An Introduction to Genetic Engineering
Third Edition

Chapter 1 summary

Aims

- To define genetic engineering as it will be described in this book
- To outline the basic features of genetic engineering
- To describe the emergence of gene manipulation technology
- To outline the structure of the book

Chapter summary/learning outcomes

When you have completed this chapter you will have knowledge of:

- The scope and nature of the subject
- The steps required to clone a gene
- The emergence and early development of the technology
- Elements of the ethical debate surrounding genetic engineering

Key words

Genetic engineering, gene manipulation, gene cloning, recombinant DNA technology, genetic modification, new genetics, molecular agriculture, genethics, DNA ligase, restriction enzyme, plasmid, extrachromosomal element, replicon, text box, aims, chapter summary, learning outcome, concept map.

Chapter 1

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 that may be performed. Over the past 35 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-funded research laboratory. Although the technology has become much more large-scale in recent years as genome sequencing projects have been established, it is still accessible by almost all of the bioscience community in some form or other.

Several terms may be used to describe the technologies involved in manipulating genes.

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.

Genetic material provides a rich resource in the form of information encoded by the sequence of bases in the DNA.

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

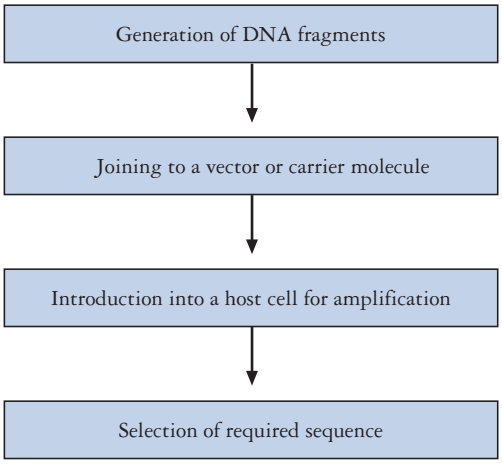

Fig. 1.1 The four steps in a gene cloning experiment. The term 'clone' comes from the colonies of identical host cells produced during amplification of the cloned fragments. Gene cloning is sometimes referred to as 'molecular cloning' to distinguish the process from the cloning of whole organisms.

many areas in which genetic manipulation is of value, including the following:

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

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

Gene cloning enables isolation and identification of individual genes.

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. Even in the era of large-scale sequencing projects, this ability to isolate a particular gene sequence is still a major aspect of gene manipulation carried out on a day-to-day basis in research laboratories worldwide.

As well as technical and scientific challenges, modern genetics poses many moral and ethical questions.

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. Recent developments in genetically modified foods have provoked a public backlash against the technology. Additional developments in

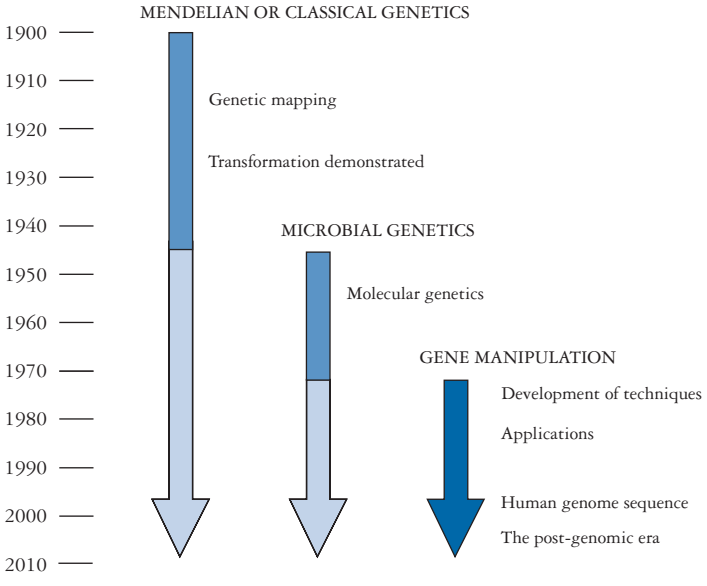

Fig. 1.2 The history of genetics since 1900. Shaded areas represent the periods of major development in each branch of the subject.

the cloning of organisms, and in areas such as *in vitro* fertilisation and xenotransplantation, raise further questions. Although organismal cloning is not strictly part of gene manipulation technology, we will consider aspects of it later in this book, because this is an area of much concern and can be considered genetic engineering in its broadest sense. Research on stem cells, and the potential therapeutic benefits that this research may bring, is another area of concern that is part of the general advance in genetic technology.

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.

Gregor Mendel is often considered the 'father' of genetics.

Watson and Crick's double helix is perhaps the most 'famous' and most easily recognised molecule in the world.

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.

1.3 First steps

By the end of the 1960s most of the essential requirements for the emergence of gene technology were in place.

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 that 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.

The key to gene cloning is to ensure that the target sequence is replicated in a suitable host cell.

The first recombinant DNA molecules were generated at Stanford University in 1972, utilising 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**; that is, 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 that became known as gene cloning (Fig. 1.3).

The discoveries in 1972 and 1973 triggered what is perhaps the biggest scientific revolution of all – the new genetics. The use of the new technology spread very quickly, and a sense of urgency and

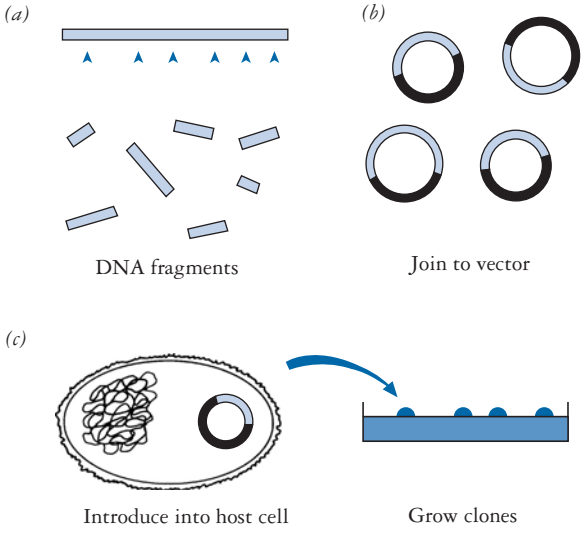

Fig. 1.3 Cloning DNA fragments. (*a*) The source DNA is isolated and fragmented into suitably sized pieces. (*b*) The fragments are then joined to a carrier molecule or vector to produce recombinant DNA molecules. In this case, a plasmid vector is shown. (*c*) The recombinant DNA molecules are then introduced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

excitement prevailed. This was dampened somewhat by the realisation that the new technology could give rise to potentially harmful organisms exhibiting undesirable characteristics. It is to the credit of the biological community that measures were adopted to regulate the use of gene manipulation and that progress in contentious areas was limited until more information became available regarding the possible consequences of the inadvertent release of organisms containing recombinant DNA. However, the development of genetically modified organisms (GMOs), particularly crop plants, has re-opened the debate about the safety of these organisms and the consequences of releasing GMOs into the environment. In addition, many of the potential medical benefits of gene manipulation, genetics, and cell biology pose ethical questions that may not be easy to answer. We will come across some of these issues later in the book.

The development and use of genetically modified organisms (GMOs) pose some difficult ethical questions that do not arise in other areas such as gene cloning.

1.4 What's in store?

In preparing the third edition of this book, I have retained the general organisation of the second edition. The content has been updated to better reflect the current applications of DNA technology and genetics, and several new subsections have been added to most of the chapters. However, I have again retained introductory material on molecular biology, on working with nucleic acids, and on the basic methodology of gene manipulation. I hope that this edition will

therefore continue to serve as a technical introduction to the subject, whilst also giving a much broader appreciation of the applications of this exciting range of technologies.

The text is organised into three parts.

Part I (*The basis of genetic engineering*; Chapters 2–4) deals with a basic introduction to the field and the techniques underpinning the science. Chapter 2 (*Introducing molecular biology*) and Chapter 3 (*Working with nucleic acids*) provide background information about DNA and the techniques used when working with it. Chapter 4 (*The tools of the trade*) looks at the range of enzymes needed for gene manipulation.

Part II (*The methodology of gene manipulation*; Chapters 5–9) outlines the techniques and strategies needed to clone and identify genes. Chapter 5 (*Host cells and vectors*) and Chapter 6 (*Cloning strategies*) describe the various systems and protocols that may be used to clone DNA. Chapter 7 is dedicated to the polymerase chain reaction, which has now become established as a major part of modern molecular biology. Chapter 8 (*Selection, screening, and analysis of recombinants*) describes how particular DNA sequences can be selected from collections of cloned fragments. Chapter 9 (*Bioinformatics*) is a new chapter that has been added to deal with the emergence of this topic.

Part III (*Genetic engineering in action*; Chapters 10–15) deals with the applications of gene manipulation and associated technologies. Chapters include *Understanding genes, genomes, and 'otheromes'* (Chapter 10), *Genetic engineering and biotechnology* (Chapter 11), *Medical and forensic applications of gene manipulation* (Chapter 12), and *Transgenic plants and animals* (Chapter 13). Organismal cloning is examined in Chapter 14 (*The other sort of cloning*), and the moral and ethical considerations of genetic engineering are considered in Chapter 15 (*Brave new world or genetic nightmare?*).

Each chapter is supplemented with some study guidelines to enable the student to use the text productively.

In the third edition I have expanded the range of features that should be useful as study aids where the text is used to support a particular academic course. There are now **text boxes** sprinkled throughout the chapters. The text boxes highlight key points on the way through the text and can be used as a means of summarising the content. At the start of each chapter the **aims** of the chapter are presented, along with a **chapter summary** in the form of **learning outcomes**. These have been written quite generally, so that an instructor can modify them to suit the level of detail required. A list of the **key words** in each chapter is also provided for reference. As in the first and second editions, a **concept map** is given, covering the main points of the chapter. Concept mapping is a technique that can be used to structure information and provide links between various topics. The concept maps provided here are essentially summaries of the chapters and may be examined either before or after reading

the chapter. I hope that these support 'tools' continue to be a useful addition to the text for the student of genetic engineering.

Suggestions for further reading are given at the end of the book, along with tips for using the Internet and World Wide Web. No reference has been made to the primary (research) literature, as this is accessible from the books and articles mentioned in the further reading section and by searching literature databases. Many research journals are also now available online. A glossary of terms has also been provided; this may be particularly useful for readers who may be unfamiliar with the terminology used in molecular biology.