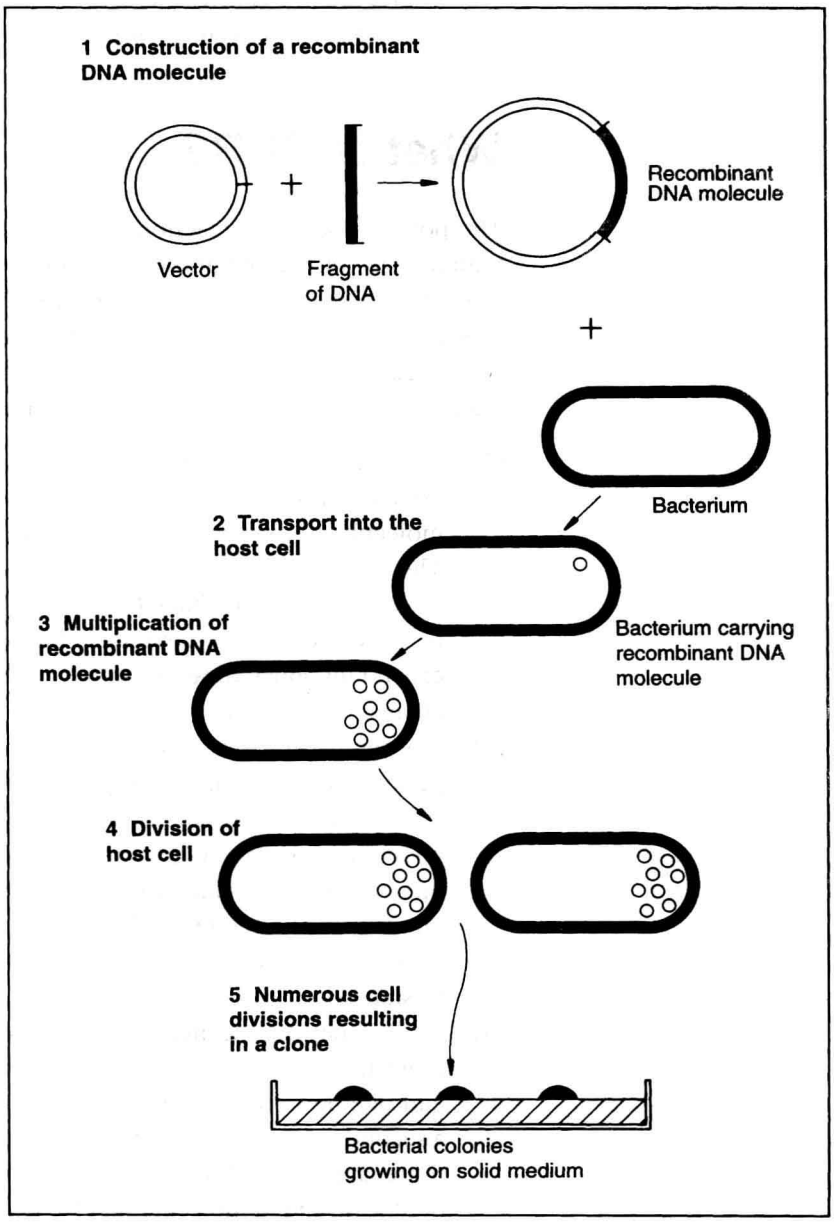

Figure 1.1 The basic steps in gene cloning.