1 Why gene cloning is important

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 (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

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 the third great age of genetics. Twenty-five years later we are still riding the rollercoaster set in motion by the gene cloning 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.

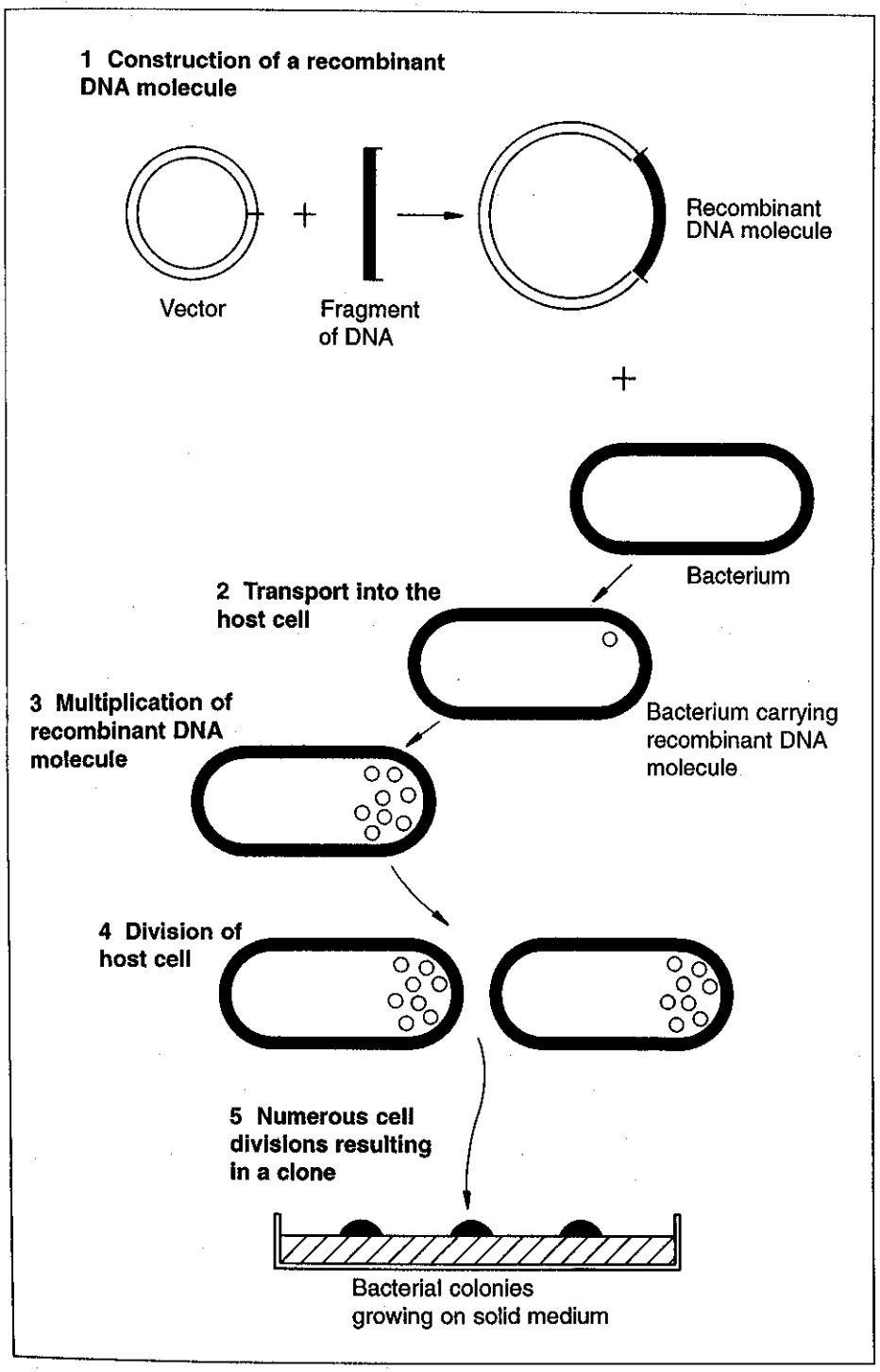


Figure 1.1 The basic steps in gene cloning.

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:

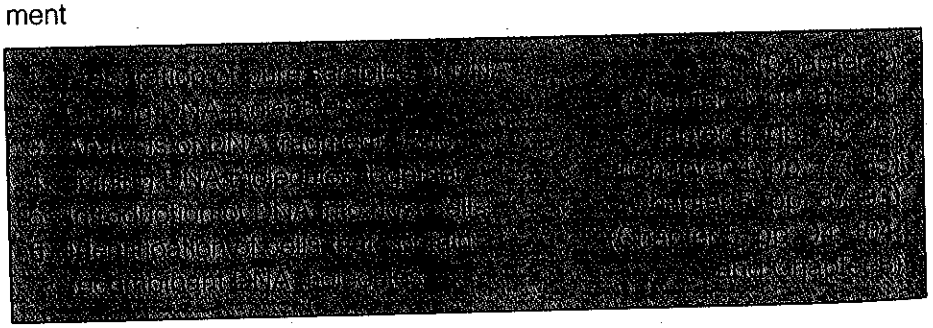
1. **Plasmids**, which are small circles of DNA found in bacteria and some other organisms. Plasmids can replicate independently of the host cell chromosome.
2. **Virus chromosomes**, in particular the chromosomes of **bacteriophages**, which are viruses that specifically infect bacteria. During infection 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.

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



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.

1.4.3 The diversity of cloning vectors

Although gene cloning is relatively new, it has nevertheless developed into a very sophisticated technology. Today a wide variety of different cloning vectors are available. Almost all of these are derived from naturally occurring plasmids or viruses, but most have been modified in various ways so that each one is suited for a particular type of cloning experiment. In Chapters 6 and 7 the most important types of vector are described, and their uses examined.

1.5 WHY GENE CLONING IS SO IMPORTANT

As you can see from Figure 1.1, gene cloning is a relatively straightforward procedure. Why then has it assumed such importance in biology? The answer is largely because cloning can provide a pure sample of an individual gene, separated from all the other genes that it normally shares the cell with.

To understand exactly how this works, consider a gene cloning experiment drawn in a slightly different way (Figure 1.2). In this example the DNA fragment to be cloned is one member of a mixture of many different fragments, each carrying a different gene or part of a gene. This mixture could indeed be the entire genetic complement of an organism, a human for instance. All these fragments will become inserted into different vector molecules to produce a family of recombinant DNA molecules, one of which carries the gene of interest. Usually only one recombinant DNA molecule will be transported into any single host cell, so that although the final set of clones may contain many different recombinant DNA molecules, each individual clone contains multiple copies of just one molecule. The gene is now separated away from all the other genes in the original mixture, and its specific features can be studied in detail.

In practice, the key to the success or failure of a cloning experiment is the ability to identify the particular clone of interest from the many different ones that are obtained. If we consider the

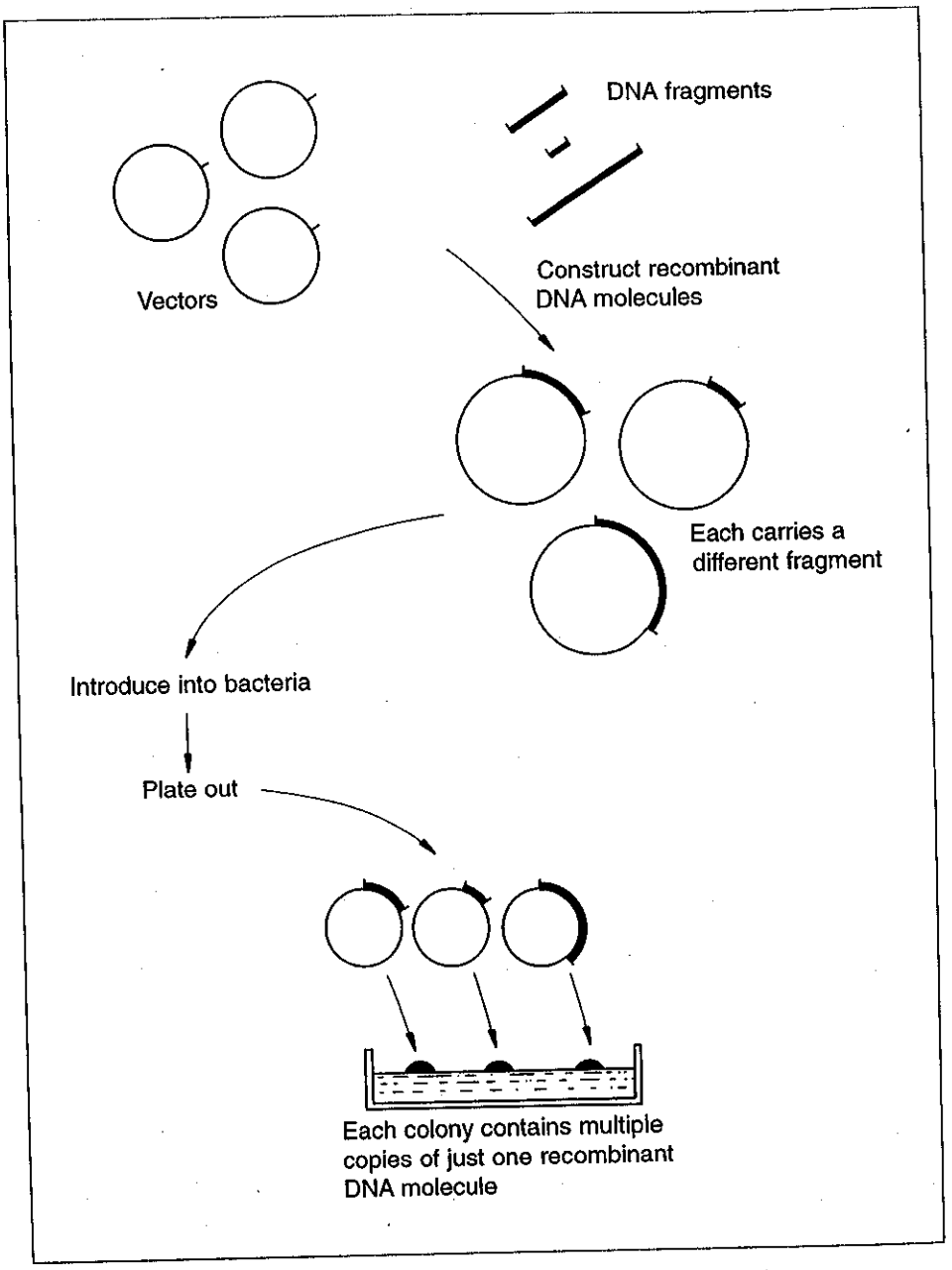


Figure 1.2 Cloning allows individual fragments of DNA to be purified.

genome of the bacterium *Escherichia coli*, which contains something in the region of 4000 different genes, we might at first despair of being able to find just one gene among all the possible clones (Figure 1.3). The problem becomes even more overwhelming when we remember that bacteria are relatively simple organisms and that the human genome contains about 20 times as many genes. However, as explained in Chapter 8, a variety of different strategies can be used to ensure that the correct gene can be obtained at the end of the cloning experiment. Some of these strategies involve modifications to the basic cloning procedure, so that only cells containing the desired recombinant DNA molecule can divide and the clone of interest is automatically **selected**. Other methods involve

techniques that enable the desired clone to be identified from a mixture of lots of different clones.

Once a gene has been cloned there is almost no limit to the information that can be obtained about the structure and expression of that gene. The availability of cloned material has stimulated the development of analytical methods for studying genes, with new techniques being introduced all the time. Methods for studying the structure and expression of a cloned gene are described in Chapters 9 and 10 respectively.

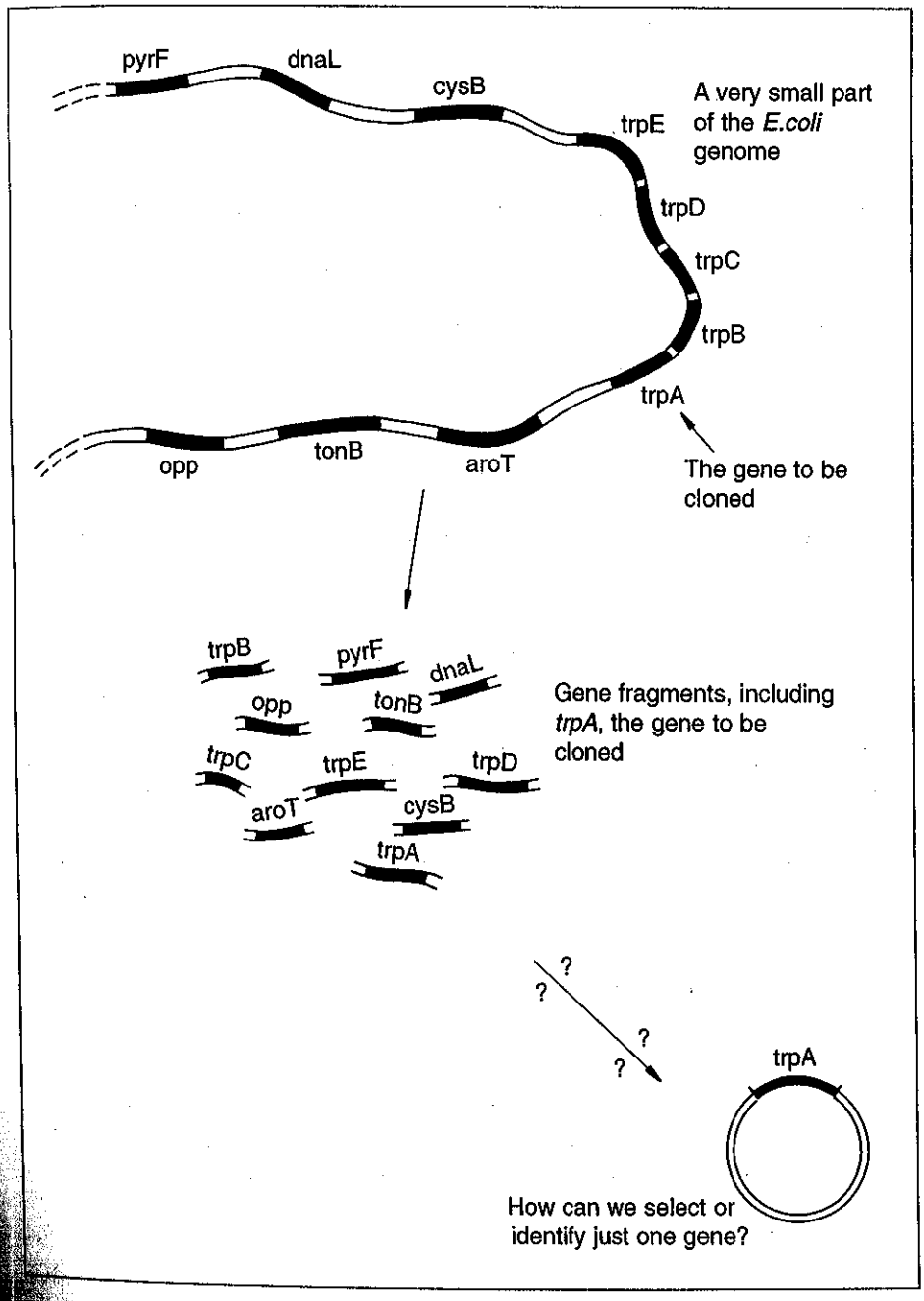


Figure 1.3 The problem of selection.

1.6 WHY THE POLYMERASE CHAIN REACTION IS ALSO IMPORTANT

Until the late 1980s cloning was the only means of obtaining a pure sample of an individual gene. This is no longer the case as the exquisitely simple but extraordinarily powerful **polymerase chain reaction (PCR)** provides today's biologists with a second approach to gene isolation. In a PCR experiment a single segment of a DNA molecule is copied many times, resulting in an amplified DNA fragment (Figure 1.4). The experiment is designed so that the segment of DNA that is amplified is one that carries a gene of interest. The result is therefore the same as with cloning: a pure sample of a single gene is obtained. PCR is a rapid technique, much less complicated than gene cloning, and can amplify millions of copies of a gene from just one starting molecule. This tremendous sensitivity means that PCR can be used to isolate genes from single cells, or from material such as dried bloodstains or even the bones of long-dead humans. The polymerase chain reaction and its many applications are covered in Chapter 11.

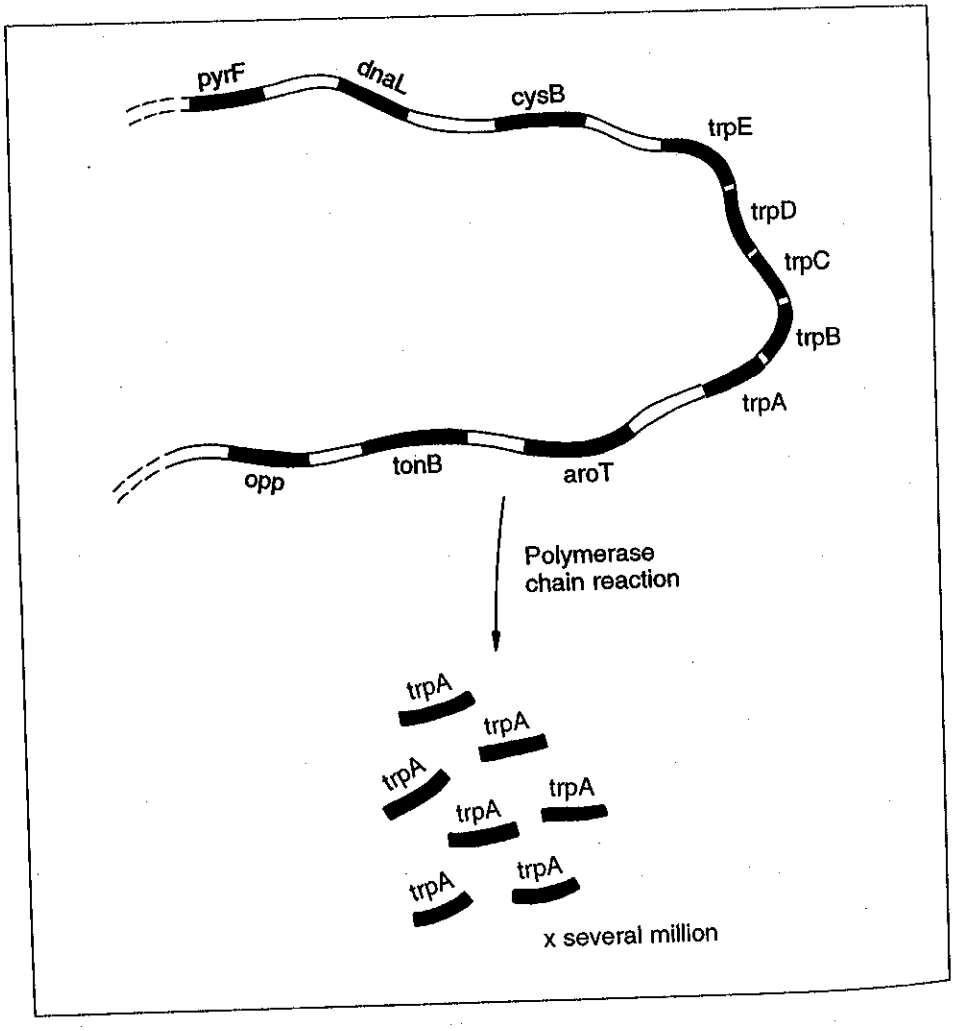


Figure 1.4 The polymerase chain reaction enables an individual gene to be amplified.

1.7 THE IMPACT OF RECOMBINANT DNA TECHNIQUES ON RESEARCH AND BIOTECHNOLOGY

There are very few areas of biological research that have not been touched by gene cloning, PCR, and the recombinant DNA techniques that these procedures have made possible. In industry, for example, the ability to clone genes has led to far-reaching advances in **biotechnology**. For many years microorganisms have been used as living factories for the production of useful compounds. Examples are provided by antibiotics, such as penicillin, which is synthesized by a fungus called *Penicillium*, and streptomycin, produced by the bacterium *Streptomyces griseus*. Gene cloning has revolutionized biotechnology, most notably in providing a way in which mammalian proteins can be produced in bacterial cells. A remarkable property of a cloned gene is that it can often be made to function in an organism totally unrelated to that in which it is normally found. For example, an animal gene can be transferred by cloning into a bacterium and then induced by some careful modifications to carry on working as though nothing had happened (Figure 1.5). The implications are enormous. Genes controlling the

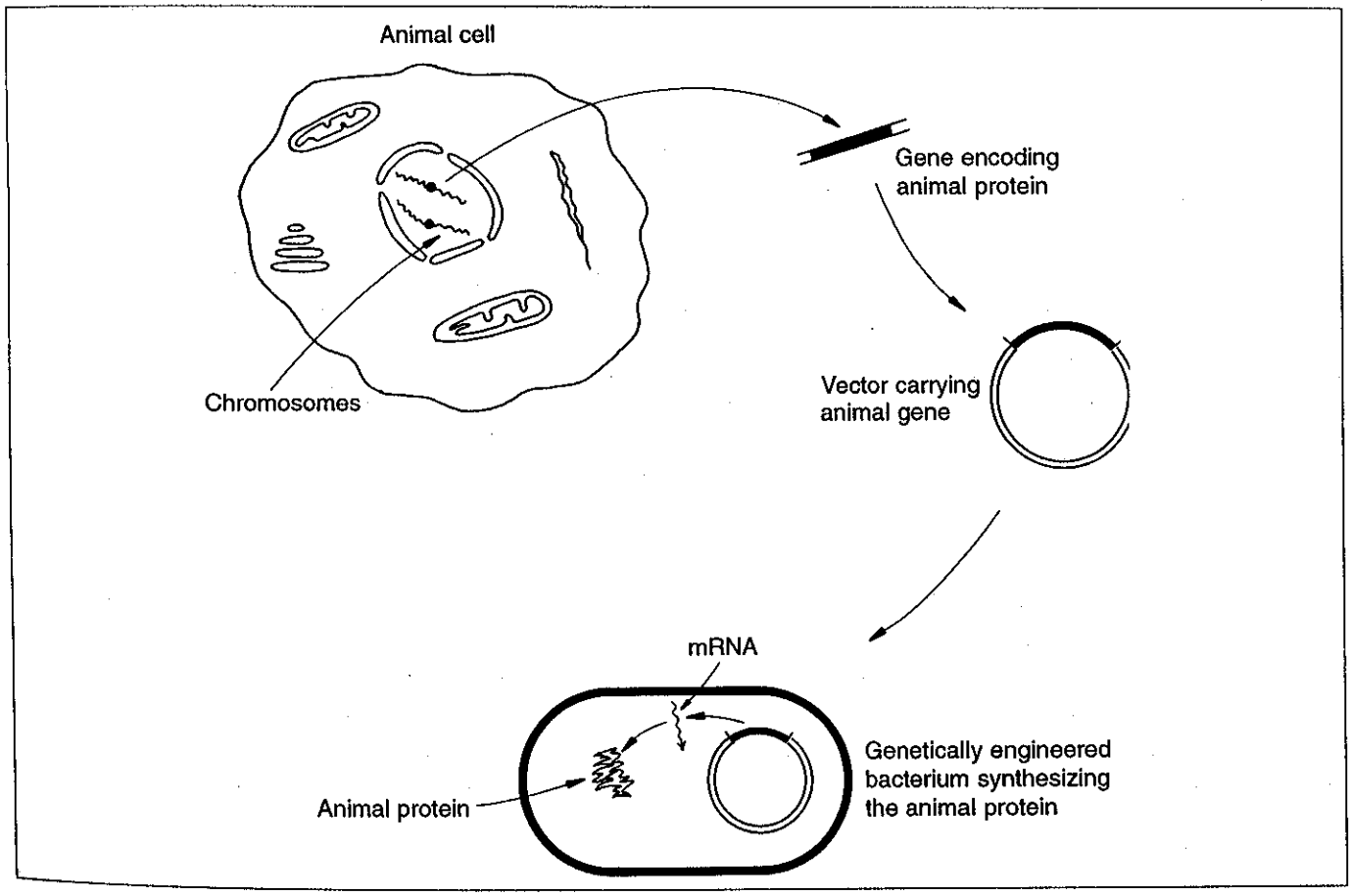


Figure 1.5 A possible scheme for the production of an animal protein by a bacterium.

synthesis of important pharmaceuticals, such as drugs and hormones, can be taken from the organism in which they occur naturally, but from which they may be costly and difficult to prepare, and placed in a bacterium or other type of organism, from which the product can be recovered conveniently and in large quantities. A number of successes have been notched up by biotechnologists, with recombinant insulin being the most noteworthy achievement so far. Production of protein by cloned genes is described in Chapter 12.

Medical and agricultural research have also received important boosts from gene cloning. New types of vaccines, providing protection against diseases for which vaccination was previously impossible, have been developed thanks to the ability to clone genes. Many inherited diseases can now be diagnosed in an unborn child, and recent research has led to the hope that cystic fibrosis, breast cancer and other heart-rending diseases will soon be treatable. In agriculture, equally important problems are being addressed through the development of genetically engineered crops able to withstand the ravages of insects. The ways in which gene cloning is being applied in these areas of research are described in the final two chapters of this book.

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