国外优秀生命科学教学用书

An Introduction to Genetic Engineering

遗传工程导论

影印版

Second Edition

• Desmond S. T. Nicholl

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

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An Introduction to Genetic Engineering (2nd ed.) 遗传工程导论

Essential Biology 生物学导论

Preface to the second edition

Advances in genetics continue to be made at an ever increasing rate, which makes writing an introductory text somewhat difficult. In the few years since the first edition was published, many new applications of gene manipulation technology have been developed, covering a diverse range of disciplines. The temptation in preparing this second edition was to concentrate on the applications, and ignore the fundamental principles of the technology. However, I wished to retain many of the features of the first edition, in which a basic technical introduction to the subject was the main aim of the text. Thus some of the original methods used in gene manipulation have been kept as examples of how the technology developed, even though some of these have become little used or even obsolete. From the educational point of view, this should help the reader cope with more advanced information about the subject – a sound grasp of the basic principles is an important part of any introduction to genetic engineering. I have been gratified by the many positive comments about the first edition, and I hope that this new edition is as well received.

In trying to strike a balance between the methodology and the applications of gene manipulation, I have divided the text into three sections. Part I deals with basic molecular biology, Part II with the methods used to manipulate genes, and Part III with the applications. These sections may be taken out of order if desired, depending on the level of background knowledge. Apart from a general revision of chapters retained from the first edition, there have been some more extensive changes made. The increasing importance of the polymerase chain reaction is recognised by a new chapter devoted to this topic. In Part III there are now five separate chapters dealing with the applications of gene manipulation, as opposed to a single chapter in the first

edition. I hope that the changes have produced a balanced treatment of the field, whilst retaining the introductory nature of the text and keeping it to a reasonable length.

My thanks go to my colleagues Simon Hettle, John McLean, Ros Brett and Anne Dickson for comments on various parts of the manuscript. Their help has made the book better; any errors of fact or interpretation remain my own responsibility. My final and biggest thank you goes to my wife Linda and to Charlotte, Thomas and Anna. They have suffered with me during the writing, and have put up with more than they should have had to. I dedicate this edition to them.

Desmond S. T. Nicholl
Paisley

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Introduction

1.1 What is genetic engineering?

Progress in any scientific discipline is dependent on the availability of techniques and methods that extend the range and sophistication of experiments which may be performed. Over the last 30 years or so this has been demonstrated in a spectacular way by the emergence of genetic engineering. This field has grown rapidly to the point where, in many laboratories around the world, it is now routine practice to isolate a specific DNA fragment from the genome of an organism, determine its base sequence, and assess its function. The technology is also now used in many other applications, including forensic analysis of scene-of-crime samples, paternity disputes, medical diagnosis, genome mapping and sequencing, and the biotechnology industry. What is particularly striking about the technology of gene manipulation is that it is readily accessible by individual scientists, without the need for large-scale equipment or resources outside the scope of a reasonably well-found research laboratory.

The term **genetic engineering** is often thought to be rather emotive or even trivial, yet it is probably the label that most people would recognise. However, there are several other terms that can be used to describe the technology, including **gene manipulation**, **gene cloning**, **recombinant DNA technology**, **genetic modification**, and the **new genetics**. There are also legal definitions used in administering regulatory mechanisms in countries where genetic engineering is practised.

Although there are many diverse and complex techniques involved, the basic principles of genetic manipulation are reasonably simple. The premise

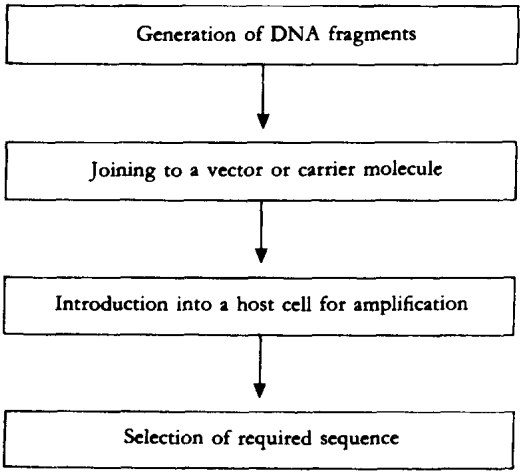

Fig. 1.1. The four steps in a gene cloning experiment. The term *clone* comes from the colonies of identical host cells produced during amplification of the cloned fragments. Gene cloning is sometimes referred to as *molecular cloning*, to distinguish the process from the cloning of whole organisms.

on which the technology is based is that genetic information, encoded by DNA and arranged in the form of genes, is a resource that can be manipulated in various ways to achieve certain goals in both pure and applied science and medicine. There are many areas in which genetic manipulation is of value, including:

- Basic research on gene structure and function
- Production of useful proteins by novel methods
- Generation of transgenic plants and animals
- Medical diagnosis and treatment.

In later chapters I look at some of the ways in which genetic manipulation has contributed to these areas.

The mainstay of genetic manipulation is the ability to isolate a single DNA sequence from the genome. This is the essence of **gene cloning**, and can be considered as a series of four steps (Fig. 1.1). Successful completion of these steps provides the genetic engineer with a specific DNA sequence, which may then be used for a variety of purposes. A useful analogy is to consider gene cloning as a form of **molecular agriculture**, enabling the production of large amounts (in genetic engineering this means micrograms or milligrams) of a particular DNA sequence.

One aspect of the new genetics that has given cause for concern is the

debate surrounding the potential applications of the technology. The term **genethics** has been coined to describe the ethical problems that exist in modern genetics, which are likely to increase in both number and complexity as genetic engineering technology becomes more sophisticated. The use of transgenic plants and animals, investigation of the human genome, gene therapy, and many other topics are of concern not just to the scientist but to the population as a whole. The recent developments in genetically modified foods have provoked a public backlash against the technology. Additional developments in the cloning of organisms, and in areas such as *in vitro* fertilisation and xenotransplantation, raise further questions. Although not strictly part of gene manipulation technology, I will consider aspects of organismal cloning later in this book, as this is an area of much concern and can be considered as genetic engineering in its broadest sense.

Taking all the potential costs and benefits into account, it remains to be seen if we can use genetic engineering for the overall benefit of mankind, and avoid the misuse of technology that often accompanies scientific achievement.

1.2 Laying the foundations

Although the techniques used in gene manipulation are relatively new, it should be remembered that development of these techniques was dependent on the knowledge and expertise provided by microbial geneticists. We can consider the development of genetics as falling into three main eras (Fig. 1.2). The science of genetics really began with the rediscovery of Gregor Mendel's work at the turn of the century, and the next 40 years or so saw the elucidation of the principles of inheritance and genetic mapping. Microbial genetics became established in the mid-1940s, and the role of DNA as the genetic material was confirmed. During this period great advances were made in understanding the mechanisms of gene transfer between bacteria, and a broad knowledge base was established from which later developments would emerge.

The discovery of the structure of DNA by James Watson and Francis Crick in 1953 provided the stimulus for the development of genetics at the molecular level, and the next few years saw a period of intense activity and excitement as the main features of the gene and its expression were determined. This work culminated with the establishment of the complete genetic code in 1966 – the stage was now set for the appearance of the new genetics.

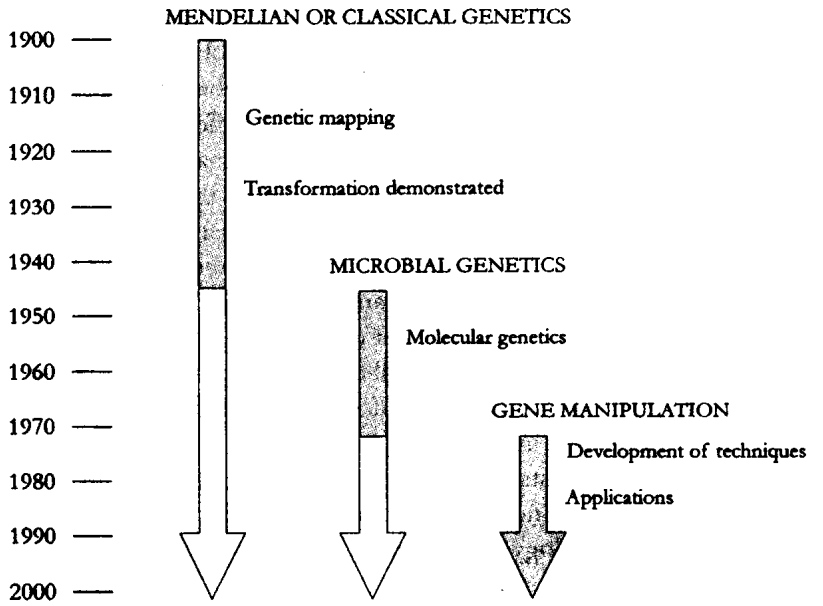

Fig. 1.2. The history of genetics since 1900. Shaded areas represent the periods of major development in each branch of the subject.

1.3 First steps

In the late 1960s there was a sense of frustration among scientists working in the field of molecular biology. Research had developed to the point where progress was being hampered by technical constraints, as the elegant experiments that had helped to decipher the genetic code could not be extended to investigate the gene in more detail. However, a number of developments provided the necessary stimulus for gene manipulation to become a reality. In 1967 the enzyme **DNA ligase** was isolated. This enzyme can join two strands of DNA together, a prerequisite for the construction of recombinant molecules, and can be regarded as a sort of molecular glue. This was followed by the isolation of the first **restriction enzyme** in 1970, a major milestone in the development of genetic engineering. Restriction enzymes are essentially molecular scissors, which cut DNA at precisely defined sequences. Such enzymes can be used to produce fragments of DNA that are suitable for joining to other fragments. Thus, by 1970, the basic tools required for the construction of recombinant DNA were available.

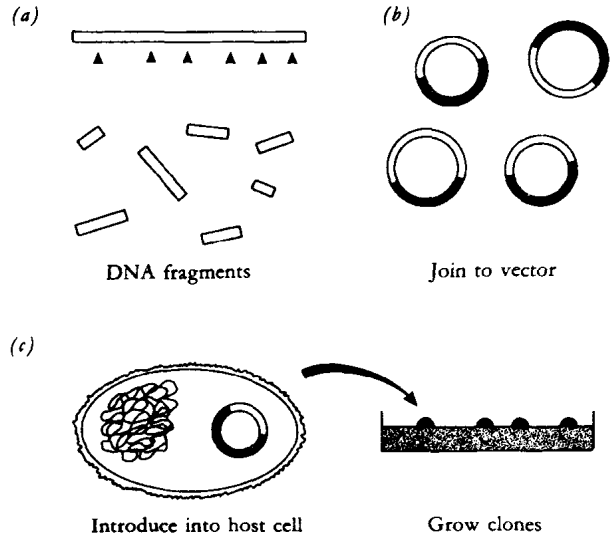

Fig. 1.3. Cloning DNA fragments. (a) The source DNA is isolated and fragmented into suitably sized pieces. (*b*) The fragments are then joined to a carrier molecule or vector to produce recombinant DNA molecules. In this case, a plasmid vector is shown. (*c*) The recombinant DNA molecules are then introduced into a host cell (a bacterial cell in this example) for propagation as clones.

The first recombinant DNA molecules were generated at Stanford University in 1972, utilizing the cleavage properties of restriction enzymes (scissors) and the ability of DNA ligase to join DNA strands together (glue). The importance of these first tentative experiments cannot be overestimated. Scientists could now join different DNA molecules together, and could link the DNA of one organism to that of a completely different organism. The methodology was extended in 1973 by joining DNA fragments to the plasmid pSC101, which is an **extrachromosomal element** isolated from the bacterium *Escherichia coli*. These recombinant molecules behaved as **replicons**, i.e. they could replicate when introduced into *E. coli* cells. Thus, by creating recombinant molecules *in vitro*, and placing the construct in a bacterial cell where it could replicate *in vivo*, specific fragments of DNA could be isolated from bacterial colonies that formed clones (colonies formed from a single cell, in which all cells are identical) when grown on agar plates. This development marked the emergence of the technology which became known as **gene cloning** (Fig. 1.3).

The discoveries of 1972 and 1973 triggered off what is perhaps the biggest scientific revolution of all – the new genetics. The use of the new technology