19 Gene technology

The discovery of the structure of DNA by Watson and Crick in the early 1950s and discoveries since have led to many applications of gene technology in areas of medicine, agriculture and forensic science. This topic relies heavily on prior knowledge of DNA structure and protein synthesis studied in the topic on Nucleic acids and protein synthesis. Where possible, students should carry out practical work using electrophoresis, either with DNA or specially prepared dyes used to represent DNA or proteins.

19.1 Principles of genetic technology

Genetic engineering involves the manipulation of naturally occurring processes and enzymes.

Genome sequencing gives information about the location of genes and provides evidence for the evolutionary links between organisms.

By the end of this section you should be able to:

a) define the term recombinant DNA
b) explain that genetic engineering involves the extraction of genes from one organism, or the synthesis of genes, in order to place them in another organism (of the same or another species) such that the receiving organism expresses the gene product
c) describe the principles of the polymerase chain reaction (PCR) to clone and amplify DNA (the role of *Taq* polymerase should be emphasised)
d) describe and explain how gel electrophoresis is used to analyse proteins and nucleic acids, and to distinguish between the alleles of a gene
e) describe the properties of plasmids that allow them to be used in gene cloning
f) explain why promoters and other control sequences may have to be transferred as well as the desired gene
g) explain the use of genes for fluorescent or easily stained substances as markers in gene technology
h) explain the roles of restriction endonucleases, reverse transcriptase and ligases in genetic engineering
i) explain, in outline, how microarrays are used in the analysis of genomes and in detecting mRNA in studies of gene expression

Introducing genetic engineering

Genetic engineering is the technique of changing the genetic constitution of an organism, brought about by means other than conventional breeding and which usually would not occur in nature. It involves the transfer of a gene or genes and other sequences from one species to another – often an unrelated species, so that the host organism is able to express a new gene product. The outcome is new varieties of organisms, often (but not exclusively) of microorganisms. These are described as genetically modified (GM) or **transgenic** organisms containing **recombinant DNA**. This type of DNA is formed by laboratory techniques, bringing together genetic material from different sources. Transgenic organisms produce, in their cells, proteins that were not previously part of their species **proteome**. The proteome is the complete set of proteins that a cell or organism can make. These additional proteins, many of them enzymes, have been engineered with the intention of bringing about significant change in the host organism, usually with a specific purpose in mind.

The techniques of the genetic engineer offer numerous practical benefits in biotechnology, medicine and agriculture, and also to science and the study of how genes operate in the control of cells of organisms. At the same time, outcomes of genetic engineering may generate environmental and **ethical issues** of concern to society. We will return to this issue later.

Other applications

Today, in addition to the creation of GM organisms, gene technologies are applied to:

- **DNA sequencing** – the creation of genomic libraries of the precise sequence of nucleotides in samples of DNA of individual organisms. The nucleotide sequence in the whole human genome was the product of the Human Genome Project. Today, other genomes have been completely sequenced, including those of some viruses, bacteria, fungi and many other eukaryotes.
- **Genetic fingerprinting** – in which DNA is analysed in order to identify the individual from which the DNA was taken to establish identity and the genetic relatedness of individuals. It is now commonly used in forensic science (for example to identify someone from a sample of blood or other body fluid). It is also used to determine whether individuals of endangered species in captivity have been bred or captured in the wild.

The gene technologist's tool kit

It is the discovery and isolation of four naturally-occurring enzymes that make possible the manipulation of individual genes. We have already met these enzymes in other contexts, but it is significant that, in this context, some of the enzymes used are now obtained from bacteria we call extremophiles (page 431). In these organisms, cells (and therefore their enzymes) are adapted to function at optimum rates under some extreme conditions, including, for example, very high or low temperatures. We shall discuss how these enzymes work shortly. They are introduced in Table 19.1.

Question

1 You have studied how the virus called HIV infects a human cell (page 205). What is the role of reverse transcriptase in this process?

Table 19.1 The genetic technologist's toolkit of enzymes

These enzymes are extracted mainly from microorganisms or viruses and are used to manipulate nucleic acids in very precise ways		
Enzyme	**Natural source**	**Application in genetic engineering**
restriction enzyme (restriction endonuclease)	cytoplasm of bacteria (combats viral infection by breaking up viral DNA)	breaks DNA molecules into shorter lengths, at specific nucleotide sequences
DNA ligase	with nucleic acid in the nucleus of *all* organisms	joins together DNA molecules during replication of DNA
DNA polymerase	with nucleic acid in the nucleus of *all* organisms	synthesises nucleic acid strands, guided by a template strand of nucleic acid
reverse transcriptase	in retroviruses only	synthesises a DNA strand (cDNA) complementary to an existing RNA strand

Gene technology–an early break through

One of the earliest successful applications of these techniques was when the human genes for **insulin** production were transferred to a strain of the bacterium *Escherichia coli*. Insulin consists of two short polypeptides linked together by sulfide bonds. Once the hormone is assembled from its component polypeptides, it enables body cells to regulate blood sugar levels (page 293). Regular supplies of insulin are required to treat insulin-dependent diabetes. Cultures of *E. coli* have been 'engineered' to manufacture and secrete human insulin when cultured in a bulk fermenter with appropriate nutrients. The insulin is extracted and made available for clinical use.

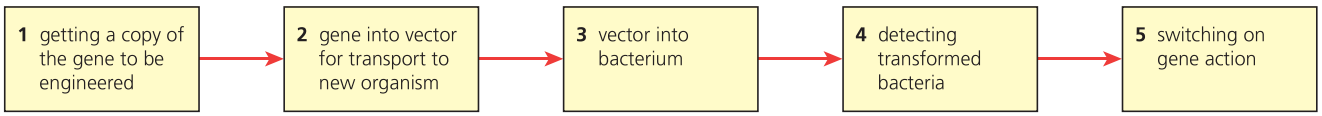


Figure 19.1 The steps in the genetic engineering of *E. coli* for insulin production

The steps in the genetic engineering of *E. coli* for insulin production

Step 1: Obtaining a copy of the human insulin gene by isolating mRNA

Question

2 Outline
- **a** why we describe DNA as double stranded
- **b** where DNA polymerase occurs and its role in the cell.

One way to obtain a copy of the gene for insulin involves starting with messenger RNA, rather than searching for the gene itself among the chromosomes. The human pancreas contains patches of cells (the islets of Langerhans, Figure 14.7, page 293) where insulin is produced. Here the relevant gene in the nuclei of the cells is transcribed to produce messenger RNA. This passes out of the nucleus to the ribosomes in the cytoplasm. Here the base sequence of the RNA is translated into the linear sequence of amino acids of the insulin protein.

There is a particular advantage in making a copy of a gene from the messenger RNA it codes for. This is explained later in this topic. First, however, the process. This involves:

- messenger RNA for insulin being isolated from a sample of tissue from a human pancreas
- the use of the enzyme **reverse transcriptase** (obtained from a retrovirus other than HIV), alongside the isolated messenger RNA to form a single strand of DNA
- the conversion of this DNA into double-stranded DNA using **DNA polymerase** (Figure 19.2). In this way the gene is manufactured. This form of an isolated gene is known as **complementary DNA (cDNA)**.

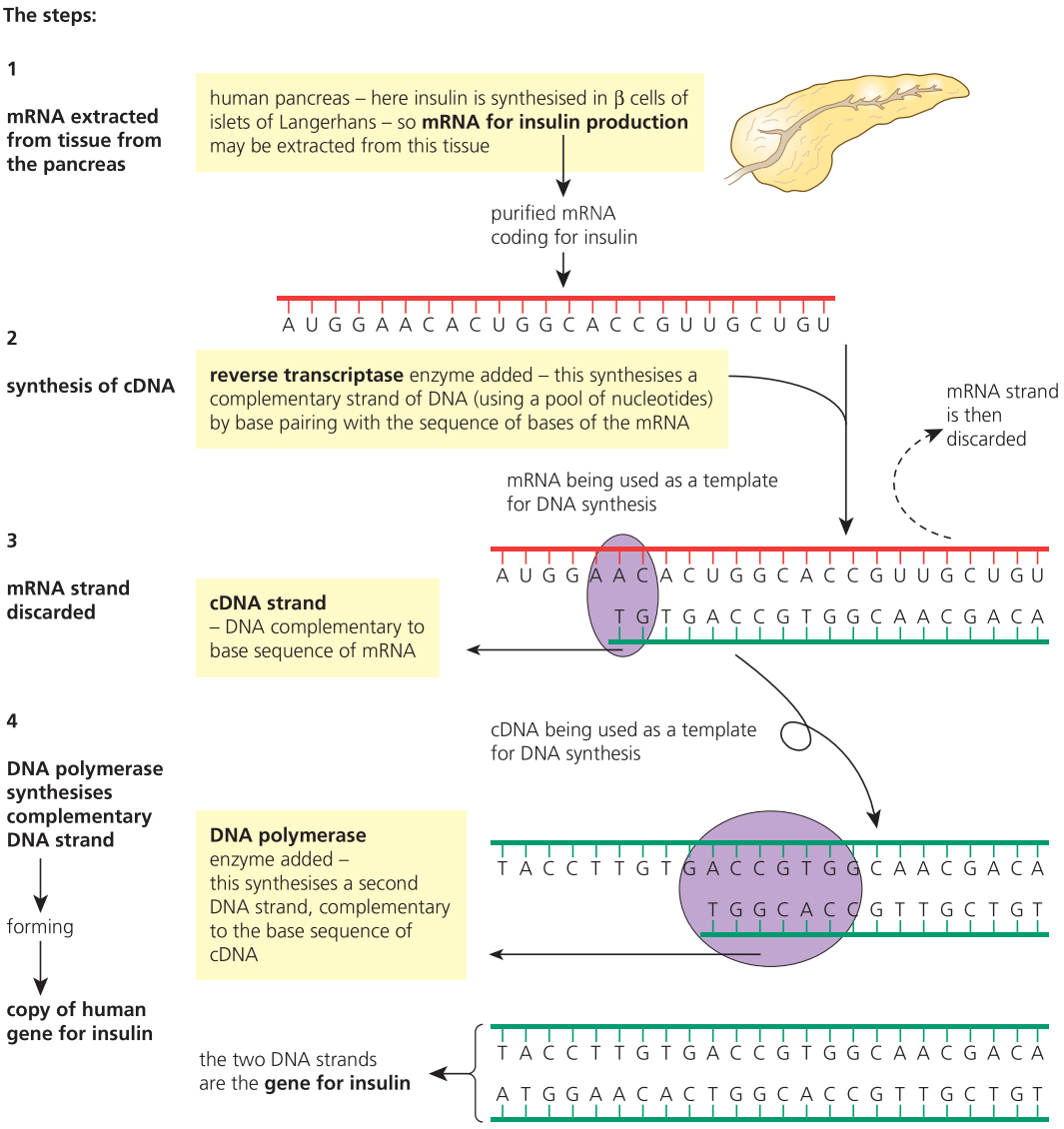


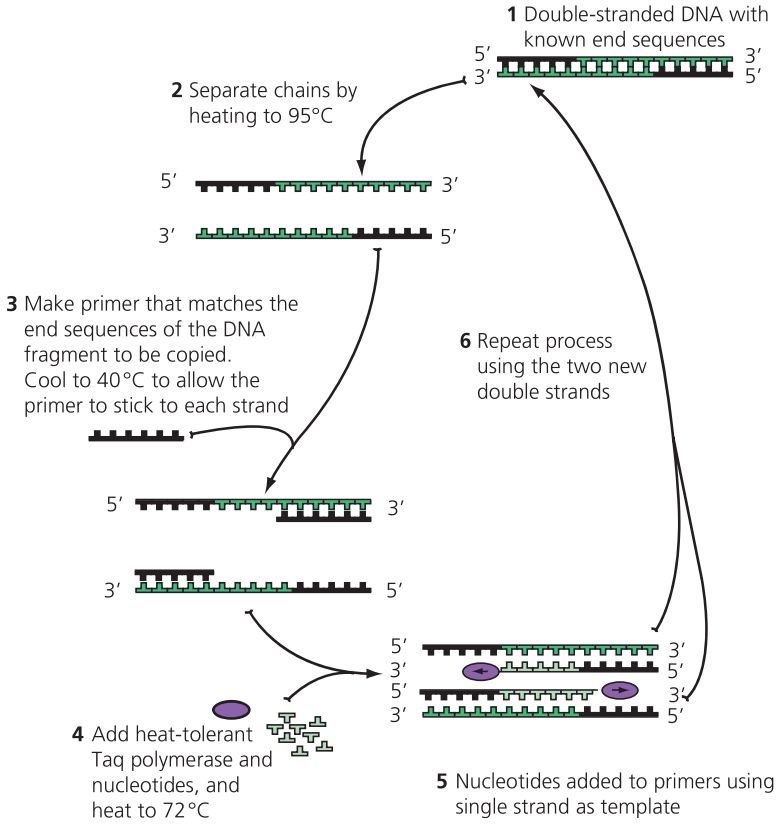
Figure 19.2 Using reverse transcriptase to build the gene for human insulin

Once the gene is isolated, the double-stranded DNA undergoes amplification; many copies are made. There are various situations in which a genetic engineer is able to produce or recover only a very small amount of DNA (such as at a crime scene). It is now possible to submit such minute DNA samples to a process known as the **polymerase chain reaction (PCR)**. In the polymerase chain reaction, DNA is replicated in an entirely automated process, *in vitro,* to produce a large number of copies. A single molecule is sufficient as the starting material, should this be all that is available. The products are always exact copies. The heat-resistant polymerase enzyme used in this process is known as ***Taq* polymerase** after the thermophilic bacterium, *Thermus aquaticus*, found in hot springs and hydrothermal vents, from which it was originally isolated. This protein is able to remain an effective catalyst at a temperature of 97.5 °C for the length of time required in the PCR.

The polymerase chain reaction

The steps to the polymerase chain reaction are of special interest because they show us how important to genetic engineering was the discovery of the extremophiles with their uniquely adapted enzymes.

The polymerase chain reaction involves a series of steps, each taking a matter of minutes. The process involves a heating and cooling cycle and is automated.
Each time it is repeated in the presence of excess nucleotides, the number of copies of the original DNA strand is doubled.



Note: 'Primers' are short sequences of single-stranded DNA made syntheically with base sequences complimentary to one end (the 3′ end) of DNA.
Remember: DNA polymerase synthesises a DNA strand in the 3′ to 5′ direction.

Figure 19.3 The polymerase chain reaction

Question

3 **a** What is meant by *sticky ends*?
b How does a sticky end attach to a complementary sticky end?

Step 2: Inserting the DNA into a plasmid vector

In genetic engineering it is the **plasmids** of bacteria that are commonly used as the vector for the transference of amplified genes. Many bacterial cells have plasmids in addition to their chromosome. These are small, circular, double-stranded DNA molecules that are passed on to daughter cells when the bacterium divides. They were seen in Figure 1.23 (page 23) and are shown in Figure 19.5. Plasmids are isolated from the cytoplasm of a sample of the strain of bacteria being used for the amplification process.

The DNA of the plasmid is cut open using a **restriction enzyme** (restriction endonuclease). The restriction enzyme chosen cuts the DNA at a specific sequence of bases, known as the restriction site. The restriction enzyme selected leaves exposed specific DNA sequences, referred to as sticky ends. These are short lengths of unpaired bases and are formed at each cut end (Figure 19.4).

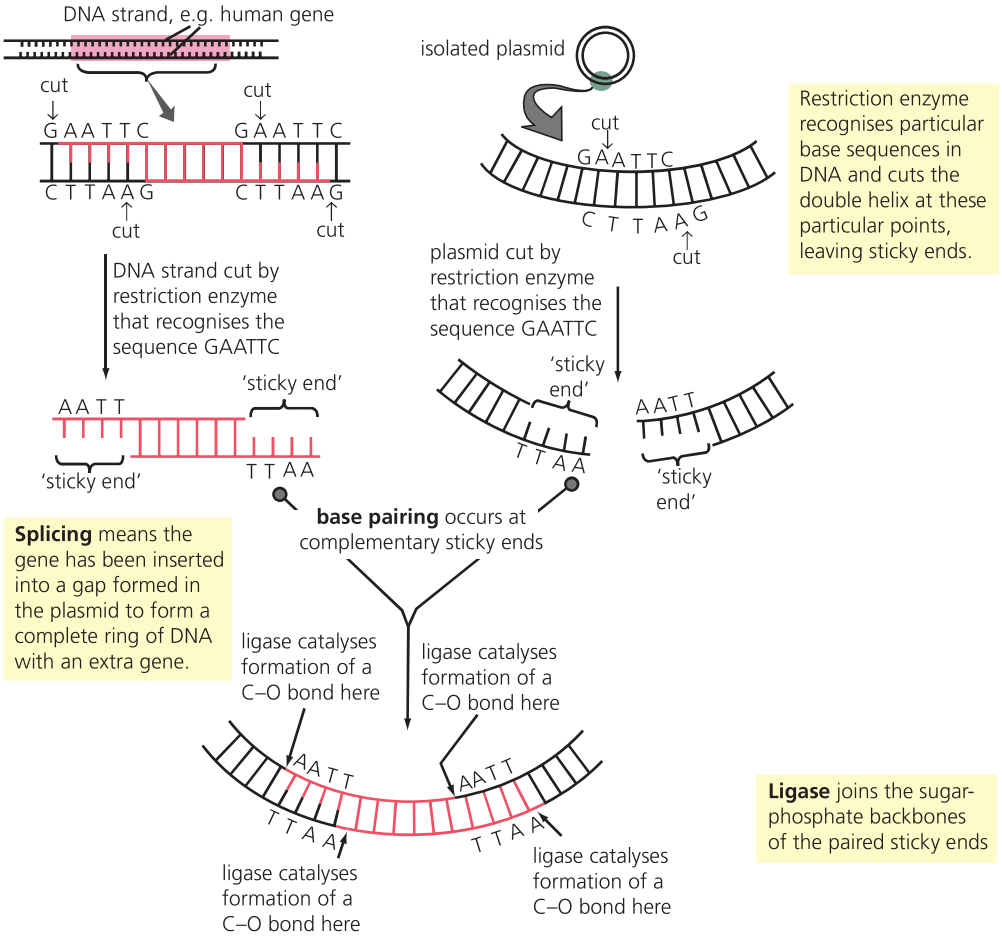


Figure 19.4 Gene splicing – the role of the restriction enzyme and ligase

Sticky ends with the same sequence as those on the plasmid are also created on the isolated insulin gene. This is done by adding short lengths of DNA that are then 'trimmed' with the same restriction enzyme. In this way complementary 'sticky ends' now exist at the free ends of the cut plasmids and the cDNA of the insulin gene, making it possible for them to be 'spliced' together into one continuous ring of DNA. The enzyme **ligase** catalyses the formation of a new C–O bond between ribose and phosphate of the DNA backbones being joined together. Energy from ATP is needed to bring the reaction about. The steps are summarised in Figure 19.5.

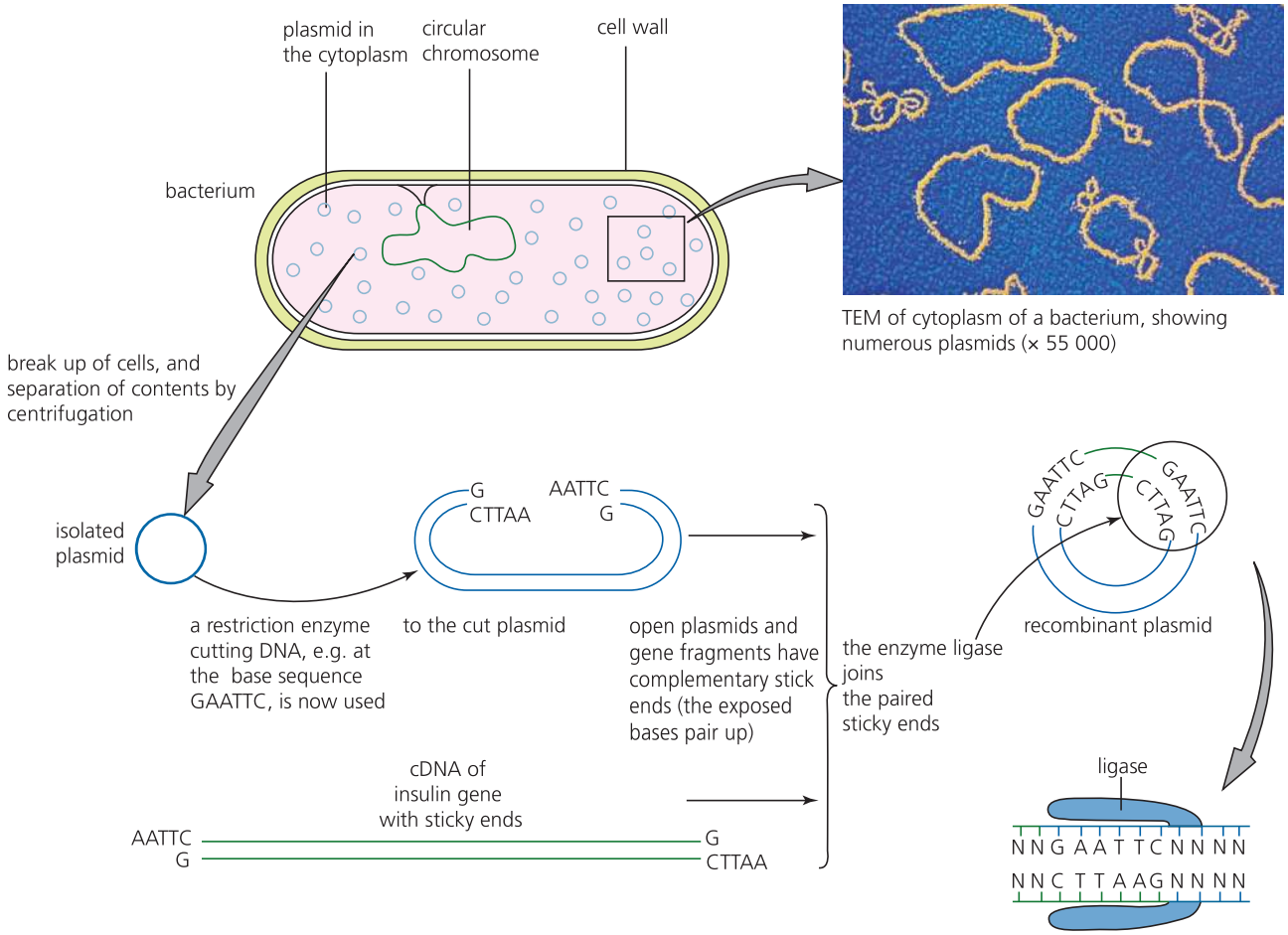
Extension

A note on restriction enzymes

Restriction enzymes occur naturally in bacteria. They are believed to have evolved as a defence mechanism against invading viruses because inside the bacterial cell they selectively cut up foreign DNA. Many different restriction enzymes have been identified as useful by genetic engineers. They are named after the bacterium from which they are isolated (genus, species and strain).

Table 19.2 Some commonly employed restriction enzymes

Name	Source organism	Recognition sequence
*Eco*RI	*Escherichia coli*	GAATTC
*Bam*HI	*Bacillus amyloliquefaciens*	GGATCC
*Hind*III	*Haemophilus influenza*	AAGCTT



TEM of cytoplasm of a bacterium, showing numerous plasmids (× 55 000)

Figure 19.5 Using a plasmid as the vector for the insulin gene

Step 3: Inserting the plasmid vector into the host bacterium

In the next step, recombinant plasmids have to be returned to bacterial cells. This is a challenge, since the cell wall is a barrier to entry. The ways this may be done are shown in Figure 19.6. Once this has been brought about, the bacteria are described as **transformed**.

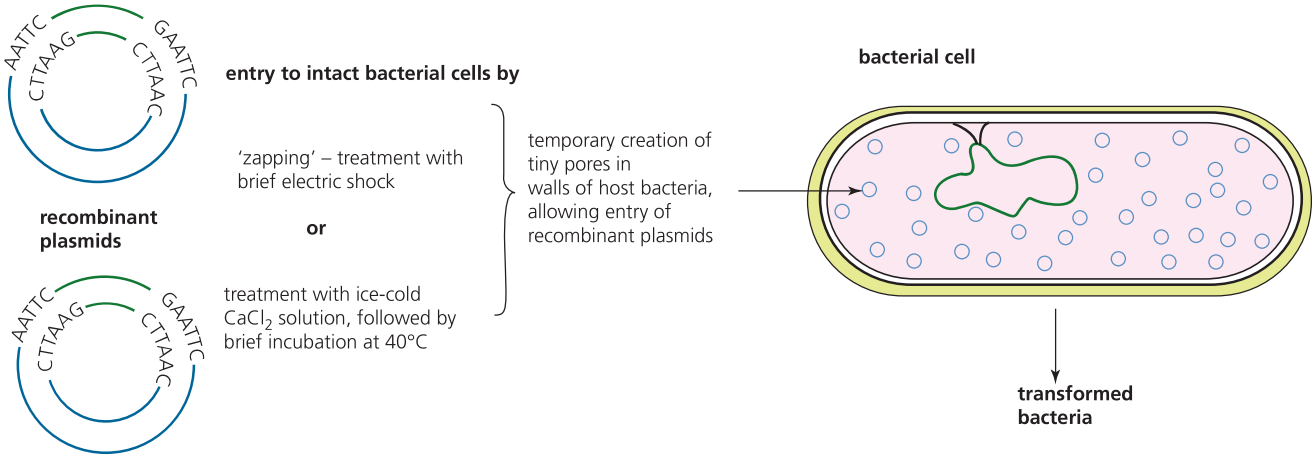


Figure 19.6 The return of recombinant plasmids to the bacterium

Step 4: Identifying transformed bacteria prior to cloning

Unfortunately, only a limited number of the cells of a culture of bacteria, treated as shown in Figure 19.6, will have successfully taken up recombinant plasmids and thus be able to synthesise insulin. The proportion of transformed bacteria may be as low as 1 per cent. It is only these bacteria that should be cloned and then cultured in a fermenter if a significant amount of product (insulin) is to be produced. One way they have been selected is by the use of **plasmids with antibiotic resistance genes (R-plasmids)**. The process is summarised in Figure 19.7.

Note that whilst the antibiotic resistance genes of R-plasmids were the first used markers, because of specific long-term safety issues that their use raises, other markers have been developed. We return to this issue later.

Step 5: Switching on gene action – why promoters are needed

The next stage is for the transformed bacteria to be cloned and then cultured in a fermenter. At this point the transferred gene needs to be 'switched on' so that the required protein – in this case, insulin – is produced in significant quantities. This product may then be extracted from the medium and purified.

In the living cell, some genes are expressed throughout the life of the cells, normally at quite low concentrations. We can assume in these cases that the proteins coded for are required constantly.

Other genes have to be activated, or switched on, first. One very efficient switch mechanism is the lac promoter, a promoter found in prokaryotic cells activated by the presence of lactose in the growth medium. A human gene such as the insulin gene, which is from a eukaryotic cell, has to be transferred into an **expression vector**, such as a plasmid so that a lac promoter occurs alongside it. The result is that, when lactose is present in the medium, recombinant bacteria containing this expression vector will be activated to produce large quantities of the desired polypeptides, insulin in this case, which can then be extracted.

Quaternary insulin production

We have already noted that although insulin is a relatively small protein, it has quite a complex quaternary structure (page 49). Insulin actually consists of two polypeptide chains, A and B, joined together by covalent disulfide bonds ($–CH_2–S–S–CH_2–$). Two separate genes code for the two polypeptides that make up insulin.

As a consequence, separate plasmids have to be engineered for the two chains of insulin, A and B. Genetically engineered *E coli* containing both types of plasmid are then selected.

A 'start' codon (ATG, also the code for methionine – see Figure 6.10, page 119) has to be inserted appropriately to the cDNA for both chain A and B. To stop the transcription process at the correct place, the 'stop' triplet codes (TAA followed by TAG) are added at the end of the cDNA, too. In this adjusted condition, both genes were added into plasmids with lac promoters.

Finally, when the two polypeptide chains are separated from the bacteria they are treated first with cyanogen bromide to cut them free from the longer polypeptide chain at the methionine amino acid. Then they are further treated to promote disulfide bond formation and development of a quaternary structure.

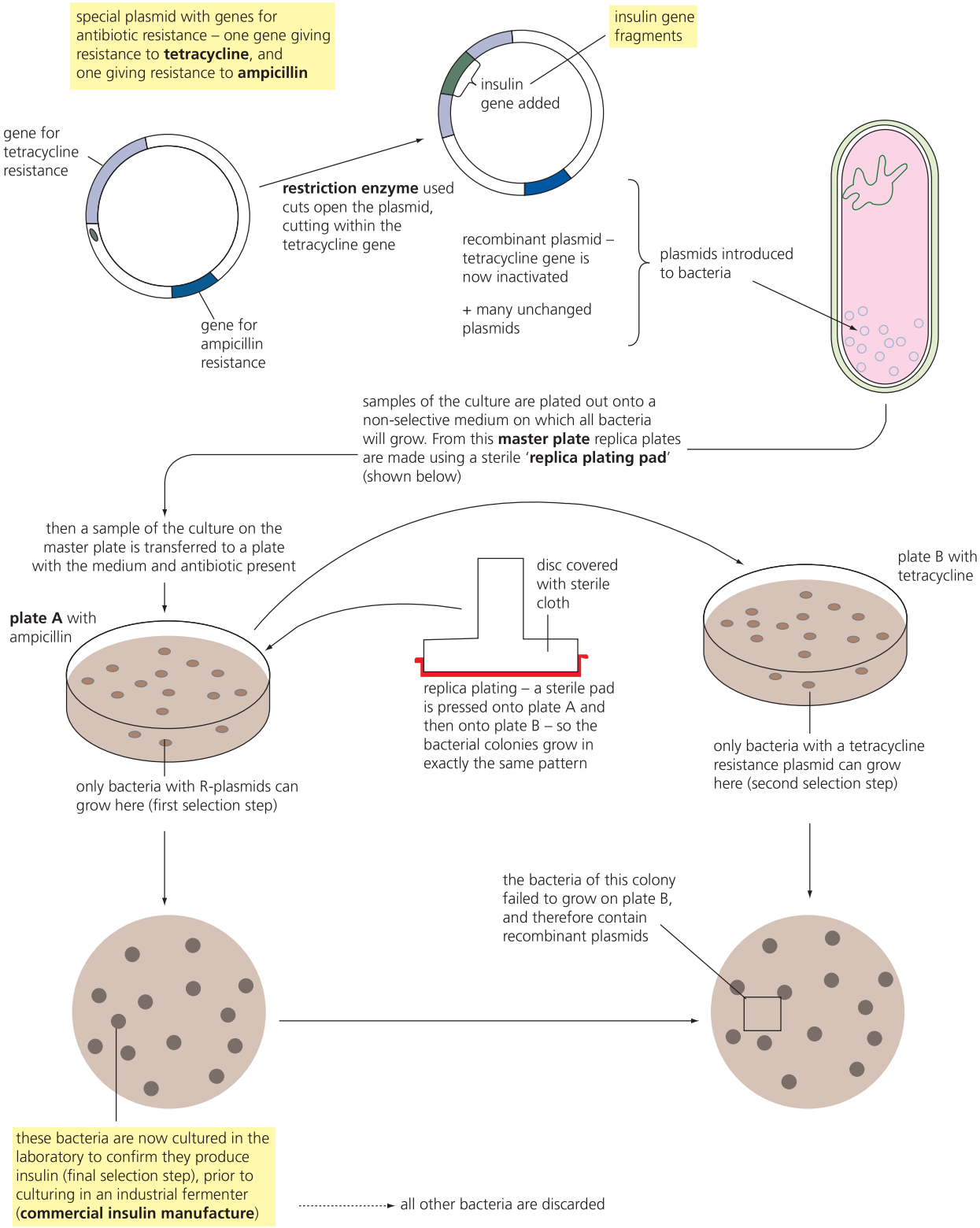


Figure 19.7 The use of R-plasmids for selecting genetically modified bacteria

A new development in insulin production

All this was part of the *original* process of producing human insulin from *E. coli*. The complications of producing human insulin from transformed bacteria have now been overcome by genetically engineering yeast cells for insulin production, rather than *E. coli*. Remember, yeast is a eukaryotic cell with a range of organelles, but it also contains plasmids. In eukaryotic cells, proteins for secretion from the cell are manufactured by ribosomes of the rough endoplasmic reticulum and transferred to the Golgi apparatus (Figure 1.16, page 18). Here, insulin is converted into its normal quaternary structure prior to discharge from the transformed yeast cells. However, since yeast is a eukaryote, the *lac* operon must be replaced by genes for promoters and other control sequences, in addition to the insulin genes in the 'engineered' plasmids. *Remind yourself of the control of gene expression in eukaryotes, on page 374.*

Alternative sources of insulin for clinic use

Before genetically engineered human insulin was available for clinic use, insulin was extracted and purified from the pancreases of pigs or cattle obtained from abattoirs after their slaughter. Insulin from both animal sources was used to treat insulin-dependent diabetes, although neither was exactly identical to human insulin.

Today, it is argued that genetically engineered human insulin has the following advantages:

- it is chemically identical to the body's own insulin and triggers no immune response as 'foreign' proteins tend to do when introduced into a patient's body
- it is acceptable to patients who previously declined 'pig' or 'cow' insulin for religious regions or because, as vegetarians, the use of animal products was unacceptable
- an immediate response is generated by the genetically engineered insulin because it exactly 'locks on to' the insulin receptors in the relevant cell surface membranes; the response brought about by insulin from other species is slower.

However, animal sources remain available and may be used in preference, if required.

Question

4 Distinguish between
- **a** genotype and genome
- **b** restriction endonuclease and ligase
- **c** a bacterial chromosome and a plasmid.

Genetic modification of eukaryotes

Manipulating genes in eukaryotes is a more difficult process than it is in prokaryotes. The reasons for this include:

- plasmids, the most useful vehicle for moving genes, do not occur in eukaryotes (except in yeasts) and, if introduced, may not survive and be replicated there
- eukaryotes are diploid organisms so each gene occurs twice in the nucleus of a cell, once on each of the homologous chromosomes, and may occur in different forms (alleles). Prokaryotes have a single, circular 'chromosome', so only one gene has to be engineered into their chromosome
- the transcription of eukaryotic DNA to messenger RNA is more complex than in prokaryotes; it involves the removal of short lengths of 'non-informative' DNA sequences, called 'introns'
- the mechanism for triggering gene expression in eukaryotes is more complex than in prokaryotes. Genes for the promoter and other control sequences have to be transferred, as well as the desired gene.

Despite these difficulties, several varieties of transgenic plants and animals have been produced.

Question

5 Why are the genes of prokaryotes generally easier to modify than those of eukaryotes?

Alternative markers for genetic engineering

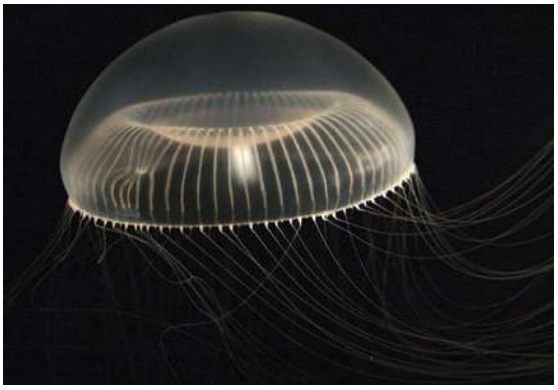
A marker is a gene that is deliberately transferred along with the required gene during the process of genetic engineering. It is easily recognised and used to identify those cells to which the gene has been successfully transferred. In the production of insulin in *E. coli* the original markers were antibiotic resistance genes (Figure 19.7).

Now alternative markers are preferred. This is because of the potential danger of the antibiotic resistance genes being accidentally transferred to other bacteria, including eventually, pathogenic strains of *E. coli* or even pathogens causing other diseases. Whilst this has not been reported, the

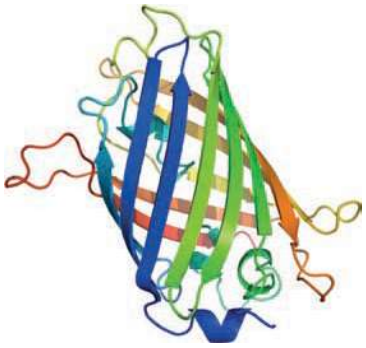
possibility must exist. The outcome would be an 'engineered' disease-causing microorganism that was resistant to one or more antibiotic.

Genes for fluorescent (or easily stained) substances are now used as markers (Figure 19.8) instead of antibiotic resistant genes, alongside the cDNA of the desired gene. These genes are then linked to a special promoter. The marker gene is expressed only when the desired gene has been successfully inserted into the genome of the host. Organisms that have been transformed in this way will glow under UV light (or can be identified by staining).

The crystal jellyfish (*Aequorea victoria*) occurs in the plankton off the west coast of North America. It shows bright green fluorescence when exposed to light in the blue to ultraviolet range.

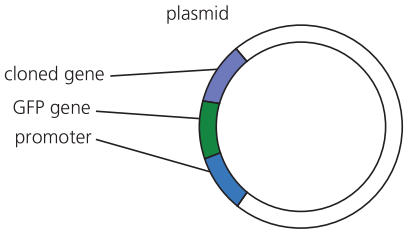


Fluorescence is due to the presence of a green fluorescent protein (GFP). This protein and the gene that codes for it have been extracted.



The tertiary structure of GFP protein

The GFP gene is now used as a marker gene in genetic engineering. For example, it may be inserted into plasmids alongside a cloned gene and its promoter. When these plasmids are returned to bacteria and the promoter is activated, the transformed bacteria will be detected by exposure to blue light.



The tertiary structure of GFP protein

The GFP gene has been introduced and maintained in the genome, and then expressed in species of bacteria, yeast, fish, plants, insects, and mammals. For example, these GM mice glow green under blue light because the GFP gene has been introduced into their DNA.

Figure 19.8 The use of fluorescent markers

Other methods of isolating genes for gene technology

Converting messenger RNA that codes for the insulin genes into cDNA, as described above, has the advantage of yielding the gene *without* the short lengths of 'nonsense' DNA (known as **introns**). After transcription of the coding strand in the nucleus this editing is a natural and essential stage. It is not easily for a genetic engineer to carry this out *in vitro*.

There are alternative methods. For example, it is possible to synthesise a copy of the gene in a laboratory if the linear sequence of amino acids of the polypeptide or protein is known. The data in the DNA genetic dictionary (Figure 6.10, page 119) gives the sequence of nucleotides from which to construct a copy of the gene.

As a result of the **Human Genome Project** (HGP), the location of each human gene and the base sequence within its DNA structure are known. Now, where a single-stranded DNA probe, complementary to a particular gene can be produced and made radioactive or fluorescent in ultraviolet light, original genes can be located and isolated. To do so, the DNA has first to be cut into fragments. Fragments are then denatured into single strands by heating, separated by means of electrophoresis (Figure 19.9), and then treated with the probe. The fragments of DNA containing the gene are located.

Both of these methods are currently applied to isolate genes required for other gene technology projects.

Electrophoresis

Electrophoresis is a process used to separate molecules such as proteins and fragments of nucleic acids. It is widely applied in many studies of DNA. For example, it is central to the investigation of the sequence of bases in particular lengths of DNA, known as **DNA sequencing**. It is also used in the identification of individual organisms and species known as **genetic fingerprinting**. We will review these applications shortly.

The principle and practice of electrophoresis

In **electrophoresis**, proteins or nucleic acid fragments (either DNA or RNA) are separated on the basis of their net charge and mass. Separation is achieved by differential migration of these molecules through a supporting medium – typically either agarose gel (a very pure form of agar) or polyacrylamide gel (PAG). In these substances the tiny pores present act as a **molecular sieve**. Through these gels, small particles can move quite quickly, whereas larger molecules move much more slowly.

Molecules separated by electrophoresis also carry an **electrical charge**. In the case of DNA, it is the phosphate groups in DNA fragments that give them a net negative charge. Consequently when these molecules are placed in an electric field they migrate towards the positive pole (anode). The distance moved in a given time will depend on the mass of the molecule or fragment – the smaller fragments moving further in a given time than the larger fragments. So, in electrophoresis, separation occurs by the **size** and by the **charge carried.** This is the double principle of electrophoretic separations.

The DNA fragments are produced by the action of a restriction enzyme (Figure 19.4). We have seen that each type of restriction enzyme cuts DNA at a particular base sequence, as and when these occur along the length of the DNA molecules. Consequently, the fragments are of different lengths. A series of wells are cut close to one end of the gel and the gel is submersed in a salt solution that conducts electricity. Then a small quantity of a mixture to be separated is placed in a well. Several different mixtures can be separated in a single gel at the same time (Figure 19.9). An animation of electrophoresis is available at **www.dnalc.org/resources/animations/gelelectrophoresis.html**.

After separation the fragments are not immediately visible because they are tiny and transparent. Their presence is identified either by **staining** or by the use of a **gene probe**.

Gene probes are single-stranded DNA with a base sequence that is complementary to that of a particular fragment or gene whose position or presence is sought. The probe must be made radioactive so that when the treated gel is exposed to X-ray film the presence of that particular probe and the fragment it attached to will be disclosed. Alternatively, the probe must have a fluorescent stain attached. It will then fluoresce distinctively in ultraviolet light, thereby disclosing the presence of the particular fragment or gene sought.

The alternative approach, demonstrated in Figure 19.9 involves the use of a stain to locate the position of all DNA fragments.

Table 19.3 Stains used in electrophoresis

Ethidium bromide	DNA fragments fluoresce in short wave UV radiation
Methylene blue	Stains gel and DNA but colour fades quickly
Nile blue A	DNA visible in light and gel not stained

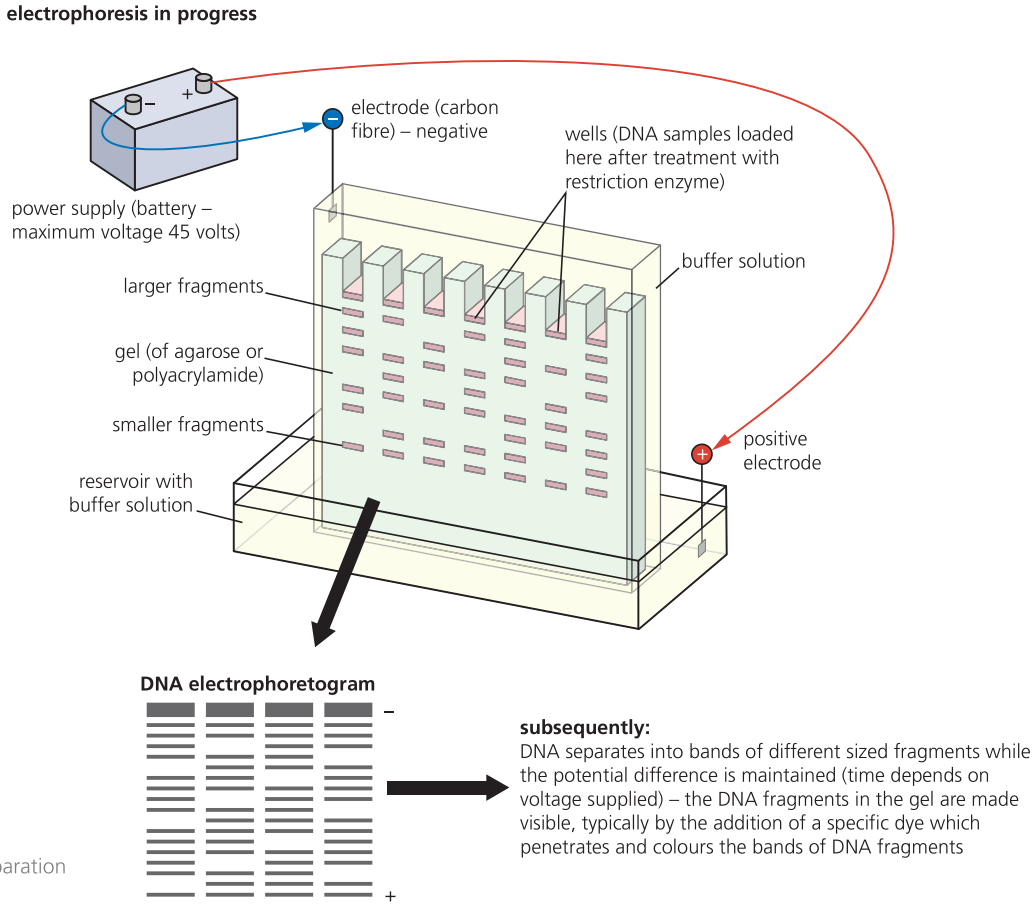


Figure 19.9 Electrophoresis – the separation of DNA fragments

Figure 19.10 Microarray analysis machine with microarray being inserted

DNA microarrays

DNA microarrays are small, solid supports, typically made of glass, silicon or nylon, each about the size of a microscope slide. Onto this surface, DNA probes that correspond to thousands of different genes are attached in a regular pattern of rows and columns. Each dot on the array contains a DNA sequence that is unique to a given gene. The microarray is then used to measure changes in expression levels of individual genes from cells under investigation.

The DNA that is to be tested originates as mRNA being expressed by a particular cell. By the action of reverse transcriptase this mRNA is converted to cDNA. As the cDNA is being synthesised it is simultaneously linked to fluorescent dye. Then the DNA probes of the microarray are exposed to this cDNA. Any complementary sequences present will bind to the fixed probes. After this, the microarray is rinsed free of any cDNA that has not hybridised and become bound. Finally, the microarray is exposed to laser light that causes fluorescence where there has been hybridisation between cDNA and a DNA probe, and the results recorded. The brighter the light emitted the higher the level of expression of a particular gene in the cell from which the original mRNA was obtained.

A full human genome microarray would have as many as 30 000 spots. Others are made with clusters of genes that relate to particular conditions, such as cancers, for example.

Analysis of a simple microarray

A DNA microarray might be used to investigate the level of gene expression in a cancerous cell compared to that in a healthy (control) cell, for example. The steps to such an investigation are:

1 mRNA is extracted from the cancerous cells and the control cells.
2 cDNA is created from both samples of mRNA, that from the cancerous cell is tagged with a red fluorescent dye, whilst that from the control cell is tagged with green fluorescent dye.
3 The two samples are then mixed together and hybridised to the microarray.
4 At each probe location the red- and green-labelled cDNA compete to bind to the gene-specific probe.
5 When subsequently excited by laser light, each dot will fluoresce red if it has bound more red than green cDNA. This will occur if that particular gene is expressed more strongly in the cancerous tissue compared to the control tissue.
6 Conversely, a dot will fluoresce green if that particular gene is expressed more strongly in the control tissue.
7 Yellow dots indicate equal amounts of red and green cDNA have bound to that spot – meaning the level of expression is the same in both tissues.

The analysis is undertaken by a fluorescence detector (microscope and camera) to produce a digital image of the microarray. From this data a computer quantifies the red to green fluorescence ratio of each spot. Then the degree of difference in expression of each gene between the cancerous and the control tissues is calculated.

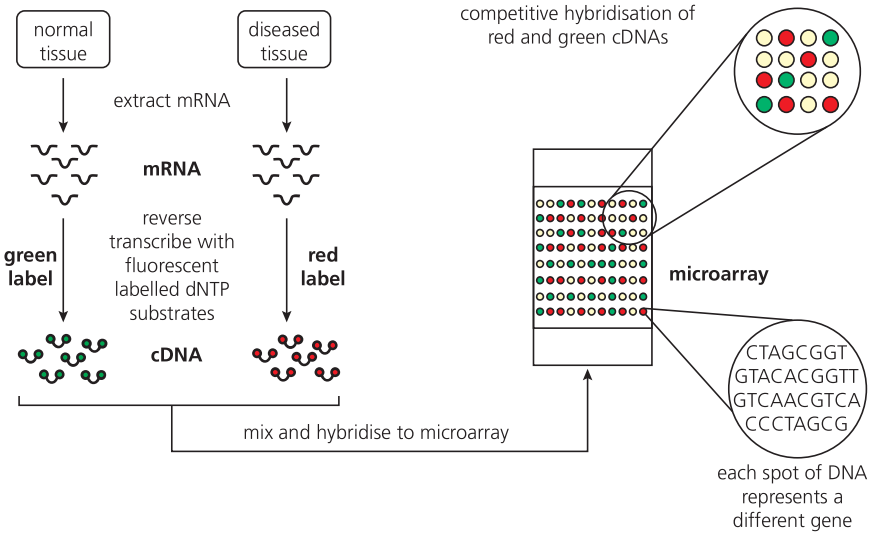


Figure 19.11 Microarray analysis of differential gene expression

19.2 Genetic technology applied to medicine

Bioinformatics combines knowledge from gene mapping, protein analysis and modern biotechnology in the service of science and society.

By the end of this section you should be able to:

a) define the term bioinformatics
b) outline the role of bioinformatics following the sequencing of genomes, such as those of humans and parasites, e.g. *Plasmodium*
c) explain the advantages of producing human proteins by recombinant DNA techniques
d) outline the advantages of screening for genetic conditions
e) outline how genetic diseases can be treated with gene therapy and discuss the challenges in choosing appropriate vectors, such as viruses, liposomes and naked DNA
f) discuss the social and ethical considerations of using gene testing and gene therapy in medicine
g) outline the use of PCR and DNA testing in forensic medicine and criminal investigations

Bioinformatics

Bioinformatics is a new discipline that combines three major components:

- **genomics** – the mapping of genomes
- **proteomics** – the analysis of the entire sets of proteins expressed by genomes
- **biotechnology** – the industrial and commercial application of biological science, particularly of microbiology, enzymology and genetics.

Developments in bioinformatics have created powerful techniques that allow scientists to find similarities in nucleotide sequences and discover the function of proteins among organisms of similar or different species. Nowadays, data is collected around the globe and from different research groups. The purpose of the resulting databases is to facilitate the sharing of this information, and to allow the widest possible exploitation of a huge and ever-growing body of knowledge and skills.

A flavour of the scale of developments can be gleamed from the following link to the 180 complete genomes sequenced:

www.genomenewsnetwork.org/resources/sequenced_genomes/genome_guide_p1.shtml

1 Knowledge of the genome of parasites and disease prevention

Malaria, the most significant insect-borne disease, poses a threat to 40 per cent of the world's population. The majority of the world's malaria cases are found in Africa, south of the Sahara, and it is here that 90 per cent of the fatalities due to this disease occur. Malaria is caused by *Plasmodium*, a protoctistan, which is transmitted from an infected person to another by blood-sucking mosquitoes of the genus *Anopheles*. Of the four species of *Plasmodium*, only one (*P. falciparum*) causes severe illness.

The search for a vaccine, begun in the 1980s, was initially unsuccessful. Now the situation is more hopeful. For example, the entire genome sequence of *P. falciparum* has been analysed. It consists of 14 chromosomes encoding approximately 5300 genes. This development is a major step in the attempts to design a successful vaccine, and one candidate is undergoing advanced trials. However, the complex parasitic life-style of *Plasmodium* (page 197) makes it a wily foe. *Plasmodium* has quickly developed drug resistance against each currently effective drug. The development of an effective malaria vaccine remains a major international challenge.

2 Human proteins and the treatment of disease

The production of a human protein by recombinant DNA techniques for the treatment of disease was an early success in the development of genetic engineering. The human genes for **insulin** were initially transferred to the bacterium, *E. coli* (and later more usefully to yeast) and human insulin was made available to treat Type I diabetes (page 295).

Haemophilia, a rare genetically determined condition in which the blood fails to clot normally, results from a failure to produce adequate amounts of blood proteins known as **clotting factors**. These are glycoproteins normally released from cells at the site of a haemorrhage (Figure 19.12). Today, haemophilia may be effectively treated by the administration of the specific clotting factor a patient lacks. For example, haemophilia A is due to a deficiency of functionally active **factor VIII**, normally the product of a gene on the X chromosome. The human gene for factor VIII has been transferred to the genome of cells obtained from hamster kidney tissue. These genetically engineered cells are then grown *in vitro*, using an animal cell culture medium. The factor VIII protein is then isolated, purified and administered to patients.

SEM of blood clot
showing meshwork of fibrin fibres and trapped blood cells

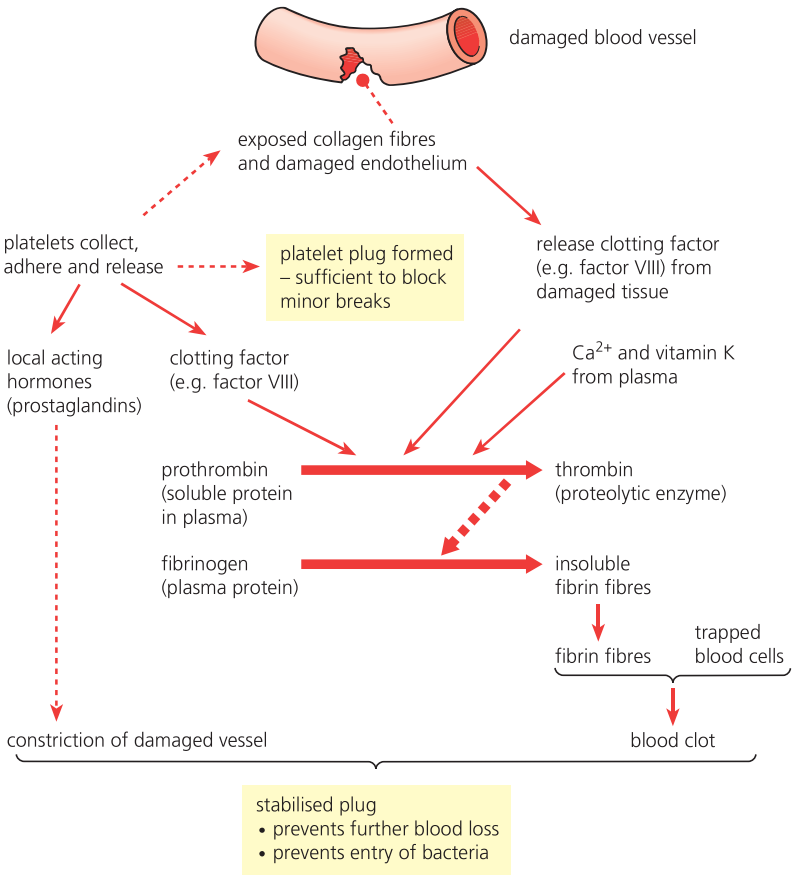


Figure 19.12 The blood-clotting mechanism

Severe combined immunodeficiency (**SCID**) is an inherited disorder due to absent or malfunctioning T- and B-lymphocytes of the immune system. The result is a defective antibody response. In SCID patients the immune system is so compromised it is effectively absent, and victims are extremely vulnerable to infectious disease from a very early age.

One form of SCID is due to a lack of the enzyme **adenosine deaminase** (**AD**), coded for by a gene on chromosome 20. In the presence of a defective form of the gene, the substrate of the enzyme this gene codes for accumulates in cells. Immature cells of the immune system are sensitive to the toxic effects of this substrate molecule, and they fail to mature.

Now stem cells, harvested from the umbilical cord blood at birth, have been genetically modified with the normal human AD gene (cDNA), introduced by retovirus. These GM stem cells are then transfused into the neonate for successful gene therapy.

So, in summary, gene therapy is an application of genetic engineering with the aim of supplying a missing gene to body cells in such a way that it remains permanently functional, when this is thought safe and ethically sound. Gene therapy is a very recent, and highly experimental, science. The steps to the process of gene therapy are discussed below.

3 Screening for genetic diseases

Genetic disorders are heritable conditions that are caused by a specific defect in a gene or genes. Most arise from a mutation involving a single gene. The mutant alleles that cause these conditions are commonly **recessive**. In these cases a person must be homozygous for the mutant gene for the condition to be expressed. However, people with a single mutant allele are '**carriers**' of that genetic disorder. Quite surprising numbers of us are carriers for one or more such conditions.

An exception is Huntington's disorder, due to a **dominant** allele on chromosome 4. In this case, an individual will be affected even if they have a single allele (they will be heterozygous for the defective gene). The disease is extremely rare (about 1 case per 20 000 live births).

A tendency to suffer from certain cancers also runs in families. An example is breast cancer. In this case, there are two genes (known as BRCA1 and BRCA2), each with one mutant allele, which can be inherited. There is also a 60 per cent probability that the healthy allele will mutate at some stage, before the person reaches the age of 50 years, thereby triggering breast cancer. Both BRCA1 and BRCA2 are natural tumour-suppressing genes when present in the homozygous state.

Genetic disorders generally afflict about 1–2 per cent of the human population. These include sickle-cell disease, Duchene muscular dystrophy, haemophilia (page 371), severe combined immunodeficiency (SCID), and **cystic fibrosis** (**CF**) – on which we focus here.

The incidence of cystic fibrosis varies around the world. The available evidence suggests that in Asia it is quite rare, but in Europe and the USA it occurs in roughly one newborn person in every 3000–3500 births. In the UK cystic fibrosis is the most common genetic disorder. Here, one person in 25 is a carrier.

Causes of cystic fibrosis

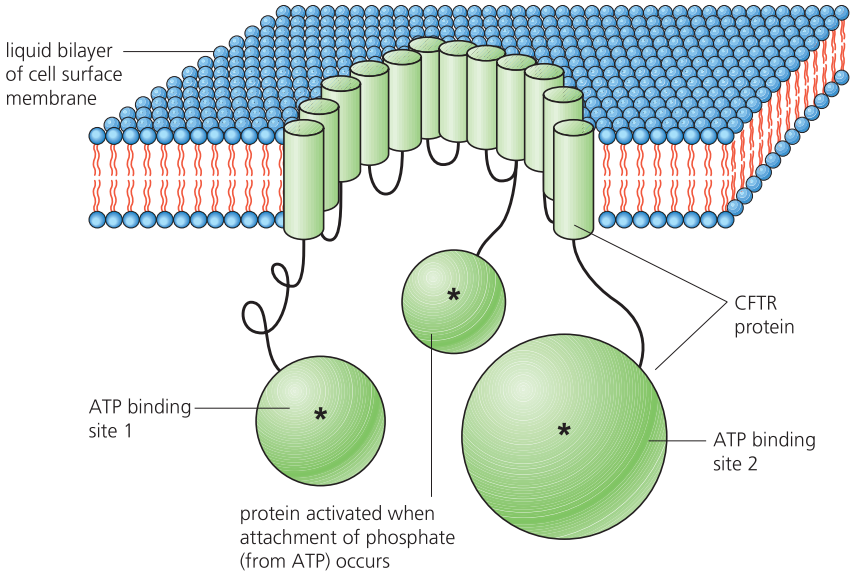
Cystic fibrosis is due to a mutation of a single gene on chromosome 7, and it affects the epithelial cells of the body. The CF gene is 180 000 base pairs long. This codes for a protein known as CFTR, which functions as an ion pump (Figure 19.13). The pump transports chloride ions across membranes and water follows the ions, so epithelia are kept smooth and moist when this protein is present and working.

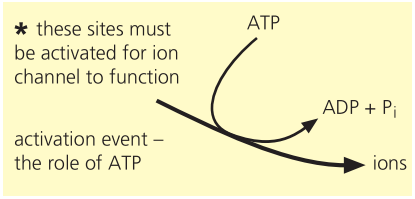
In cystic fibrosis the most common mutation involves a deletion of just three of the gene's nucleotides and results in the loss of an amino acid (phenylalanine) at one location along a protein built from almost 1500 amino acids in total. The mutated gene codes for no protein or for a faulty protein. The result is epithelium cells that remain dry and a build-up of thick, sticky mucus.

Question

6 What do you understand by the term *mutant allele*?

CFTR protein *in situ* in plasma membrane





How CFTR regulates water content in mucus

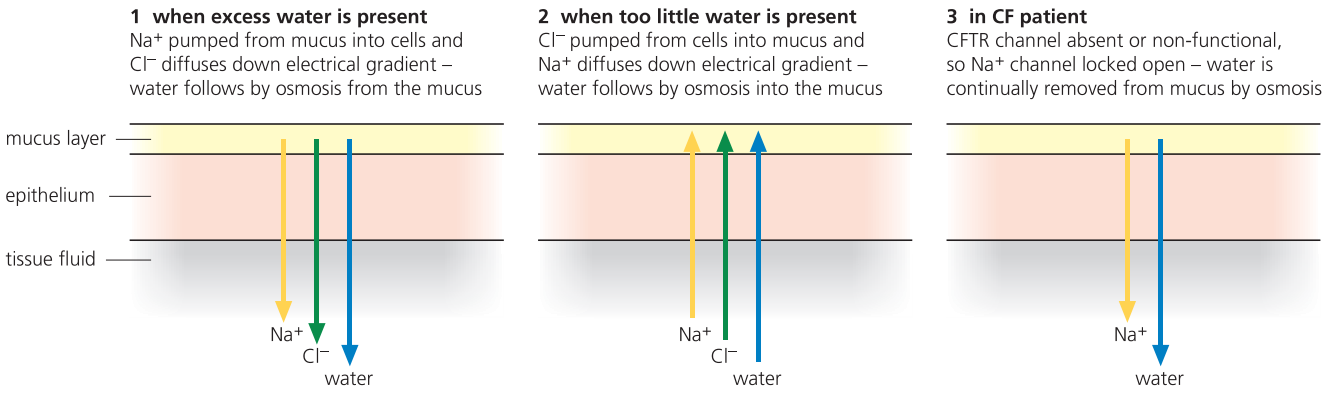


Figure 19.13 The CFTR protein – a channel protein

The signs and symptoms of cystic fibrosis

The effects of faulty CFTR ion pumps are felt:

- in the pancreas – secretion of digestive juices by the gland cells in the pancreas is interrupted by blocked ducts. Digestion is hindered.
- in the sweat glands – salty sweat is formed. This is used in the diagnosis of cystic fibrosis.
- in the lungs – these become blocked by mucus and are prone to infection. This effect is most quickly life-threatening if not treated promptly.

In adult patients, the membranes of epithelium cells in their reproductive organs are affected too. Infertility frequently results. In women with cystic fibrosis, the mucus naturally present in the cervix becomes a dense plug in the vagina, restricting entry of sperm into the uterus. In men with cystic fibrosis, dense mucus in the sperm ducts blocks these tubes, significantly reducing the sperm count when semen is ejaculated.

The process of genetic screening

We can now locate genes responsible for human genetic diseases, if we choose. This is one outcome of the Human Genome Project. For example, the gene that codes for cystic fibrosis (CFTR) was sequenced in 1989. This made possible the screening of human cells for the presence of the mutated *CFTR* gene (Figure 19.14). So it is possible for couples to be genetically screened to assess how likely they are to have children who will have cystic fibrosis (because both are 'carriers'), for example. Alternatively, it is possible for all members of a family group be offered screening where there is a history of a particular condition.

Prenatal screening is used to test for the presence of a number of genetic conditions in the unborn child. This allows the detection of:

- chromosomes abnormalities such as Down syndrome (trisomy 21), trisomy 13 and trisomy 18. In the latter two abnormalities, only very rarely do affected infants survive their first year.
- single gene disorders, such as haemophilia, sickle cell anaemia and cystic fibrosis.
- neural tube defects, such as spina bifida and anencephaly.

Prenatal screening may be carried out by:

- **chorionic villus sampling** – undertaken at weeks 11–13 of the pregnancy, a sample from the placenta is taken.
- **amniocentesis** – undertaken at weeks 15–20 of the pregnancy, the fetal cells in the amniotic fluid are examined.

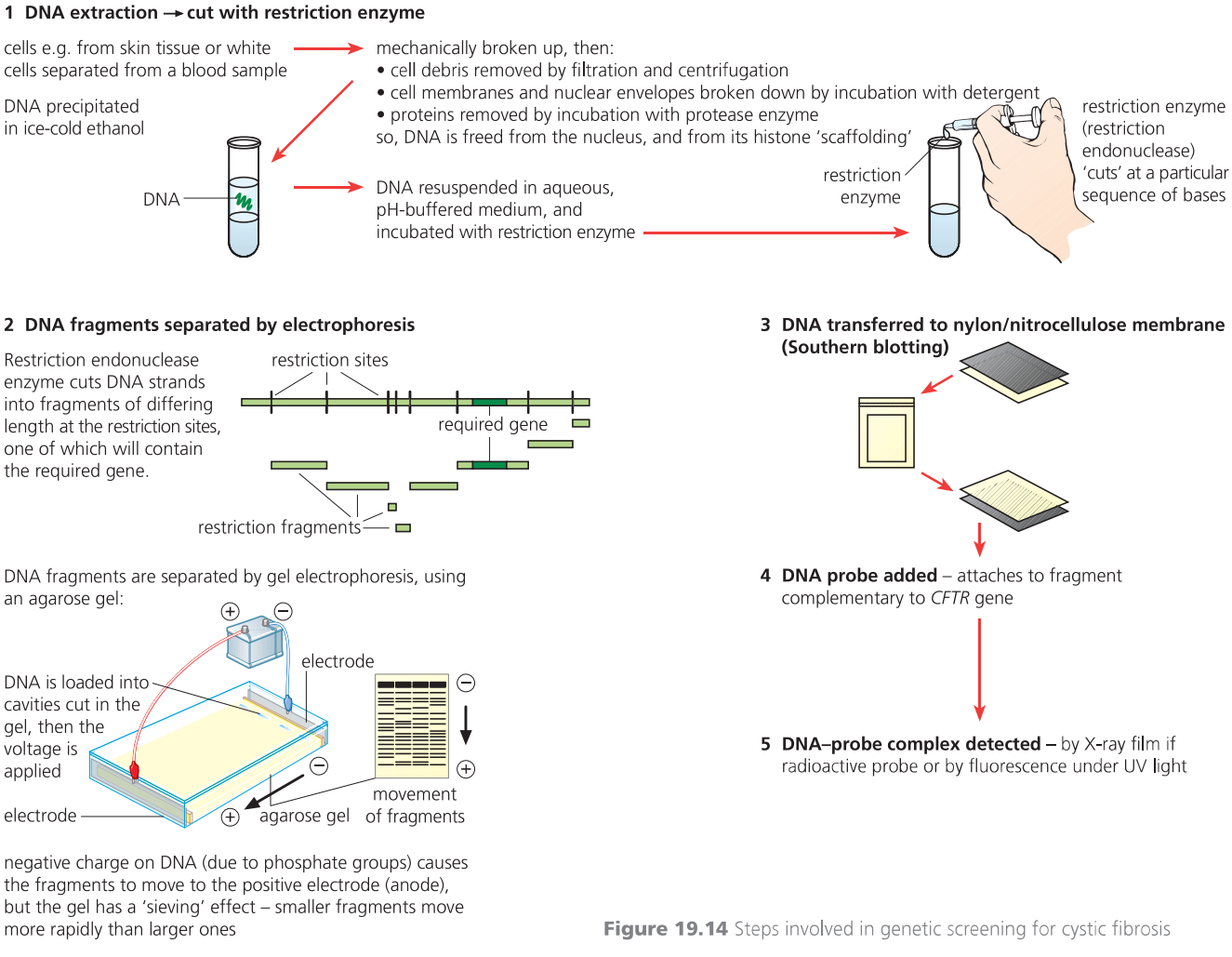


Figure 19.14 Steps involved in genetic screening for cystic fibrosis

Question

7 How does the development of a genetic disorder differ for a person with a single recessive allele for cystic fibrosis and a person with a single dominant allele for Huntington's disorder?

Newborn screening is undertaken in some countries, to detect conditions such as **phenylketonuria (PKU)**. Phenylketonuria, an error of protein metabolism, is caused by a mutation in a gene coding for the enzyme that converts the amino acid phenylalanine into tyrosine. In infants born with this mutation, the phenylalanine naturally obtained in the diet and not immediately used in the synthesis of new proteins builds up in the blood. An excess of phenylalanine in the body causes dangerous side effects, including growth deficiency and, eventually, severe mental retardation. Phenylketonuria can be detected at birth by means of a simple blood test and the symptoms can largely be avoided by adjusting the diet.

chorionic villus sampling – withdrawal of a sample of the fetal tissue part-buried in the wall of the uterus in the period 11–13 weeks into the pregnancy; the tiny sample is of cells that are actively dividing and can be analysed quickly.

amniocentesis – withdrawal of a sample of amniotic fluid in the period 15–20 weeks of gestation; the fluid contains cells from the surface of the embryo

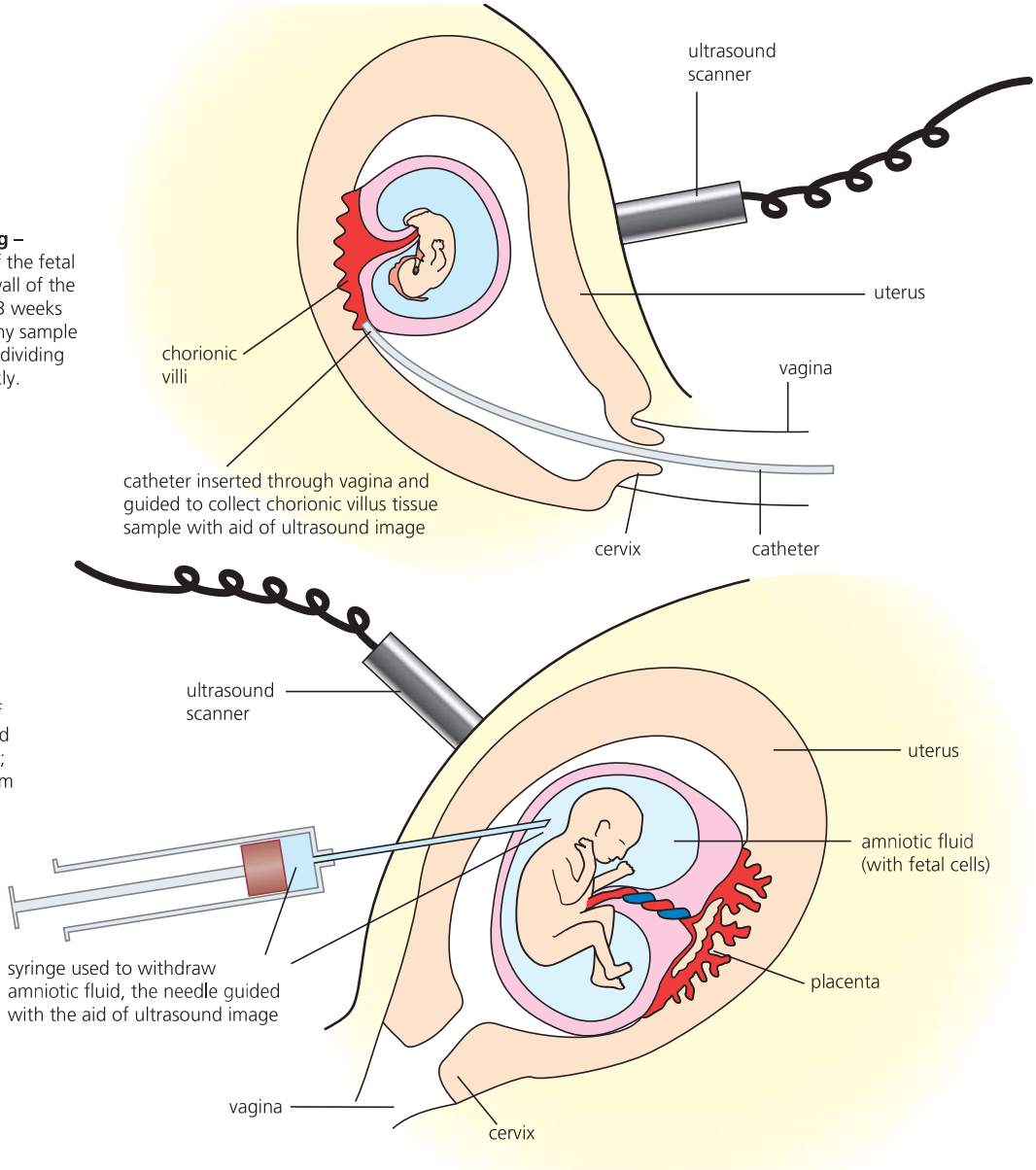


Figure 19.15 Prenatal screening

Extension

The outcome of screening – genetic counselling

The outcome of genetic screening frequently does not necessarily allay a person's (or their partner's) fears. They are likely to be referred to a **genetic counsellor**. The role of the counsellor is to take a detailed case history of the person and their immediate family. A counsellor may then research the **family pedigree**.

Figure 19.16 A genetic counselling session

Reasons for referral to a genetic counsellor

- Screening has confirmed that one or both partners are carriers.
- One or both partners have a relative with a genetic disease.
- The partners are first cousins.
- One or both partners belong to an ethnic group with an above-average risk of a genetic disease.
- There is a history of earlier pregnancies ending in a miscarriage.
- The partners are aged 38 or over.

In these cases people need to know the risk of their offspring inheriting the defective allele or actually developing the disorder. One outcome may be the possibility of further treatment in cases where a defective allele has been detected. There may be consequences for future health, life expectancy, the appropriateness of future education and training and future employment. These are all issues where information and reassurance may be needed. Sometimes, genetic conditions affect the chance of obtaining life insurance. The role of the counsellor is to provide information in a way which allows their clients to make their own decisions.

A case study: investigating haemophilia by family pedigree

In the blood circulatory system of a mammal, if a break occurs there is a risk of uncontrolled bleeding. This is normally overcome by the blood clotting mechanism which causes any gap to be plugged. Haemophilia is a rare, genetically determined, condition in which the blood does not clot normally. The result is frequent, excessive bleeding.

There are two forms of haemophilia, known as haemophilia A and haemophilia B. They are due to a failure to produce adequate amounts of particular blood proteins (factors VIII and IX), both essential to the complex blood clotting mechanism. Today, haemophilia is effectively treated by the administration of the clotting factor the patient lacks.

Haemophilia is a sex-linked condition because the genes controlling production of the blood proteins concerned are located on the X chromosome. Haemophilia is caused by a recessive allele. As a result, haemophilia is largely a disease of the male since in him a single X chromosome carrying the defective allele ($\mathbf{X^hY}$) will result in disease. For a female to have the disease, she must be homozygous for the recessive gene ($\mathbf{X^hX^h}$), but this condition is usually fatal *in utero*, typically resulting in a miscarriage.

So, a family tree where haemophilia occurs typically shows no female haemophiliacs. However, there will also be female carriers ($\mathbf{X^HX^h}$), with one X chromosome with the recessive allele. Female carriers have normal blood clotting, but for any children they have with a normal male, there is a 50 per cent chance of the daughters being carriers and a 50 per cent chance of the sons being haemophiliac.

The British queen, Queen Victoria (1819–1901) was a carrier of haemophilia. She transmitted the allele to three of her nine children and it was passed on to at least seven of her grandchildren. Look at the pattern of haemophiliac males and female carriers in this extensive royal family (Figure 19.17).

The transmission of this 'royal' haemophilia, which has had political and social consequences in the past 150 years, particularly in Russia and Spain, is well documented.

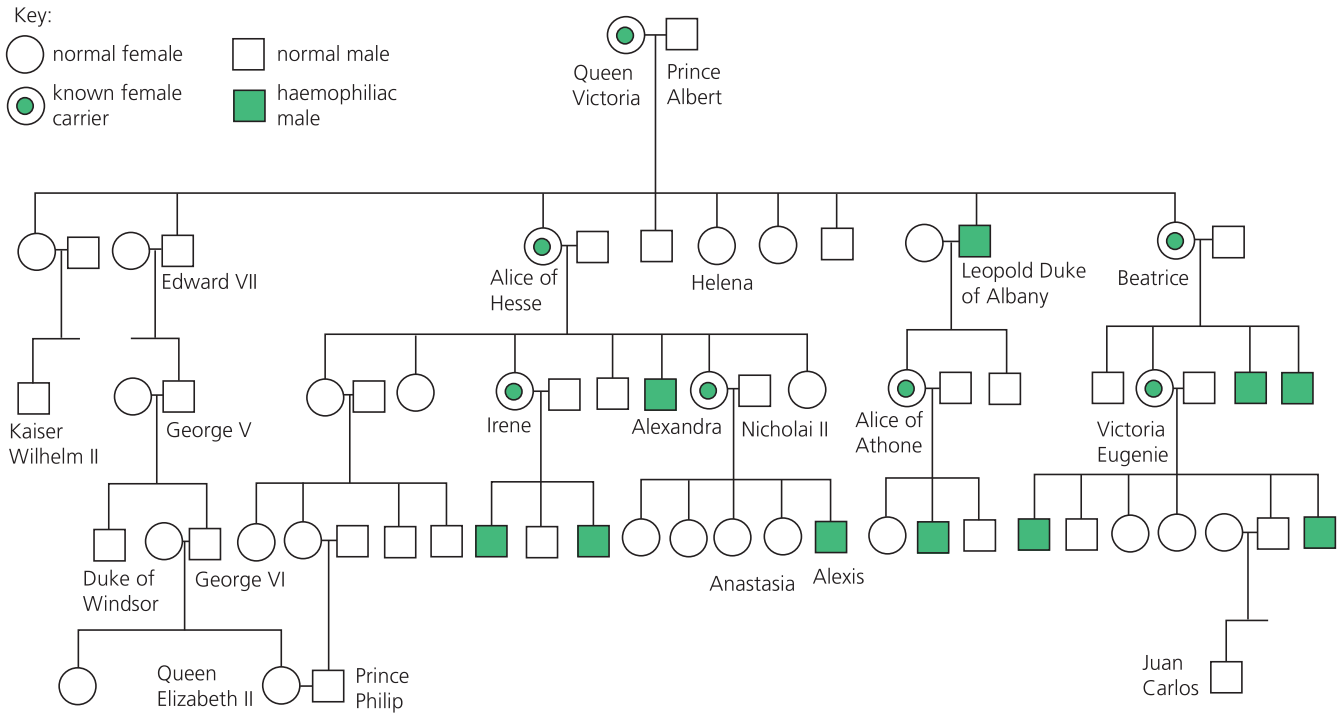


Figure 19.17 Queen Victoria and her heirs – a celebrated case!

4 Gene therapy

Somatic gene therapy applied to cystic fibrosis

Since the healthy *CFTR* gene codes for a protein that functions as an ion pump, the gene therapy initiative has involved getting copies of the healthy gene to the cells of the lung epithelium. This can be attempted in a number of ways.

The *CFTR* gene may be delivered in an aerosol spray. The spray contains tiny lipid bilayer droplets called liposomes to which copies of the healthy gene are attached. The liposomes fuse with cell membrane lipid and deliver the gene into the epithelium cell (Figure 15.16). In trials, the treatment is effective but the epithelium cells are routinely replaced so the beneficial effects only last a few days and the treatment has to be regularly repeated. The cure can only be more permanent when it is targeted on the cells that make epithelium cells and unfortunately this approach has failed to deliver the healthy gene effectively.

An alternative approach is based on a viral delivery of the healthy *CFTR* gene. Viruses such as the Adenoviruses have been used in place of liposomes. This virus is capable of entering and infecting lung epithelium cells. However, the virus is first genetically engineered to inactivate or remove from its genome the alleles that cause disease. Additionally, healthy *CFTR* alleles are added in. Unfortunately the treatment has not been successful. Viral vectors have triggered allergic or other immune responses and have had to be discontinued.

Other vectors for gene therapy

We saw that the vector for gene therapy in cystic fibrosis was a lipid envelope (a liposome). There are alternative vectors:

- **Viruses**, including retroviruses, have been used to transfer DNA into cells, as shown in Figure 19.18. Since a retrovirus is an RNA virus, the enzyme reverse transcriptase that the virus carries has a key role in the insertion of recombinant DNA in the host liver cells. Familial hypercholesterolaenia (FM) is a genetically inherited disorder in which low-density lipoproteins accumulate in the blood plasma, and instead of being absorbed into liver cells, contribute to the lipid being deposited as cholesterol in the arteries, leading to coronary heart disease. A retrovirus has also been used in the treatment of SCID. Adenovirus and vaccinia virus have also been successful vectors, and the conditions treated include inherited eye disease.

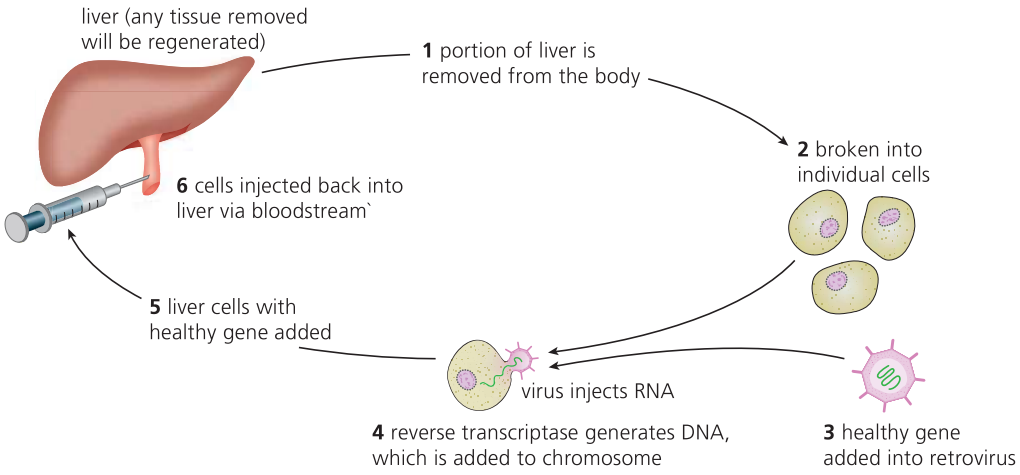
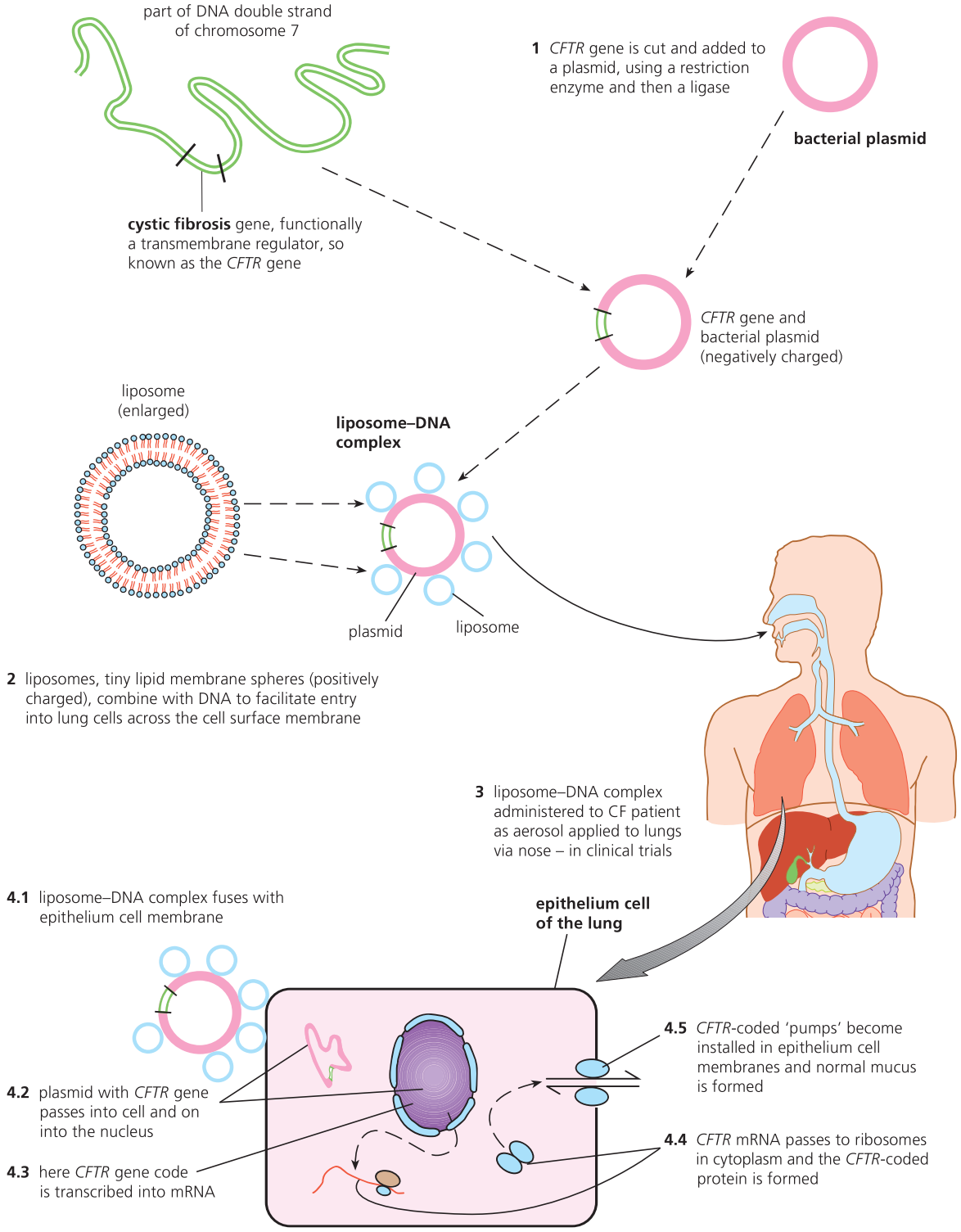


Figure 19.18 Gene therapy using a virus as vector

- **Naked DNA**. Genes have also been transferred by direct take-up of DNA from the environment. The host cells have to be capable of taking up the DNA – a condition described as 'competent'. Calcium chloride treatment has proved to be one of the best methods of preparing competent cells. The genetic diseases treated in this way include SCID.

The cystic fibrosis gene codes for a membrane protein that occurs widely in body cells, and pumps ions (e.g. Cl^-) across cell membranes.



In recent clinical trials some 20% of epithelium cells of CF patients were *temporarily* modified (i.e. accepted the *CFTR* gene), but the effects were relatively short-lived. This is because our epithelium cells are continually replaced at a steady rate, and in CF patients the genetically engineered cells are replaced with cells without *CFTR*-coded pumps. Patients would require periodic treatment with the liposome–DNA complex aerosol to maintain the effect permanently.

Figure 19.19 Somatic gene therapy: supplying the healthy *CFTR* gene to the lungs

Social and ethical aspects of genetic engineering and gene therapy

The benefits and hazards of gene technology

Genetically modified organisms have been produced for specific purposes, relatively quickly, where this proves possible. They offer enormous benefits However, geneticists are really producing new organisms when genes are transferred so there may be hazards as well as benefits.

Questions

8 Suggest what advantage would result from the transfer of genes for nitrogen fixation from nodules in leguminous plants to cereals such as wheat.

9 Occasionally newspaper articles make criticisms of genetic modification of food organisms, sometimes with alarmist headlines. Search the web for articles that give differing views on this. Prepare the following to use in a discussion with your peers.

- **a** a concise summary of the criticisms frequently made, avoiding extremist language or unnecessary exaggeration
- **b** a list of balancing arguments in favour of genetic modification of food organisms
- **c** a concise statement of your own view on this issue

Table 19.4 The benefits and potential hazards of gene technology

The benefits so far	Hazards that have been anticipated
• In addition to human insulin production by modified *E. coli*, human growth hormone, and blood clotting factor VIII have been produced by genetically modified bacteria.	• Could a harmless organism such as the human gut bacterium *E. coli* be transformed into a harmful pathogen that escapes from the laboratory into the human population?
• Another application of genetically engineering is bioremediation. This is the removal of toxic compounds, carcinogens and pollutants, such as industrial solvents, contaminating ground waters and crude oil spills by genetically engineered bacteria which degrade these substances into safer molecules. The process is slow.	• Genes move between bacterial populations with time. Could the antibiotic-resistance genes in plasmid vectors become accidentally transferred into a pathogenic organism?
• Sugar beet plants have been genetically modified to be 'tolerant' of glyphosate herbicide applications. These plants are able to inactivate the herbicide when it is sprayed over them, but the surrounding weeds cannot and are killed. (This is discussed further in this topic.)	• The accidental transfer of herbicide-resistance from crop plants, such as sugar beet, to a related wild plant and then on to other plants is a possibility which could result in 'super weeds' whose spread would be hard to prevent.
• Similarly, natural resistance to attack by chewing insect has resulted from the addition of the genes for Bt toxin into crop plants (potato, cotton, and maize) by genetic engineering. This reduces the need for extensive aerial spraying of expensive insecticides which are harmful to wildlife.	• Natural insecticidal toxin engineered into crop plants, whilst an effective protection from browsing insects, might harm pollinating species such as bees and butterflies too.
• There are on-going attempts to use gene technology to treat genetic diseases, such as cystic fibrosis (page 469) and severe combined immunodeficiency (SCID). It is hoped that some cancers may yield to this approach in the future.	• The presence of the gene for the Bt toxin in the environment may lead to insects with resistance, too.
	• There are well publicised anxieties about food products from genetically-engineered species. Might the food become toxic or trigger some allergic reactions. So far there has been no evidence of these.

The social implications of gene technology

Some of the **social implications** of gene technology are apparent from the list of benefits and potential hazards in Table 19.4. We can summarise them as follows.

The **advantages** of gene technology for society may be:

- improved, cheaper medicines
- improved food supplies
- improved nutritional quality of foods
- a cleaner environment
- improved treatment of genetic diseases.

The **disadvantages** of gene technology for society may be:

- unexpected reductions in crop yields due to ecological disturbance
- farmers made dependent on specific varieties, needing fresh seed annually and expensive fertilisers
- reduced natural biodiversity resulting in a reduced possibility of new varieties arising
- a reduced effectiveness of antibiotics as more bacteria become resistant.

Ethical issues in gene technology

Ethical implications depend on wider values. Ethics are the moral principles that we feel ought to govern or influence the conduct of a society. Ethics are concerned with how we decide **what is right and what is wrong**. Today, developments in science and technology influence many aspects of people's lives. This certainly throws up increasingly complex ethical issues which we need to examine and respond to. Gene technology is just one case in point.

There are important forces in our lives concerned with right and wrong in various ways, but they are not themselves ethics (Table 19.5). After all, what is considered right and what is considered wrong varies across the world.

Table 19.5 Defining ethics by recognising what they are not

The law	Our laws are made by governments and may or may not be ethically-based. (In some cases, they may even only be a view held by a minority.)
Religion	In our societies there are followers of different religions and of none, yet ethics apply to us all.
Cultural norms	Many of these are little more than 'fashions' that seemed acceptable at one time and which may still be held uncritically.
Science	Whilst this may seek to give us an understanding of the origins of our world and life, and how these work, it does not suggest how we should act.
Our feelings or conscience	These are likely to be the product of our early environment, general outlook and temperament, and our individual experiences.

On the other hand, the principles we do use in coming to ethical decisions are identified in Table 19.6. You can apply them to the issues that gene technology raises, after you have read them, if you can accept them as comprehensive.

Regarding the **ethics of gene technology**, perhaps there are two broad issues that societies need to address in deciding what developments they wish to fund.

- Is there an important over-riding principle to be held to, that humans should not tamper with nature in a deliberate way? Are the changes in biodiversity and genetic diversity that inadvertently flow from it completely beyond our current knowledge? Alternatively, does all that we do influence and eventually change our environment so our role is to see that our activities are conducted responsibly?
- To what extent is gene technology a costly technology that is mostly beneficial to the health and life expectancy of wealthier people or perhaps to just those of developed nations? If the funds used for gene technology were made available for the more basic problems of housing, health and nutrition in less developed countries instead, could vastly more people benefit immediately? Alternatively, if we have the ability and resources to bring about beneficial development, is it our duty to do so?

What does your group think?

Table 19.6 Ethical standards to apply in decision making

Rights We have a duty to ensure these	**Human rights have been recognised and articulated, for example:** • The United Nations Universal Declaration of Human Rights: • 'All human beings are born free and equal in dignity and rights. They are endowed with reason and conscience and should act towards one another in a spirit of brotherhood'. • The European Convention on Human Rights, which includes issues of security, liberty, political freedom and welfare, and economic and group rights. • In countries with a Bill of Rights written into their constitution, as in the USA: '*We hold these truths to be self-evident, …*'.
Justice A principle to guide actions	**The principle that all should be treated equally; the idea of fairness in our actions to each individual is considered essential.**
Utilitarianism A belief that the overall benefits should be greater than the costs	**The principle of the greatest good for the greatest number and with actions that generate minimal harm to others' interests. In effect, this involves a cost–benefit analysis.**
Common good The core conditions that are essential to the welfare of all	**Where the life of a working, interacting community is inherently good, then all actions should prosper and support the common interest.**
Virtue The placing of importance on a need to live a 'good life'	**Ethical actions necessarily respect and embody values like truth, honesty, courage, compassion, generosity, tolerance, integrity, fairness, self-control and prudence.**
Other criteria?	Do you or your peers have any other criteria about which the whole group can be convinced?

Question

10 Using a pedigree chart showing the incidence of haemophilia, what information is needed to identify a particular carrier in any generation?

Ethical issues raised by genetic screening

The nature and application of ethical principles in decision making has just been discussed.

It is possible to abort a fetus that has been identified as carrying a defective gene. Genetic counselling may result in ethical concerns for parents, medical personnel and for society as a whole. Other ethical issues that screening and counselling may present include:

- Who should decide who may be screened or tested?
- What known disorders should be considered by the medical profession or society? For example, should screening be available for disorders for which there is, as yet, no known cure?
- Who should meet the substantial costs of screening, given the escalating cost of public health provision, where this exists?
- Should the results be confidential? If not, who should have access to the information? Should it be available to potential employers or insurance providers?

What other issues do you think are relevant?

Specific issues to consider

The following issues are appropriate for discussion between individuals, and with peers in groups, as well as by patients and their medical teams, when they arise.

1 **Genetic disorders where treatment exists v. where none exist.**
For those in authority who supervise the allocation of public funds, and for the scientific and medical workers who are required to make professional decisions in the laboratory or clinic about the direction and emphasis on future developments, this issue will arise. *How should they respond?*

2 **Infertility and IVF.**
Fertilisation followed by implantation of an embryo does not always proceed in the normal way. Either the male or the female or both may be infertile, due to a number of different causes.

In **males** these may include:

- failure to achieve or to maintain an erect penis
- structurally abnormal sperm, sperm of poor mobility, short-lived sperm or too few sperm
- a blocked sperm duct, which prevents semen containing sperm from being ejaculated.

In **females** these may include:

- conditions in the cervix that cause death of sperm
- conditions in the uterus that prevent implantation of the blastocyst
- blocked or damaged oviducts, which prevent the egg from reaching sperm
- eggs that fail to mature or fail to be released.

In some cases, a couple's infertility may be treated by the process of fertilisation of eggs outside the body – ***in-vitro* fertilisation** (**IVF**). The key step in *in-vitro* fertilisation is the successful removal of sufficient egg cells from the ovaries. To achieve this, the normal menstrual cycle is temporarily suspended with hormone-based drugs. Then the ovaries are induced to produce a large number of eggs simultaneously, at a time controlled by the doctors. In this way the correct moment to collect the eggs can be known accurately. Eggs cells are then isolated from the surrounding follicle cells and mixed with sperm. If fertilisation occurs, the fertilised egg cells are incubated so that embryos at the eight-cell stage may be placed in the uterus. If one (or more) imbed there, then a normal pregnancy may follow.

The first baby created in this way, called a 'test tube baby', was born in 1978. The process of *in-vitro* fertilisation is illustrated in Figure 19.20. Today, the procedure is regarded as a routine one.

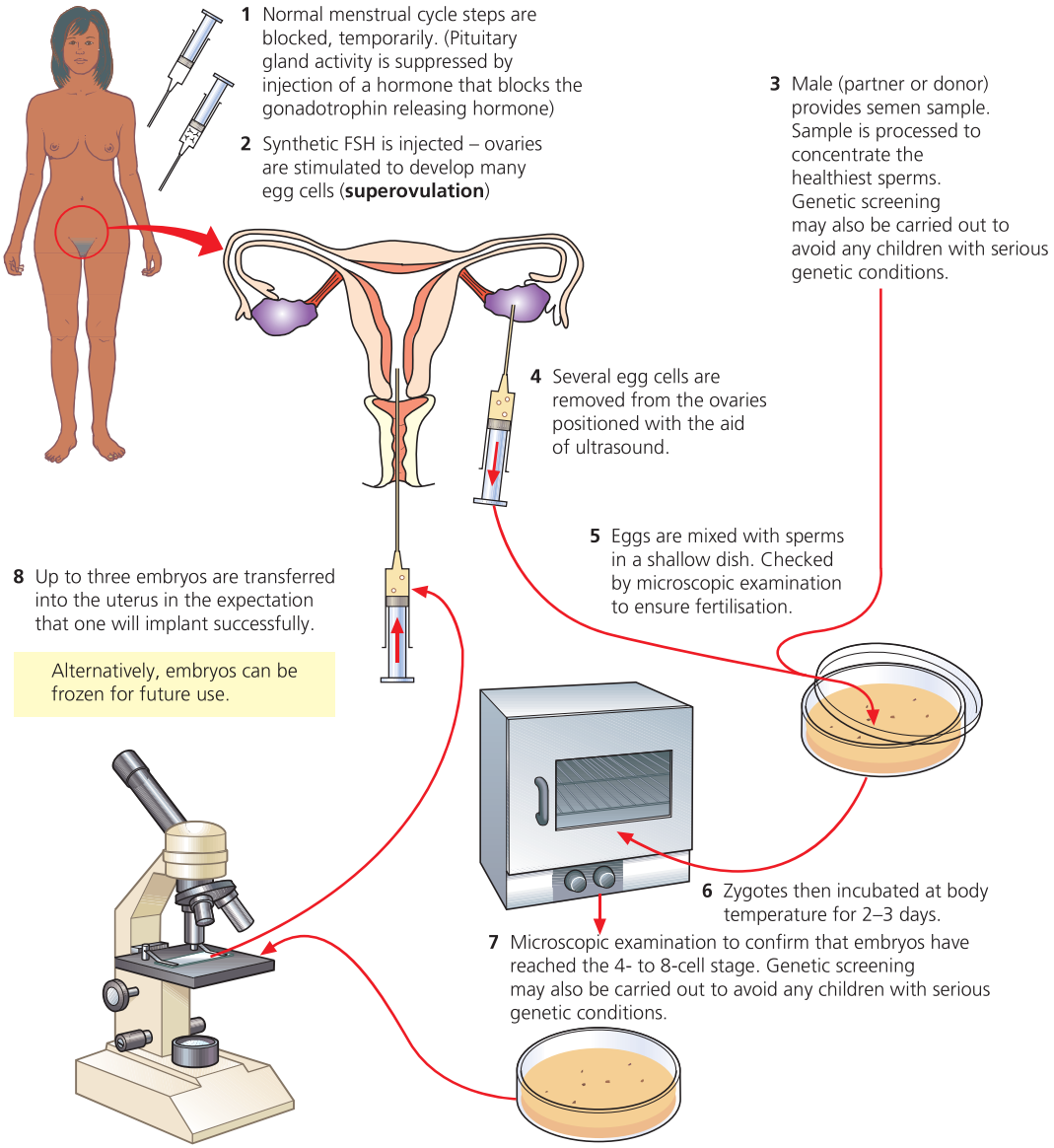


Figure 19.20 The process of *in-vitro* fertilisation

In-vitro fertilisation raises many issues for society. Infertility is a personal problem that generates stress and unhappiness in those affected. Infertile couples may seek assistance and a cure. The medical treatments involved are expensive and their deployment inevitably deflects finite resources away from other needs. Success rates are sometimes low. In the face of the world's booming human population, some argue against these sorts of development in reproduction technology.

Another controversial issue is that current fertility treatments tend to create several embryos, all with the potential to become new people. However, the embryos that are not selected for implantation ultimately must be destroyed – a stressful task in itself.

The ethical implications of *in-vitro* fertilisation are presented in Table 19.7.

Table 19.7 The ethical implications of *in-vitro* fertilisation

Arguments for IVF	Arguments against IVF
• For some otherwise childless couples, desired parenthood may be achieved. • Allows men and women surviving cancer treatments the possibility of having children later, using gametes harvested prior to radiation or chemotherapy treatments. • Permits screening and selection of embryos before implantation to avoid an inherited disease. • Offspring produced by IVF are much longed-for children who are more certain to be loved and cared for. • IVF can be used to treat the medical problem of infertility. Nobody should be allowed to deny treatment of a problem that can be cured.	• Allows infertility due to inherited defects to be passed on (unwittingly) to the offspring, who may then experience the same problem in adulthood. • Excess embryos are produced to ensure success and so an embryologist has to select some new embryo(s) to live and to allow the later destruction of other, potential human lives. • Multiple pregnancies have been a common outcome, sometimes producing triplets, quads or sextuplets, leading to increased risk to the mother's health, and also increased risks of premature birth and babies with cerebral palsy, for example. • It can be argued that infertility is not always strictly a health problem; it may have arisen in older parents who choose to delay having a family (a lifestyle issue). • There is an excess of unwanted children, cared for in orphanages or in foster homes. These children may have benefited from adoption by couples, childless or otherwise, keen to be caring 'parents'.

Question

11 Outline the key points that might be put by a genetic counsellor who felt that an alternative way to establish a family was more appropriate for a particular childless couple seeking *in-vitro* fertilisation treatment.

3 Embryo biopsy, preselection and therapeutic abortions.

Biopsy is the medical procedure that involves taking small samples of tissue for further analysis. It is also applied to the tissue concerned. You can see embryo biopsy in Figure 19.15 (page 472). We have noted how this procedure provides data that may need resolving by genetic counselling. It makes it possible to perform embryo selection (such as selecting the sex of a child) as well as therapeutic abortion. A situation where some people are able to make such decisions about the fate of an embryo or fetus, raises the most pressing ethical, social and cultural issues that need to be resolved where they arise. Nevertheless, these issues also arise for any wise and caring community or society. *Your discussions will contribute to this process.*

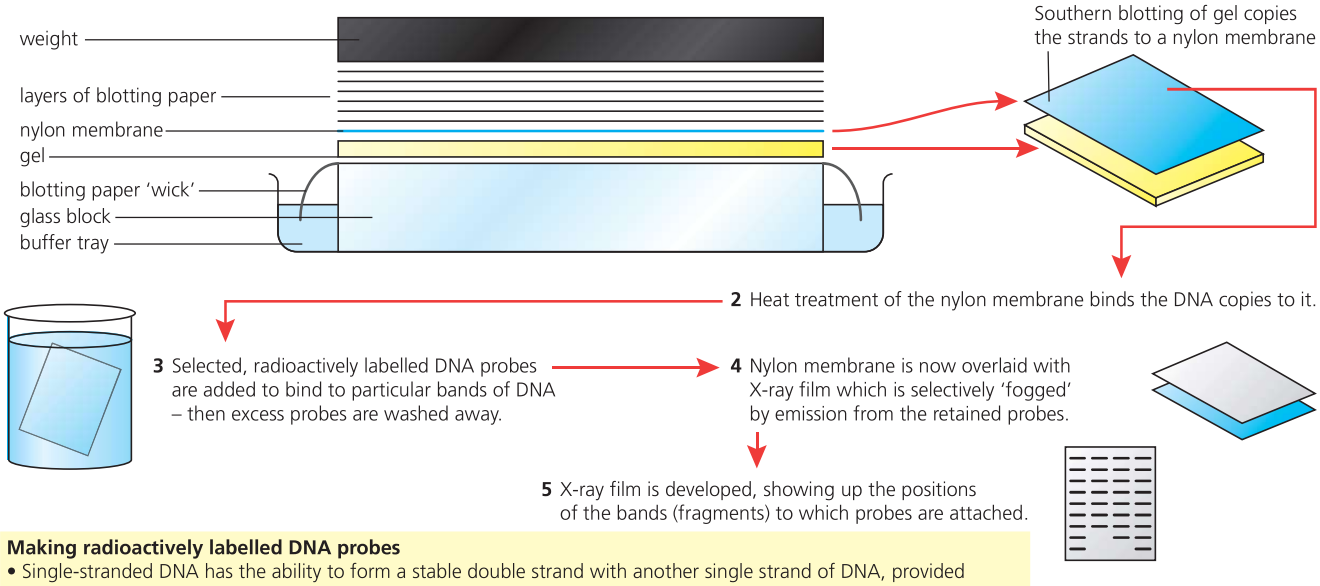
Bioinformatics in forensic medicine and criminal investigations

DNA profiling

DNA profiling exploits the techniques of genetic engineering to identify a person or organism from a sample of their DNA. The DNA of our chromosomes, which is unique, includes that of all our genes.

Southern blotting (named after the scientist who devised the routine):
- extracted DNA is cut into fragments with restriction enzyme
- the fragments are separated by electrophoresis
- fragments are made single-stranded by treatment of the gel with alkali.

1 Then a copy of the distributed DNA fragments is produced on nylon membrane:



Making radioactively labelled DNA probes
- Single-stranded DNA has the ability to form a stable double strand with another single strand of DNA, provided the bases are complementary (i.e. pair). If one strand is 'labelled', the presence of the paired strands is easily detected.
- Short lengths of single-stranded DNA are made in the laboratory for this purpose, by enzymically combining and then adding selected nucleotides one at a time, in a precise sequence.
- Consequently, the base sequence of probes is predetermined and known.
- All the nucleotides used contain radioactive phosphorus (^{32}P), or carbon (^{14}C) in the ribose of the nucleic acid backbone so the subsequent positions of the probes (and the location of a complementary strand of DNA, e.g. on a nylon membrane) can be located by autoradiography.

What a probe is and how it works

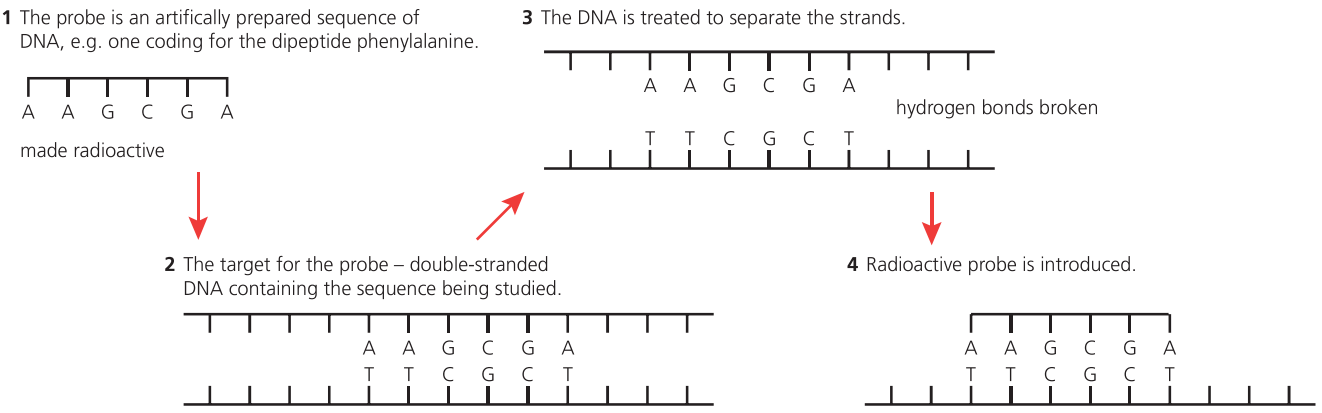


Figure 19.21 Steps in genetic profiling

The bulk of our DNA is not genes and does not code for proteins. There are extensive 'non-gene' regions consisting of short sequences of bases repeated many times. Whilst some of these sequences are scattered throughout the length of the DNA molecule, many are joined together in major clusters. It is these long lengths of non-coding, 'nonsense' DNA that are used in genetic profiling. They may be referred to as **satellite DNA** or as **variable number tandems repeats (VNTRs)**. We inherit a distinctive combination of these apparently non-functional satellite DNA, half from our mother and half from our father. Consequently, each of us has a unique sequence of nucleotides in our satellite DNA (except for identical twins, who share the same pattern).

To produce a genetic 'fingerprint', a sample of DNA is cut with a restriction enzyme which acts close to the satellite DNA regions. Electrophoresis is then used to separate pieces according to length and size and the result is a pattern of bands.

To produce a DNA 'fingerprint' or profile:

1 A sample of cells is obtained from blood, semen, hair root or other body tissues, and the DNA is extracted. (Where a tiny quantity of DNA is all that can be recovered, this is precisely copied using the polymerase chain reaction (see Figure 19.3 on page 457) to obtain sufficient DNA to analyse.)
2 The DNA is cut into small, double-stranded fragments using a particular restriction enzyme chosen because it 'cuts' close to but not within the satellite DNA.
3 The resulting DNA fragments are of varying lengths, and are separated by **gel electrophoresis** into bands which are invisible, at this stage.
4 The gel is treated to split DNA into single strands and then a copy is transferred to a membrane.
5 Selected, radioactively labelled DNA probes are added to the membrane to bind to particular bands of DNA and then the excess is washed away. Alternatively, the probes may be labelled with a fluorescent stain which shows up under ultraviolet light.
6 The membrane is now overlaid with X-ray film which becomes selectively 'fogged' by radioactive emission from the retained probes.
7 The X-ray film is developed and shows the positions of the bands (fragments) to which probes have attached. The result is a profile with the appearance of a bar code.

Steps 4 to 7 make up the technique known as Southern blotting and are illustrated in Figure 19.21.

Question

12 Explain why the composition of the DNA of identical twins challenges an underlying assumption of DNA fingerprinting, but that of non-identical twins does not?

DNA profiling has applications in forensic investigations

DNA profiles are produced from samples taken from the scene of a serious crime, such as rape attacks, both from victims and suspects as well as from others who have certainly not been involved in the crime, as a control. The greatest care has to be taken to ensure the authenticity of the sample. There must be no possibility of contamination if the outcome of subsequent testing is to be meaningful. DNA profiling helps eliminate innocent suspects, and to identify a person or people who may be responsible or related. It cannot prove with absolute certainty, anyone's guilt or connection. An example is shown in Figure 19.22.

Other crimes such as suspected murder and burglaries may be solved in cases where biological specimens are left at the scene of the crime, such as a few hairs or a tiny drop of blood. Specimens may also be collected from people suspected of being present at the crime. DNA fingerprinting can help forensic scientists identify corpses otherwise too decomposed for recognition, or where only parts of the body remain, as may occur after bomb blasts or other violent incidents, including natural disasters.

Note that there are various circumstances where the amount of DNA available or which can be recovered (such as at a crime scene) is very small indeed – apparently too little for analysis, in fact. It is now possible to submit such minute samples to the process of polymerase chain reaction (PCR) in which the DNA is replicated in an entirely automated process, *in vitro*, to produce a large amount of the sequence. A single molecule is sufficient as the starting material, should this be all that is available. The product is exact copies in quantities sufficient for analysis.

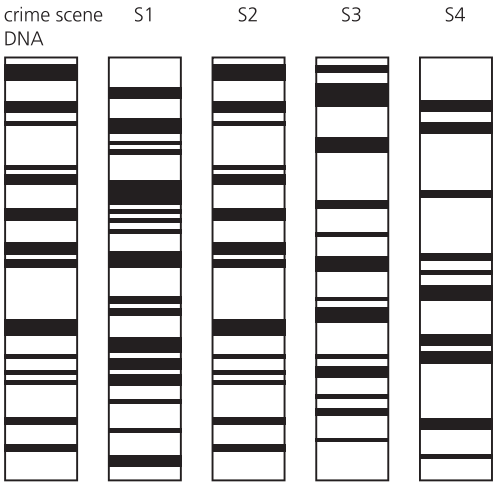
Extension

DNA profiling in forensic investigation

Identification of criminals

At the scene of a crime (such as a murder), hairs – with hair root cells attached – or blood may be recovered. If so, the resulting DNA profiles may be compared with those of DNA obtained from suspects.

Examine the DNA profiles shown to the right, and suggest which suspect should be interviewed further.



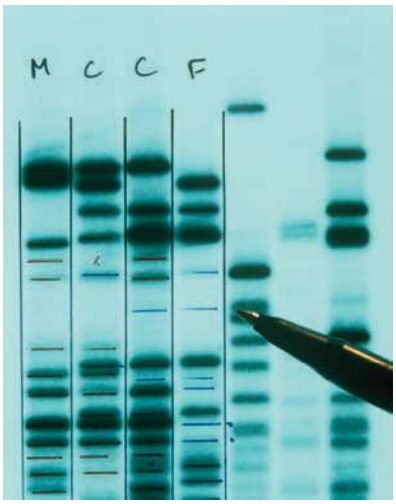
Identification in a rape crime involves the taking of vaginal swabs. Here, DNA will be present from the victim and also from the rapist. The result of DNA analysis is a complex profile that requires careful comparison with the DNA profiles of the victim and of any suspects. A rapist can be identified with a high degree of certainty, and the innocence of others established.

Identification of a corpse which is otherwise unidentifiable is achieved by taking DNA samples from body tissues and comparing their profile with those of close relatives or with DNA obtained from cells recovered from personal effects, where these are available.

DNA profiles used to establish family relationships

Is the male (♂) the parent of both children?

Examine the DNA profiles shown to the right.



Look at the children's bands (C).

Discount all those bands that correspond to bands in the mother's profile (♀).

The remaining bands match those of the biological father.

DNA fingerprinting has also been widely applied in biology. In ornithology, for example, DNA profiling of nestlings has established a degree of 'promiscuity' in breeding pairs, the male of which was assumed to be the father of the whole brood. In birds, the production of a clutch of eggs is extended over a period of days, with copulation and fertilisation preceding the laying of each egg. This provides the opportunity for different males to fertilise the female.

Figure 19.22 Applications of DNA profiling

Extension

DNA profiling has applications in determining paternity

Another very important application of DNA profiling is in issues of parentage. A range of samples of DNA of the people who are possibly related are analysed side by side. The banding patterns are then compared (Figure 19.22). Because a child inherits half its DNA from its mother and half from its father, the bands in a child's DNA fingerprint that do not match its mother's must come from the child's father.

DNA profiling also has wide applications in studies of wild animals, for example, concerning breeding behaviour, and in the identification of unrelated animals as mates in captive breeding programmes of animals in danger of extinction.

19.3 Genetically modified organisms in agriculture

The ability to manipulate genes has many potential benefits in agriculture, but the implications of releasing genetically modified organisms (GMOs) into the environment are subject to much public debate in some countries.

By the end of this section you should be able to:

a) explain the significance of genetic engineering in improving the quality and yield of crop plants and livestock in solving the demand for food in the world, e.g. Bt maize, vitamin A enhanced rice (Golden rice™) and GM salmon
b) outline the way in which the production of crops such as maize, cotton, tobacco and oil seed rape may be increased by using varieties that are genetically modified for herbicide resistance and insect resistance
c) discuss the ethical and social implications of using genetically modified organisms (GMOs) in food production

Genetic engineering and improvements in quality and yield

The World Health Organisation (WHO) has stated that hunger and related malnutrition are the greatest threat to the world's public health. Of the global population (currently 7.1 billion), some 870 million people suffer from chronic undernourishment. Most of these people live in developing countries.

The growing issue of food shortage is due to several factors:

- The size of human populations – this currently increases by 75 million people per year.
- The increasing wealth of populations in countries like China and Brazil. Growing numbers of people who once obtained the bulk of their nourishment from a diet high in grain (rice, wheat, maize) are increasing demand for meat and dairy products. Animals reared intensively require grain. It takes 5 kilos of grain to produce a single kilo of meat.
- Extreme weather conditions – climate change and water stress lessen agricultural production.
- Small farmers, whose production may sustain the needs of many of the poorest communities, have access to a shrinking land area. This is due to development of industrial farming, demands imposed by urbanisation, transport and the growth of cities, and the increasing use of land to grow alternative crops that supply raw materials for industry and urban populations. Crops grown for biofuels is just one example.

Genetically modified organisms have a part to play in worldwide improvement of agricultural production by increasing quantity and nutritional value, and by reducing loss due to pests and weed growth. However, the benefits arising from genetic modification may be at a price that the poorest (and most undernourished) are mostly unable to meet. There are urgent social and political factors to tackle too, in the battle against hunger. Nevertheless, there have been significant successes achieved by genetic modification.

Bt maize

The soil bacterium *Bacillus thuringiensis* is a natural source of an insecticide known as Cry toxin. Genes for the production of this toxin have been transferred from this bacterium to selected crops, making them toxic to certain insects if the animal should feed on the leaves, for example. The crops that have been genetically modified in this way are varieties of potato, corn and cotton. They are then referred to as Bt crops.

Bt maize has been grown in the USA since 1995. The resulting crops have been used as biofuels or in animal feed only. Subsequently, Bt potatoes and Bt cotton crops have been approved for use in USA. Bt cotton is the only genetically modified crop authorised for use in India and China. In Europe, Bt maize is grown mainly in Spain, where the crop is used as animal feed.

The advantages for Bt crops detected to date are:

- the level of toxin expression is sufficient to be toxic to pests
- expression of the Bt genes occurs within cells of the crops in such a way as to cause only insects that feed on the plant body to be killed
- toxin expression has been modulated by adding tissue-specific promoters that have limited their sites of production.

Question

13 Explain why any threat to honey bees and butterflies poses such a problem to agriculture and horticulture.

The use of these genetically modified varieties appears to have been advantageous, at least during the first 10 years. For example, the use of externally sprayed insecticides on corn and cotton crops has fallen substantially over this period.

The disadvantage has been a more recent, increasing resistance to Bt toxin among the insect pests of these crops. As a result, there has had to be a return to the use of externally-applied insecticides to maintain crop productivity.

Meanwhile, opponents of the use of Bt genetically modified crops continue to advance arguments and anxieties about their adoption (Table 19.8).

Table 19.8 Arguments advanced against the use of Bt crops and responses

Argument	Response
Pollen from Bt crops could kill butterflies, including the monarch butterfly.	This has been queried, since the pollen of Bt plants contains a very low level of toxin. Experimental evidence suggests this is not a realistic danger. Further, monarch butterflies do not consume maize pollen or even associate with the plants.
Bt genes may be transferred from Bt maize to natural maize in neighbouring plots.	A large-scale study initially failed to find evidence of contamination. Subsequent experiments have shown some small scale genetic contamination. Research continues.
Bt genes in crops may be linked to the recent disease of colony collapse in honey bee hives.	This is a recent problem and its cause, whether due to an unknown parasite or some toxin, is unknown. A study by an agricultural research group has found no evidence that Bt crops adversely affect bees, so far.

Vitamin A-enhanced rice

Vitamin A (retinol) is found only in foods of animal origin, but milk and many vegetables and fruits contain the deep orange pigment, **beta-carotene (β-carotene)**. This molecule (a photosynthetic pigment, page 264) is easily converted to retinol in the body. Beta-carotene is a good indirect source of vitamin A (Figure 19.23).

Vitamin A is essential for vision in dim light and a deficiency leads to night blindness. For children in many parts of the world a severe deficiency leads to total blindness. More recently, it has been shown that vitamin A is crucial to the functioning of our immune system. Because of this, a deficiency of vitamin A is the cause of very many childhood deaths from such common infections as diarrhoeal disease and measles.

We have already noted that rice is the staple for very many people. Whilst the green foliage of rice contains beta-carotene, the grain contains none. A genetically modified variety of rice has been produced which stores significant (and potentially life-saving) quantities of beta-carotene in the grain.

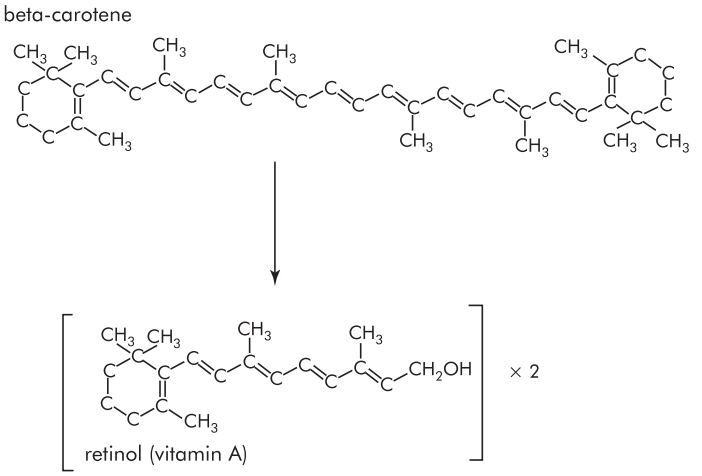


Figure 19.23 Beta-carotene as a source of vitamin A

We earlier saw that herbicide resistance had been introduced into some crops using the tumour-forming bacterium, *Agrobacterium* (page 395). This soil-inhabiting bacterium invades broad-leaved plants at the junction of stem and root, forming a huge growth called a tumour or crown gall. However, other plants, including the grasses are not attacked in this way. Consequently, a different method of delivering the genes for storage of beta-carotene in rice grain had to be used. In rice, the genes were delivered directly into the cells using extremely small gold or tungsten 'bullets' that were coated with the required DNA and then literally fired in with an appropriately sized gun. This method of genetic modification is used quite widely now.

A helping of about 300 g of these genetically modified rice grains, when cooked, contains sufficient precursor of vitamin A to meet a person's daily requirements. However, there remains a great deal of opposition to it. For example, field trials of this crop have not even been undertaken in some parts of the world, including in countries where vitamin A deficiency causes health problems. Opponents of the product argue that a more balanced diet that includes fresh green vegetables is a healthier solution. One might query whether this response is realistic, given the huge numbers of people that live in crowded urban environments where many items of a balanced diet are not available to most people. There is no doubt that genetic modification is a technology with many opponents.

GM salmon

Wild salmon (*Salmo salar*) have been genetically engineered to include growth-hormone regulating genes from Chinook salmon of the Pacific ocean. This has resulted in a much faster growth rate, and one that is maintained all the year round, rather than just in the spring and summer when growth is fastest in wild and fish-farmed salmon. These GM fish grow to market size in 18 months, rather than 3 years.

The role of crops with herbicide and disease resistance

The production of disease-resistant crops has been discussed previously (page 396).

Herbicide-resistant crops

Transgenic flowering plants may be formed using tumour-forming *Agrobacterium*. This soil-inhabiting bacterium sometimes invades broad-leaved plants at the junction of stem and root, forming a huge growth called a tumour or crown gall. The gene that induces tumour formation occurs naturally in a plasmid in the bacterium, known as a **Ti plasmid**. Useful genes may be added to the Ti plasmid (using restriction enzymes and ligase – see page 458), and the recombinant plasmid placed back into *Agrobacterium*. Then a host crop plant can be infected by the modified bacterium. The gall tissue that results may be cultured into independent plants, all of which also carry the useful gene. The plant will then make the gene product, which will be useful to humans. Herbicide-resistant sugar beet and other crop plants have been produced in this way (Figure 19.24).

Glyphosate is a powerful systemic herbicide. Once it has been absorbed by a green plant it is translocated to all tissues and organs and inhibits an enzyme essential for the production of amino acids. The outcome is that the whole plant dies. This enzyme is absent from animals, so glyphosate has very low toxicity for animal life. On reaching the soil, glyphosphate is inactivated and rendered harmless by soil bacteria.

The gene coding for the enzyme that inactivates glyphosphate has been identified in these soil bacteria, isolated and then transferred to the crop plants. These plants are now referred to as 'herbicide-resistant' because when the herbicide is applied the GM crop plant degrades the herbicide molecules and remains unharmed. Meanwhile, glyphosphate is also absorbed by weeds and kills even the largest weeds, including their extensive root systems.

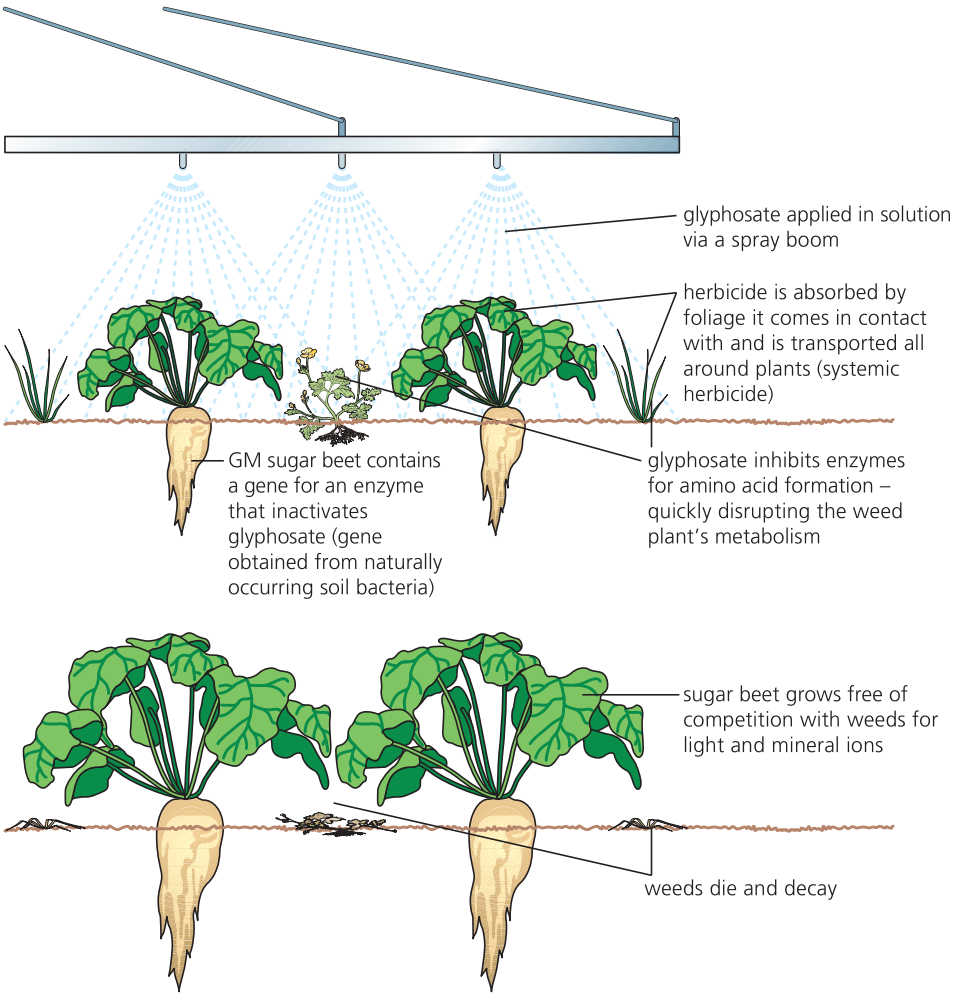


Figure 19.24 Treatment of a herbicide-resistant sugar best crop

Table 19.9 Benefits and potential dangers of growing glyphosphate-resistant crops

Benefits	Dangers
• It eradicates weeds around sugar beet crops, where loss of yield due to competition with weeds is very high. • Glyphosate herbicide is transported all around the weed plant, killing even the largest weeds with extensive roots. • Glyphosate inhibits an enzyme for the production of essential amino acids in plants. This enzyme is absent from animals, so glyphosate has very low toxicity. • An average of five sprays of herbicide are applied to sugar beet in conventional cultivation, whereas with GM beet a maximum of three spays (and normally only one or two) are needed because of the greater activity of glyphosate. • Cereals normally precede spring-sown beet. Cereal stubble is an important source of food for bird life in autumn and winter. With glyphosate herbicide applied to GM beet being so effective, there is no need for weed control in stubble the preceding autumn – leaving wild birds' food sources available longer. • Glyphosate herbicide provides good weed control, allowing cultivations by discs or tines (conservation tillage) rather than by ploughing. These systems have fewer harmful effects on soil organisms, increase moisture, improve soil structure, and reduce tillage costs. • Glyphosate herbicide is applied as large droplets from coarse nozzles, rather than as a fine mist of tiny droplets that are prone to drift onto surrounding habitats.	• Fewer weed species on farmland and longer periods without weeds growing breaks wildlife food chains. • Selection of a glyphosate-resistant crop plant ties the grower to one particular herbicide product – choice is lost. • Will genetically modified plant material, when consumed by humans, release novel toxins or otherwise adversely affect existing enzyme systems in the human digestive system? • Genetically engineered genes may be vectored in plasmids containing an antibiotic-resistance gene to facilitate processes in the lab. This latter gene might be accidentally transferred to human gut bacteria via food eaten. • 'Superweeds' may develop by cross-pollination between herbicide-tolerant crop plants and compatible weed species (fat-hen, sea beet and weed beet are naturally occurring near relatives of sugar beet). Superweeds would be difficult to eradicate from crops. • There is a possibility of cross-pollination between GM crops and conventional and organic crops. The maintenance of sufficient distance or the devising of effective barriers to prevent or reduce pollen transfers between crops may be difficult to achieve. • If glyphosate herbicide droplets do reach hedgerow plants (or further) their size and chemical activity in plants means they are more likely to do damage.

Ethical and social implications of GM organisms in food production

In the description of the development of genetically-modified organisms (GMOs) presented in this topic, specific ethical, social and scientific issues have already been raised (see pages 477–81).

In preparing a paper for discussion with peers and your tutor, the issues can be researched and your own opinion developed within the following framework:

- What social and legal controls does society need, to influence these developments?
- What effects may GMOs have on the environment and on surrounding, unrelated species?
- What health risks are raised by GMOs, and what are their effects on human health?
- Who will have access to the technology, and how will scarce resources be allocated?

Because GMO technologies have been available for a relatively short while, some possible long-term effects may not yet have emerged. Remember, 'absence of evidence is not evidence of absence'. The greatest dangers may be one or more that have not yet emerged. On the other hand, if calculated risks are not taken, no progress is possible.

Summary

- **Gene technology** includes **genetic engineering, DNA sequencing** and **genetic fingerprinting**. Gene technology has applications in biotechnology, medicine and the pharmaceuticals industries, agriculture and forensic science. Gene technology generates many benefits for humans, but there are potential hazards, too, so gene technology raises **ethical issues**.
- **Genetic engineering** involves the transfer of genes from one species to another, possibly unrelated organism. Genes are transferred by inserting DNA into a vector, typically a **plasmid**, a tiny ring of double-stranded DNA obtained from a bacterium. The gene and the vector are cut by means of the same **restriction enzyme**, forming compatible **sticky ends** at the cuts. The gene and plasmid are then brought together and joined using the enzyme **ligase**. An alternative vector is the nucleic acid of a virus.
- Bacteria that have been genetically engineered to carry a new gene are **identified** by the use of plasmids that carry genes for resistance to two antibiotics, known as **R-plasmids**. Alternatively, a **fluorescent marker** is used.
- For a gene to be expressed (transcribed into messenger RNA) a **promoter** normally needs to be attached and activated. One example is the lac promoter mechanism present in certain bacteria.
- A gene may be **constructed from the messenger RNA** that it codes for, using the enzyme **reverse transcriptase**. Alternatively, copies of genes may be built up from nucleotides in the correct sequence. This is worked out from the **amino acid sequence** of the protein the gene codes for using the **genetic code**.
- **Genetically modified bacteria** are now used to make valuable products such as human **insulin** (for the treatment of diabetics), human **growth hormone** and several other hormones and enzymes of use in medicine, agriculture and other industries.
- **Genetically modified eukaryotes** are harder to produce. They carry two copies of a gene, unlike prokaryotes, so the engineering processes are often more difficult. However, food plants with **herbicide resistance**, **insect resistance** or improved food value have been produced.
- **DNA sequencing** involves the creation of genomic libraries of the precise **sequence of nucleotides** in samples of DNA of individual organisms. The nucleotide sequence in the whole human genome was the product of the **Human Genome Project**. Many other genomes have been completely sequenced, too.
- **Genetic profiling** involves the analysis of DNA to identify the individual from which the DNA was taken. It is used to establish identity and the genetic relatedness of individuals, for example in forensic science. The DNA samples are cut by **restriction enzymes** and the fragments separated. **Electrophoresis** is a process used to separate molecules such as proteins and nucleic acid fragments (of either DNA or RNA) on the basis of their net charge and size.
- **Genetic disorders** are heritable conditions that are caused by a specific defect in a gene or genes. Most arise from a **mutation** involving a **single gene**. Genetic disorders affect about 1–2 per cent of the human population and include sickle cell anaemia, haemophilia and **cystic fibrosis**. It is possible that the symptoms of a genetic disorder like cystic fibrosis may eventually be overcome by **gene therapy**. The Human Genome Project has made it possible to **screen** for the presence of the mutant cystic fibrosis allele. Consequently, affected people and their relatives may be referred to a **genetic counsellor** whose role is to take a detailed case history and to provide information from which their clients can make choices.
- Crop improvements are also attempted by genetic engineering. Projects have included herbicide-resistant oil seed rape and sugar beet, insect-resistant maize and cotton and vitamin A enhanced rice. The results have often been cautiously and even critically received, sometimes with possible good reasons. Genetic engineering remains a technology with many opponents. Very few countries have approved genetically modified crops for commercial production.

Examination style questions

1 **a)** A husband and wife who already have a child with cystic fibrosis (CF) elected to have their second child tested for the condition while still a fetus in very early pregnancy. The results of the test, a DNA banding pattern, were discussed with a genetic counsellor.
The relevant DNA banding pattern produced by electrophoresis is shown in Fig. 1.1.

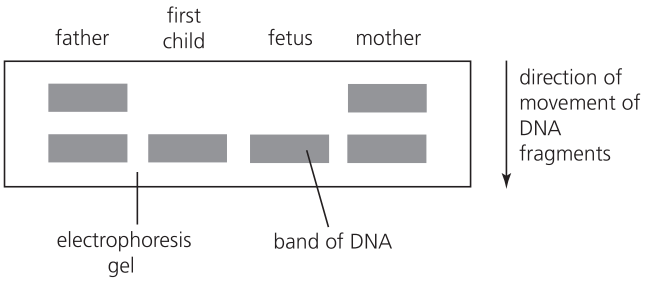


Fig. 1.1

With reference to Fig. 1.1, explain why,

ii) the fetus will develop CF [1]

ii) the positions of the bands of DNA of the first child and of the fetus indicate that the mutant allele for CF has a deletion in comparison with the normal allele. [2]

b) Explain briefly the need to discuss the result of the test with a genetic counsellor. [4]

[Total: 7]

(Cambridge International AS and A Level Biology 9700, Paper 41 Q6 November 2009)

2 In humans, the gene *RPE65* encodes a protein responsible for regenerating visual pigment in rod and cone cells after they have been exposed to light. A recessive allele of this gene causes impaired vision from birth, progressing to complete blindness in early adulthood. This condition is called LCA.

In 2008, trials were carried out into the possibility and safety of treating LCA using gene therapy.

a) Suggest and explain why LCA is suitable for treatment using gene therapy. [3]

b) Six adults with this condition were used in the study. Genetically modified adenoviruses (a type of virus that can cause respiratory infections) were used as vectors.

The vectors were injected beneath the retina of one eye of each of the participants.

Suggest two ways in which the genome of the adenoviruses used as vectors would differ from that of normal adenoviruses. [2]

c) Improvements were found in the vision of all the participants, but the small number in the trials made most of these improvements not statistically significant.

Suggest why these trials were designed to include such a small number of participants. [2]

[Total: 7]

(Cambridge International AS and A Level Biology 9700, Paper 41 Q5 November 2011)

3 **a)** Four naturally occurring enzymes make possible the genetic engineer's activities. List these enzymes, their sources, and the reaction they catalyse. [8]

b) In the *in-vitro* synthesis of human insulin, a copy of the gene has been made by first isolating mRNA from human pancreatic tissue. Outline the steps that this process involves. [6]

c) Explain the advantages gained by:

i) Making the insulin gene from mRNA compared to obtaining genes directly from relevant chromosomes. [3]

ii) The choice of insulin obtained by genetic engineering for the treatment of diabetics, compared to the use of insulin from animal sources. [3]

[Total: 20]

4 **a)** Give an illustrated account of the separation of DNA fragments by electrophoresis. [6]

b) Explain:

i) The nature and significance of 'variable number tandem repeat' sections of chromosomes in DNA fingerprinting.

ii) The steps in the production of a DNA profile. [10]

c) Outline the diverse applications of DNA profiling in science and society. [4]

[Total: 20]

5 **a)** Outline the steps to crop improvement by genetic modification with reference to canola sugar beet crops and herbicide resistance.

b) Identify both the potential benefits of genetic engineering of food crops for agricultural production and the possible hazards that may arise, illustrating your answer with reference to current examples.