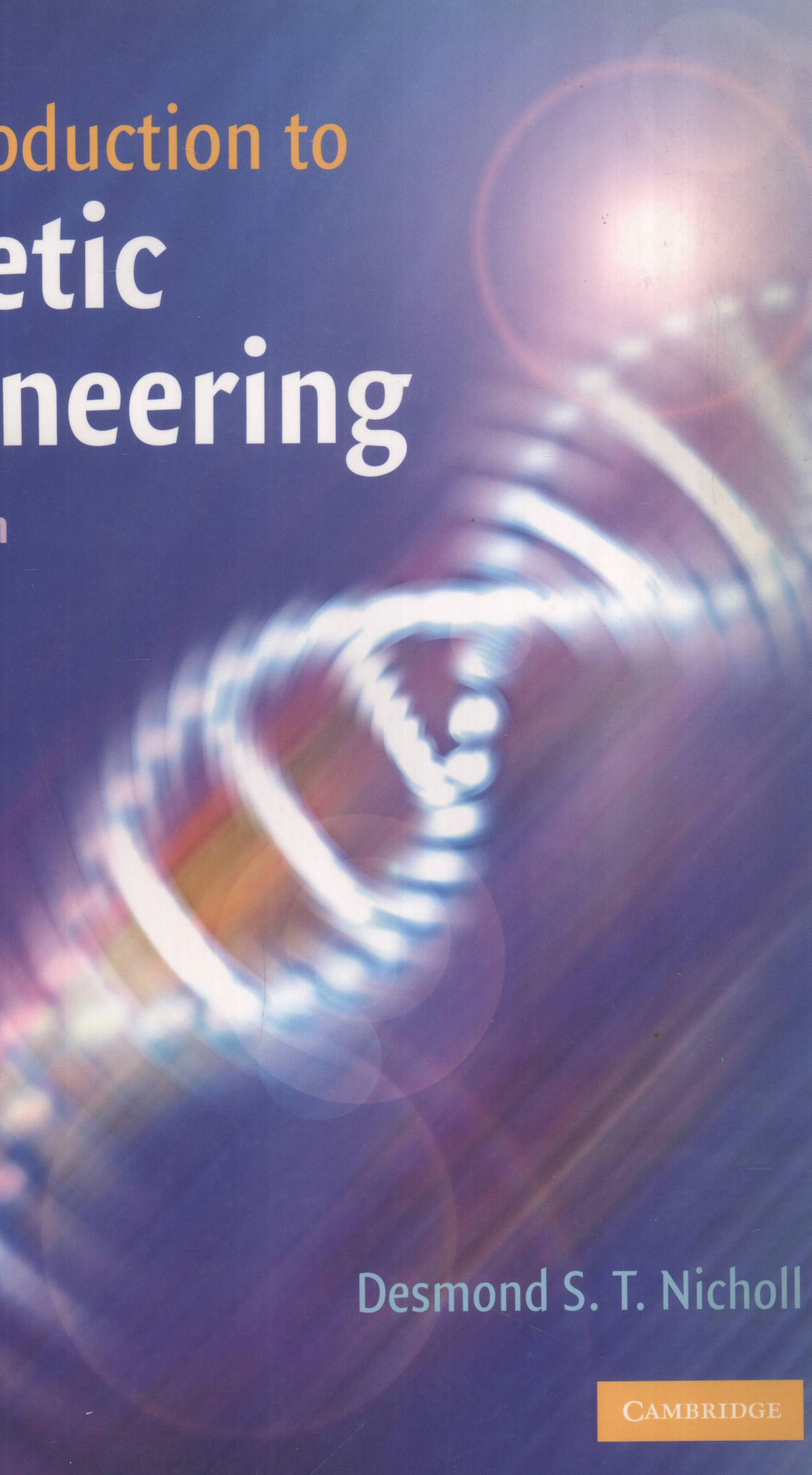
An Introduction to

# Genetic Engineering

Third Edition



Desmond S. T. Nicholl

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Desmond S. T. Nicholl
*University of the West of Scotland, Paisley, UK*



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# An Introduction to Genetic Engineering

## Third Edition

In this third edition of his popular undergraduate-level textbook, Desmond Nicholl recognises that a sound grasp of basic principles is vital in any introduction to genetic engineering. Therefore, as well as being thoroughly updated, the book also retains its focus on the fundamental principles used in gene manipulation. The text is divided into three sections: Part I provides an introduction to the relevant basic molecular biology; Part II, the methods used to manipulate genes; and Part III, applications of the technology. There is a new chapter devoted to the emerging importance of bioinformatics as a distinct discipline. Other additional features include text boxes, which highlight important aspects of topics discussed, and chapter summaries, which include aims and learning outcomes. These, along with key word listings, concept maps, and a glossary, will enable students to tailor their studies to suit their own learning styles and ultimately gain a firm grasp on this subject that students traditionally find difficult.

Desmond S. T. Nicholl is a Senior Lecturer in Biological Sciences at the University of the West of Scotland, Paisley, UK.

# Preface to the third edition

As I found when preparing the second edition of this text, advances in genetics continue to be made at an ever increasing rate, which presents something of a dilemma when writing an introductory text on the subject. In the years since the second edition was published, many new applications of gene manipulation technology have been developed, covering an increasingly diverse range of disciplines and applications. The temptation in preparing this third edition, as was the case for its predecessor, was to concentrate on the applications and ignore the fundamental principles of the technology. However, in initial preparation I was convinced that a basic technical introduction to the subject should remain the major focus 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, as a sound grasp of the basic principles is an important part of any introduction to genetic engineering. I have again been gratified by the many positive comments about the second edition, and I hope that this new edition continues to serve a useful purpose as part of the introductory literature on this fascinating subject.

In trying to strike a balance between the methodology and the applications of gene manipulation, I have retained the division of the text into three sections. **Part I** deals with an introduction to 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 second edition, there have been some additional changes made. The emerging importance of bioinformatics as a distinct discipline is recognised by a new chapter devoted to this topic. To help the student of genetic engineering, two additional features have been included. **Text boxes** highlight some of the important aspects of the topics, and **chapter summaries** have been provided, which include aims and learning outcomes along with a listing of **key words**. Along with the concept maps, I hope that these additions will help the reader to make sense of the topics and act as a support for studying the content. By using the summaries, key words, text boxes, and concept maps students should be able to tailor their study to suit their own individual learning styles. 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 despite an overall increase in coverage.

My thanks go to my colleagues Peter Birch and John McLean for comments on various parts of the manuscript, also to Don Powell of the Wellcome Trust Sanger Institute for advice and critical comment on Chapter 9. Their help has made the book better; any errors of fact or interpretation of course remain my own responsibility. Special thanks to Katrina Halliday and her colleagues at Cambridge University Press, and to Katie Greczylo of Aptara, Inc., for their cheerful advice and patience, which helped bring the project to its conclusion. My final and biggest thank-you goes as ever to my wife, Linda, and to Charlotte, Thomas, and Anna. They have again suffered with me during the writing, and have put up with more than they should have had to. I dedicate this new edition to them, with grateful thanks.

Desmond S. T. Nicholl
Paisley 2007

# Contents

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# 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.

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.

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

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

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

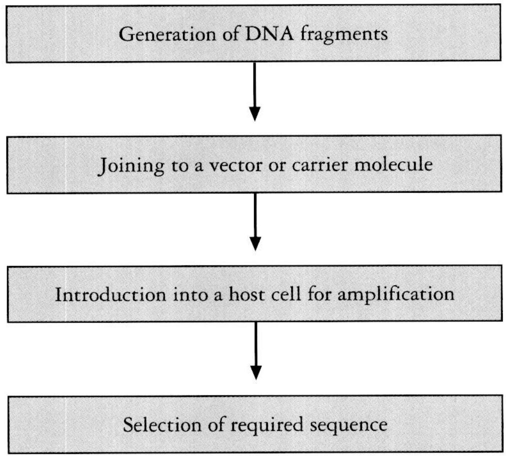


**Fig. 1.1** The four steps in a gene cloning experiment. The term 'clone' comes from the colonies of identical host cells produced during amplification of the cloned fragments. Gene cloning is sometimes referred to as 'molecular cloning' to distinguish the process from the cloning of whole organisms.

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

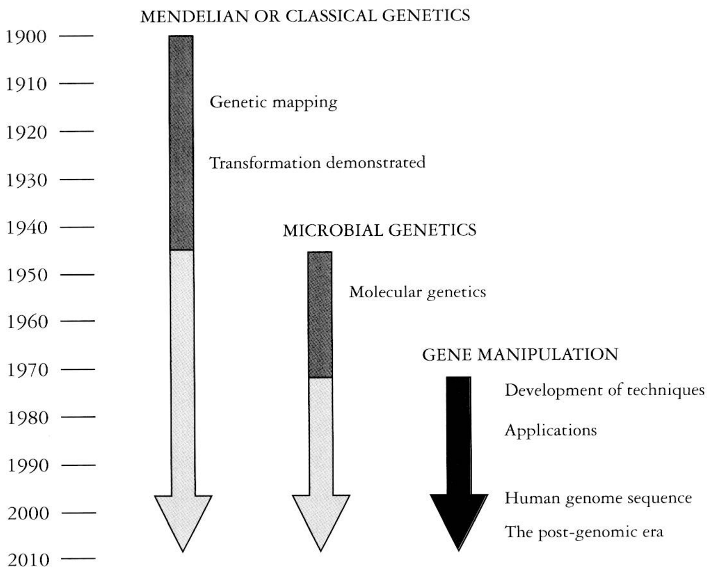


**Fig. 1.2** The history of genetics since 1900. Shaded areas represent the periods of major development in each branch of the subject.

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

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

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.