GENE CLONING

An introduction

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Second edition

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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 One, 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 Two 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 Three 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 Three 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.

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Contents

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.

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Part One

The Basic Principles of Gene Cloning

Why gene cloning is important

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Just over a century ago, 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 DNA to be the genetic material; up to 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 (Delbruck, 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

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 1971–73 genetic research was thrown back into gear by what can only be described as a revolution in modern biology. A whole new methodology was developed, allowing 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 the third great age of genetics. We are still in the midst of the boom caused by this revolution 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 **chimaera** or **recombinant DNA molecule**.
2. The vector acts as a **vehicle** that transports the gene into a host cell, which is usually a bacterium, although other types of living cell can be used.
3. Within the host cell the vector multiplies, producing numerous identical copies not only of itself but also of the gene that it carries.
4. When the host cell divides, copies of the recombinant DNA molecule are passed to the progeny and further vector replication takes place.
5. After a large number of cell divisions, a colony, or **clone**, of identical host cells is produced. Each cell in the clone contains one or more copies of the recombinant DNA molecule; the gene carried by the recombinant molecule is now said to be cloned.

1.4 GENE CLONING REQUIRES SPECIALIZED TOOLS AND TECHNIQUES

1.4.1 VEHICLES

The central component of a gene cloning experiment is the vehicle, which transports the gene into the host cell and is responsible for its replication. To act as a cloning vehicle a DNA molecule must be capable of entering a host cell and, once inside, replicating to produce multiple copies of itself. Two naturally occurring types of DNA molecule satisfy these requirements:

1. **Plasmids**, which are small circles of DNA found in bacteria and some other organisms. Plasmids can replicate independently of the host cell chromosome.

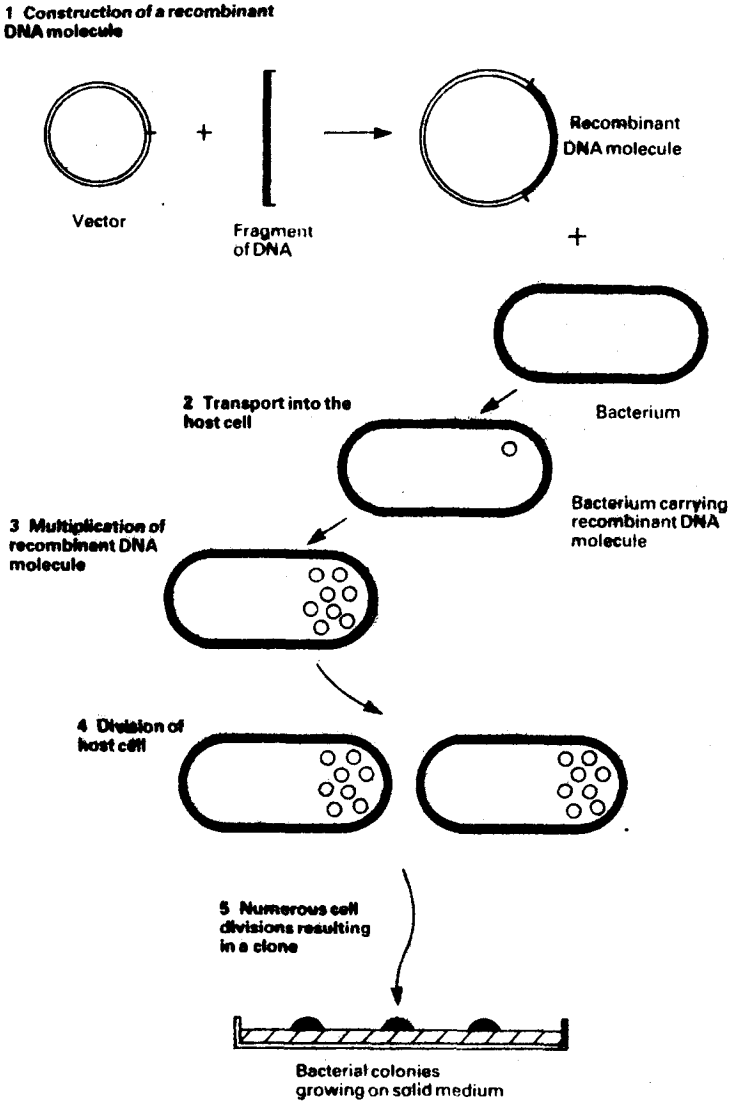

Figure 1.1 The basic steps in gene cloning.

2. **Virus chromosomes**, in particular the chromosomes of **bacteriophages**, which are viruses that specifically infect bacteria. During infection the bacteriophage DNA molecule is injected into the host cell where it undergoes replication.

Chapter 2 covers the basic features of plasmids and bacteriophage chromosomes, providing the necessary background for an understanding of how these molecules are used as cloning vehicles.

1.4.2 TECHNIQUES FOR HANDLING DNA

Plasmids and bacteriophage DNA molecules display the basic properties required of potential cloning vehicles. But this potential would be wasted without experimental techniques for handling DNA molecules in the laboratory. The fundamental steps in gene cloning, as described on p. 4 and in Figure 1.1, require several manipulative skills (Table 1.1). First, pure samples of DNA must be available, both of the cloning vehicle and of the gene to be cloned. The methods used to purify DNA from living cells are outlined in Chapter 3.

Having prepared samples of DNA, construction of a recombinant DNA molecule requires that the vector be cut at a specific point and then repaired in such a way that the gene is inserted into the vehicle. The ability to manipulate DNA in this way is an offshoot of basic research into DNA synthesis and modification within living cells. The discovery of enzymes that can cut or join DNA molecules in the cell has led to the purification of **restriction endonucleases** and **ligases**, which are now used to construct recombinant DNA molecules in the test-tube. The properties of these enzymes, and the way they are used in gene cloning experiments, are described in Chapter 4.

Once a recombinant DNA molecule has been constructed, it must be introduced into the host cell so that replication can take place. Transport into the host cell makes use of natural processes for uptake of plasmid and viral DNA molecules. These processes, and the ways they are utilized in gene cloning, are described in Chapter 5.

Table 1.1 Basic skills needed to carry out a simple gene cloning experiment

1. Preparation of pure samples of DNA	(Chapter 3)
2. Cutting DNA molecules	(Chapter 4, pp. 56/65)
3. Analysis of DNA fragment sizes	(Chapter 4, pp. 65/72)
4. Joining DNA molecules together	(Chapter 4, pp. 73/83)
5. Introduction of DNA into host cells	(Chapter 5, pp. 84/90)
6. Identification of cells that contain recombinant DNA molecules	(Chapter 5, pp. 90/100, and Chapter 8)