8 TECHNOLOGY IS USED TO TREAT DISEASES

UNIT 3 CONTENT

SCIENCE INQUIRY SKILLS

- conduct investigations, including the collection of data related to homeostasis and the use of models of disease transmission, safely, competently and methodically for the collection of valid and reliable data
- represent data in meaningful and useful ways, including the use of mean, median, range and probability; organise and analyse data to identify trends, patterns and relationships; discuss the ways in which measurement error, instrumental accuracy, the nature of the procedure and the sample size may influence uncertainty and limitations in data; and select, synthesise and use evidence to make and justify conclusions
- select, use and/or construct appropriate representations, including diagrams, models and flow charts, to communicate conceptual understanding, solve problems and make predictions

SCIENCE AS A HUMAN ENDEAVOUR

- synthetic hormones may be developed to control or treat endocrine dysfunction, including diabetes mellitus, hypothyroidism and hyperthyroidism, to improve the quality of life for individuals
- gene therapy can be used to treat a range of diseases, including diabetes mellitus
- hormones and vaccines are developed using recombinant DNA and associated biotechnological techniques
- cell replacement therapy has the potential to treat nervous system disorders including Alzheimer's and Parkinson's diseases

Source: School Curriculum and Standards Authority, Government of Western Australia

9780170449168

Do you eat bread, cheese or yoghurt? If so, you are consuming foods that are produced by biotechnology. **Biotechnology** uses cellular processes to make products that are of use to humans. For thousands of years people have been using yeasts to make bread and alcohol, and bacteria to make cheese and yoghurt.

Shutterstock.com/Jeroen Nieuwenhuisen

FIGURE 8.1 Cheese making uses ancient biotechnology methods

The science of cheese

Modern biotechnology has dramatically expanded the range of techniques and products that can be used to improve human welfare. Improvements in the treatment and prevention of disease, food production, production of clean energy and enhanced efficiency of manufacturing processes are all resulting from advances in biotechnology.

Therefore, the definition of biotechnology has more recently been expanded to include genetic testing, gene manipulation, cell replacement therapies and tissue engineering. Some of the methods and outcomes of modern biotechnology are described in this chapter.

8.1 RECOMBINANT DNA

Many methods of modern biotechnology utilise our understanding of DNA and apply it to specific processes.

DNA

You learnt about DNA in *Human Perspectives ATAR Units 1 & 2*; however, we will review it briefly here. DNA (deoxyribonucleic acid) is found in the cells of all organisms, usually in the nucleus, but there is also some in the mitochondria and, in some organisms, in the cytosol. All DNA molecules consist of two strands of nucleotides. Each nucleotide is made up of a deoxyribose sugar molecule, a phosphate group and a nitrogenous base. When these nucleotides join together, it makes a backbone of alternating deoxyribose sugar and phosphate with nitrogen bases branching at each sugar molecule. The bases from two strands are attracted to one another by hydrogen bonds, and this forms cross-links between the two strands. The DNA molecule is twisted into a spiral known as a double helix.

There are four different nitrogen bases in a DNA molecule: adenine (A), thymine (T), cytosine (C) and guanine (G). The base pairs are complementary; adenine will only pair with thymine, and cytosine will only pair with guanine. The order in which the nitrogen bases occur in the DNA molecule is the genetic information that determines the structure of the cell and the way it functions.

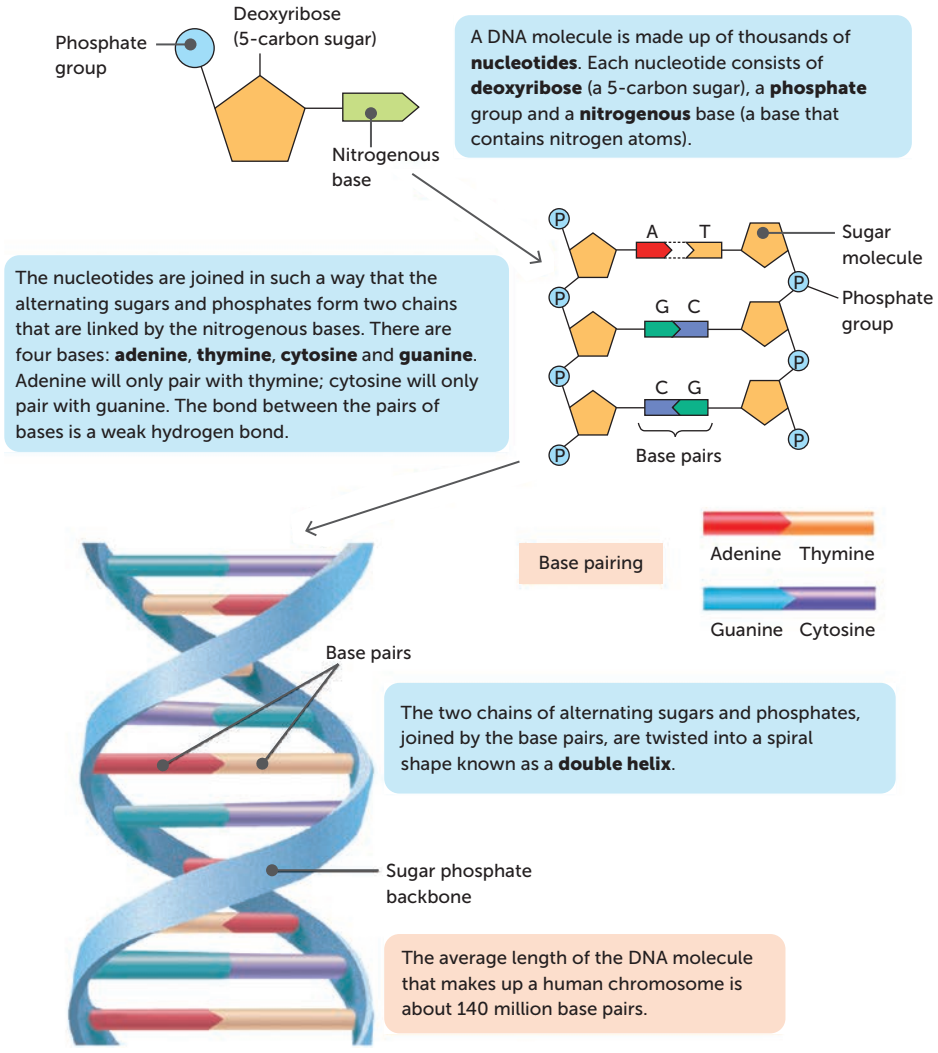


FIGURE 8.2 DNA consists of two strands of alternating sugars and phosphates with pairs of nitrogen bases forming cross-links between the chains. The two strands are twisted into a double helix

Recombinant DNA technology

Scientists have been modifying the DNA of organisms for a long time. By selecting which male and female organisms are crossed to produce offspring, we are increasing the chance of certain genes being present in the DNA of the next generation. If parents with desired traits are chosen, we can increase the chance of the gene for those phenotypes being passed on. And when parents without undesirable traits are chosen, there is a decreased chance of the genes for that phenotype occurring in the next generation. In this manner, we can either increase or decrease the incidence of certain genes. This process is known as **artificial selection** or **selective breeding**.

Artificial selection is quite a slow and inefficient process. Genes are passed on by chance and it is necessary to wait for the next generation to mature before knowing the outcome.

Shutterstock.com/Nature Art

FIGURE 8.3 Pugs are an example of selective breeding being used to increase the occurrence of genes for desirable traits

An alternative process is **genetic engineering,** which involves the artificial modification of DNA. This is also known as **recombinant DNA technology**. In this process, DNA is either added or removed from a cell. The DNA produced is called **recombinant DNA** and the organism is a **genetically modified organism (GMO)**. This technology has a wide range of possible uses, including introducing genes for desired traits into organisms, using harmless bacteria to produce proteins, and replacing faulty genes.

AAP Photos/Erik de Castro/Reuters

FIGURE 8.4 Golden Rice is named for its golden colour due to the presence of beta-carotene

Some applications of genetic engineering involve DNA from one species being introduced into a different species. The organism produced is a **transgenic organism**. The aim of transgenic organisms is to introduce a trait that is not normally present. All transgenic organisms are GMOs, but not all GMOs are transgenic. One example of a transgenic organism is Golden Rice, which was developed to address vitamin A deficiency in developing countries. The deficiency kills approximately 670 000 children under the age of five every year. Golden Rice was produced by introducing a gene from maize and a bacterium found in soil into rice. This allows the rice to produce beta-carotene, which the human body can use to synthesise vitamin A.

Golden Rice
This website provides more information about Golden Rice.

Key concept

Recombinant DNA technology, or genetic engineering, involves artificially changing DNA and produces a genetically modified organism. If this organism has DNA from another species, it is called a transgenic organism.

Development of recombinant DNA technology

Stanley Norman Cohen and Herbert Boyer invented the recombinant DNA technique in 1973. Their technique was to isolate and amplify genes or DNA segments and insert them into a bacterial cell, creating a transgenic bacterium. The introduced genes become part of the transgenic organism's DNA and can be passed on from one generation to the next.

Transgenic or GMO?
This website explains the difference between transgenic organisms and genetically modified organisms in more detail.

Restriction enzymes

For genetic engineering to be possible, the gene for the desired trait must be identified and then isolated. Next, the DNA receiving the gene must be 'opened', and the gene is then added to the recipient and joins its DNA.

A key breakthrough in genetic engineering involved viruses that infect bacterial cells. These are called **bacteriophages**, or **phages**. It was discovered that certain enzymes in bacteria are able to restrict the duplication of bacteriophages by cutting up the viral DNA. Scientists found that these enzymes always cut the DNA at a point where there is a certain sequence of bases. This sequence is known as a **recognition site** (or **recognition sequence**), and the enzyme that cuts the DNA is a **restriction enzyme** because it restricts the duplication of bacteriophages.

9780170449168

Restriction enzymes are examples of **endonucleases**, enzymes that cut within a DNA molecule by separating two nucleotides. Some restriction enzymes produce a straight cut at the sequence, while others produce a staggered cut.

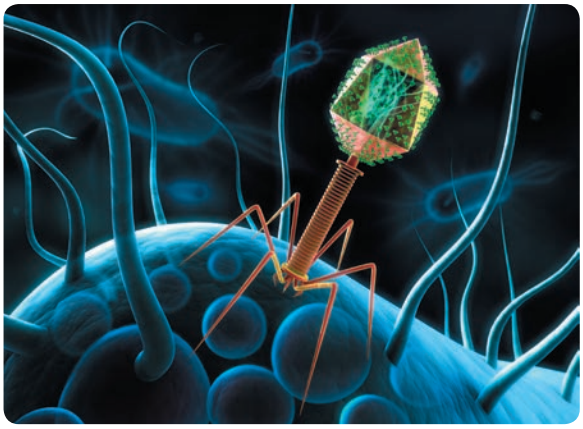
- A **straight cut** is when the restriction enzyme makes a clean break across the two strands of DNA to produce a blunt end. A **blunt end** is when both strands terminate in a base pair.
- A **staggered cut** results in fragments with sticky ends. A **sticky end** is a stretch of unpaired nucleotides in the DNA molecule that overhang at the break in the strands.

Recognition sites are four to eight base pairs in length and are **palindromic**, meaning that they have the same sequence when read both forward and backwards. This, along with the complementary nature of the bases, means that the same sequence occurs on both strands within the recognition site. Therefore, both strands will be cut, resulting in the DNA molecule forming two segments.

Each restriction enzyme will:

- recognise a certain base sequence
- cut at a certain point.

Both of these factors contribute to the type of cut. For example, the XmaI enzyme and SmaI both have the same recognition site, 5′-CCCGGG-3′. XmaI cuts between the first and second nucleotides and produces sticky ends, while SmaI cuts between the third and fourth nucleotides and produces blunt ends.



Shutterstock.com/peterschreiber.media

FIGURE 8.5 A digital image of a bacteriophage infecting a bacterium

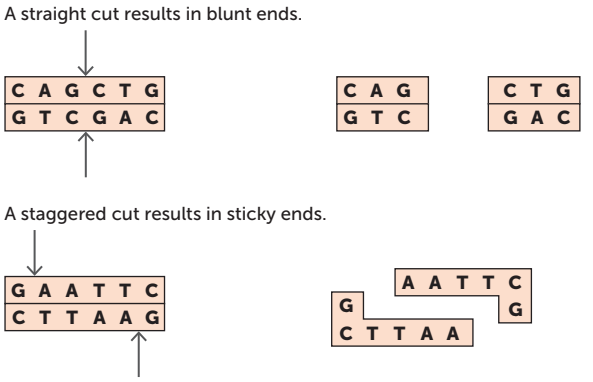
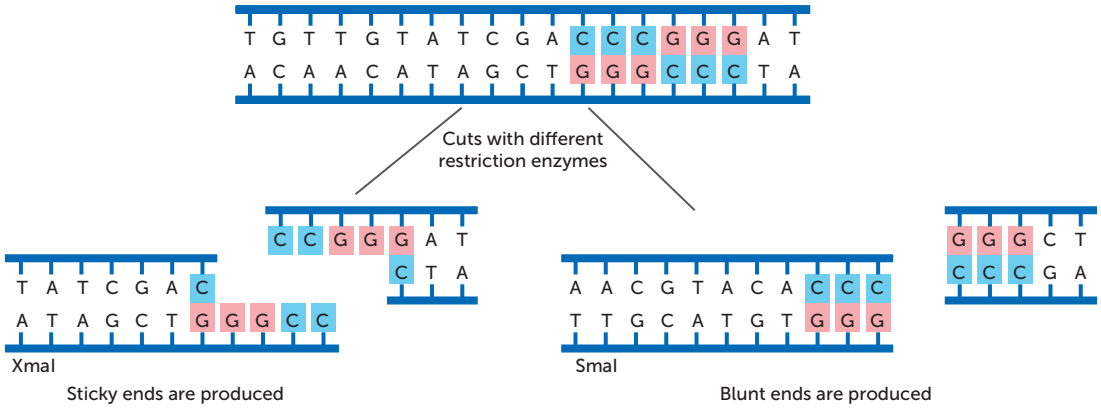


FIGURE 8.6 Cuts in DNA strands produced by restriction enzymes

FIGURE 8.7 The recognition site and position of the cut determine the types of ends produced by restriction enzymes



Sticky ends are so named because of their ability to combine with sections of DNA that have a complementary ending. These are very useful in recombinant DNA technology as they allow a single-stranded overhang from one DNA fragment to be paired with any other piece of DNA that has a corresponding sequence. This DNA could be from the same or a different organism.

Table 8.1 lists some restriction enzymes. The name of each enzyme reflects its origin.

- The first letter of the name comes from the genus of the bacterium from which it was isolated.
- The second two letters come from the species.
- The next letter refers to the strain of the bacterium.
- The roman numerals represent when the enzyme was isolated, where I is the first enzyme isolated, II is the second enzyme isolated etc.

For example, EcoRI is the first restriction enzyme isolated from the RY13 strain of the bacterium *Escherichia coli*, while HindIII is the third enzyme isolated from the R(d) strain of the *Haemophilus influenzae* bacterium.

Restriction enzymes
Use the simulation to view the cuts produced by different restriction enzymes.

Activity 8.1
Investigating restriction enzymes

TABLE 8.1 Examples of restriction enzymes

ENZYME	RECOGNITION SITE	BACTERIAL ORIGIN
BamHI	G↓GATCC CCTAG↑G	*Bacillus amyloliquefaciens*
EcoRI	G↓AATTC CTTAA↑G	*Escherichia coli*
HindIII	A↓AGCTT TTCGA↑A	*Haemophilus influenzae*
TaqI	T↓CGA AGC↑T	*Thermus aquaticus*
PvuII	CAG↓CTG GTC↑GAC	*Proteus vulgaris*

Key concept

Restriction enzymes cut DNA at a palindromic recognition site, producing either blunt ends or sticky ends in sections of DNA.

DNA ligase

Another major breakthrough in being able to modify genes was the discovery of an enzyme that was able to join, or recombine, separate pieces of DNA. This enzyme, found in the bacterium *Escherichia coli* (*E. coli*), was originally called a 'DNA-joining enzyme', but is now known as **DNA ligase**. Some version of DNA ligase is used by every living cell to 'glue' together short strands of DNA during replication, a process called **ligation**.

DNA ligase works by joining the phosphate group at the end of one strand to the sugar molecule at the end of another strand. For this to be possible, the complementary bases must first join by forming hydrogen bonds. Then the DNA ligase can join the backbone of each strand.

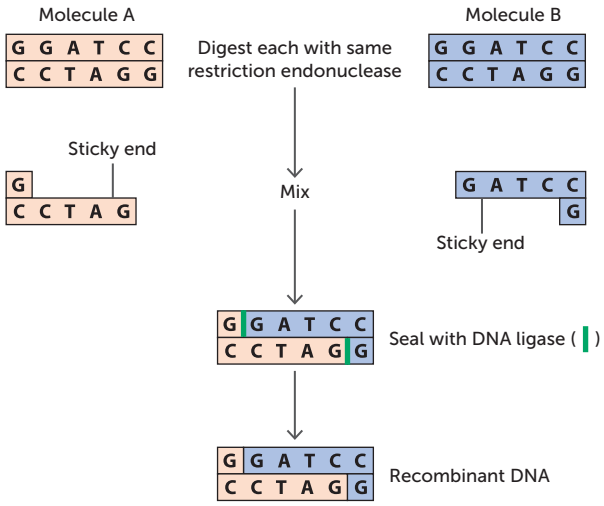


FIGURE 8.8 Making a recombinant DNA molecule

Activity 8.2
Investigating bacterial transformation

Key concept

DNA ligase is able to join sections of DNA.

Use of vectors

In genetic engineering, a **vector** is a DNA molecule that is used to carry DNA into a cell. The first step in producing an organism with recombinant DNA is to isolate the gene of interest. This gene is then inserted into a vector and cloned. This is achieved by:

1. identifying the desired gene
2. using a restriction enzyme to cut the DNA on either side of the gene
3. using the same restriction enzyme to cut the DNA of the vector
4. adding the desired gene to the vector
5. using DNA ligase to join the two sections of DNA.

Video on recombinant DNA
This website has a video showing how DNA recombination can be used in the manufacture of certain proteins.

In recombinant DNA technology, commonly used vectors are bacterial plasmids and bacteriophage viruses. **Plasmids** are usually circular, double-stranded units of cytoplasmic DNA, frequently found in bacteria, that are capable of replicating within a cell independently of the chromosomal DNA. The gene of interest is integrated into the plasmid or phage, and is referred to as recombinant DNA. Cloning of the vector then occurs so that numerous copies of the DNA are available to insert into the host cells. Once large quantities of the vector have been produced, they can be introduced into the selected host cells such as special bacterial, yeast or mammalian cells. These host cells will then produce the foreign protein using instructions in the gene in the recombinant DNA.

Bacteria into which the gene for insulin production has been introduced are now used in the manufacture of insulin for the treatment of diabetes.

Key concept

Vectors, such as plasmids or bacteriophages, can be used to transfer DNA into a host cell.

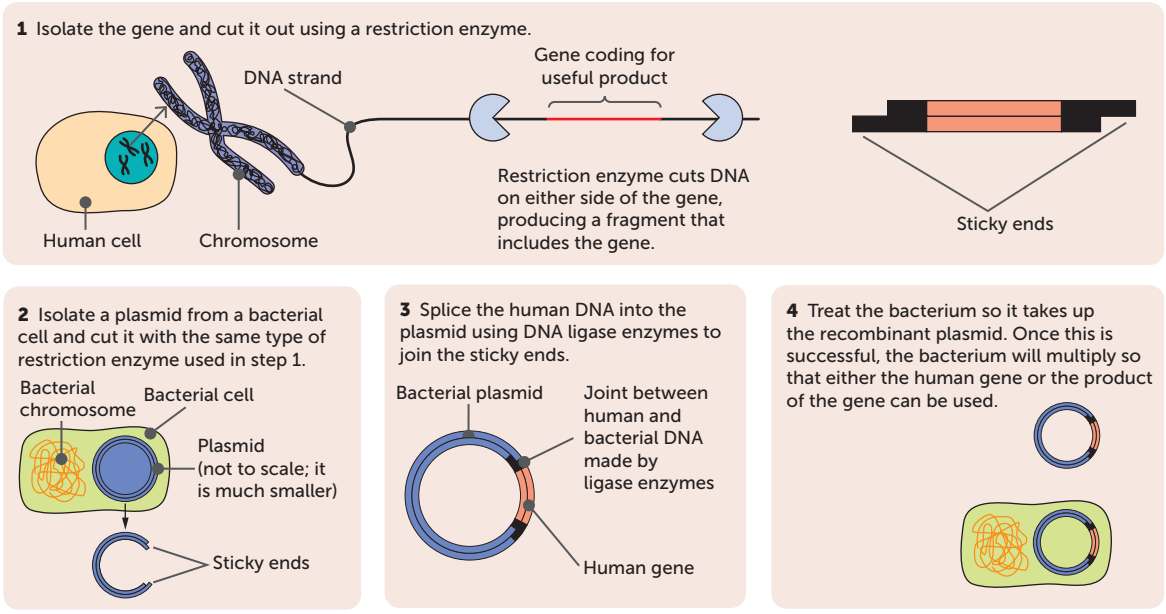


FIGURE 8.9 A simplified diagrammatic representation of recombinant DNA technology

TABLE 8.2 Terminology for recombinant DNA technology

TERM	DEFINITION
Blunt ends	The ends produced by a straight cut of a sequence of nucleotide bases
DNA ligase	An enzyme capable of combining two small components of single-stranded DNA into one single structure
Phage	Or bacteriophage; a virus that infects bacteria
Plasmid	A small circular strand of DNA distinct from the main bacterial genome; it is composed of only a few genes and is able to replicate independently within a cell
Restriction enzyme	An enzyme that cuts a strand of DNA at a specific sequence of nucleotides called the recognition site
Staggered cut	Produced when a restriction enzyme creates fragments of DNA with unpaired nucleotides that overhang at the break in the strands; called sticky ends
Straight cut	Produced when a restriction enzyme makes a clean break across the two strands of DNA so that the ends terminate in a base pair; called blunt ends
Sticky ends	The overhanging ends produced by a staggered cut of a sequence of nucleotide bases; can be called cohesive ends
Vector	A bacterial plasmid, viral phage or other such agent used to transfer genetic material from one cell to another

Examples of the use of recombinant DNA technology

Recombinant DNA technology has had an enormous impact on the diagnosis and treatment of diseases and genetic disorders. It has also enabled the manufacture of large quantities of pure protein for many medical products, including insulin, growth hormone, factor VIII and follicle-stimulating hormone (FSH). In the past these substances had to be extracted from people or animals, and they were often impure and/or of variable strength. One example was the transmission of the human variant of Creutzfeldt-Jakob disease (vCJD) by contaminated human growth hormone. This disease, a variant of 'mad cow disease', is a rare but fatal brain infection. There is evidence that some blood products used to produce the protein factor VIII were contaminated with vCJD.

Shutterstock.com/Varvara Vomarovska

FIGURE 8.10 GloFish®, the first commercially available transgenic organism, are a type of zebra fish that have been modified through the insertion of a gene that codes for the production of a protein that glows with a green fluorescence

Designer hens
This website shows how designer hens are able to lay eggs containing proteins to fight human diseases.

Recombinant DNA and vaccines

The first vaccine for human use that was produced using recombinant DNA technology was the hepatitis B vaccine, introduced in 1986. It was produced by inserting a gene from the hepatitis B virus into the cowpox virus.

Most of the vaccines currently being investigated are focused on using recombinant bacterium *E. coli* or cells from mammals, insects or yeast to produce protein antigens. These can be introduced to the body, where they will elicit an immune response. Vaccines produced using recombinant DNA are known as **recombinant vaccines**.

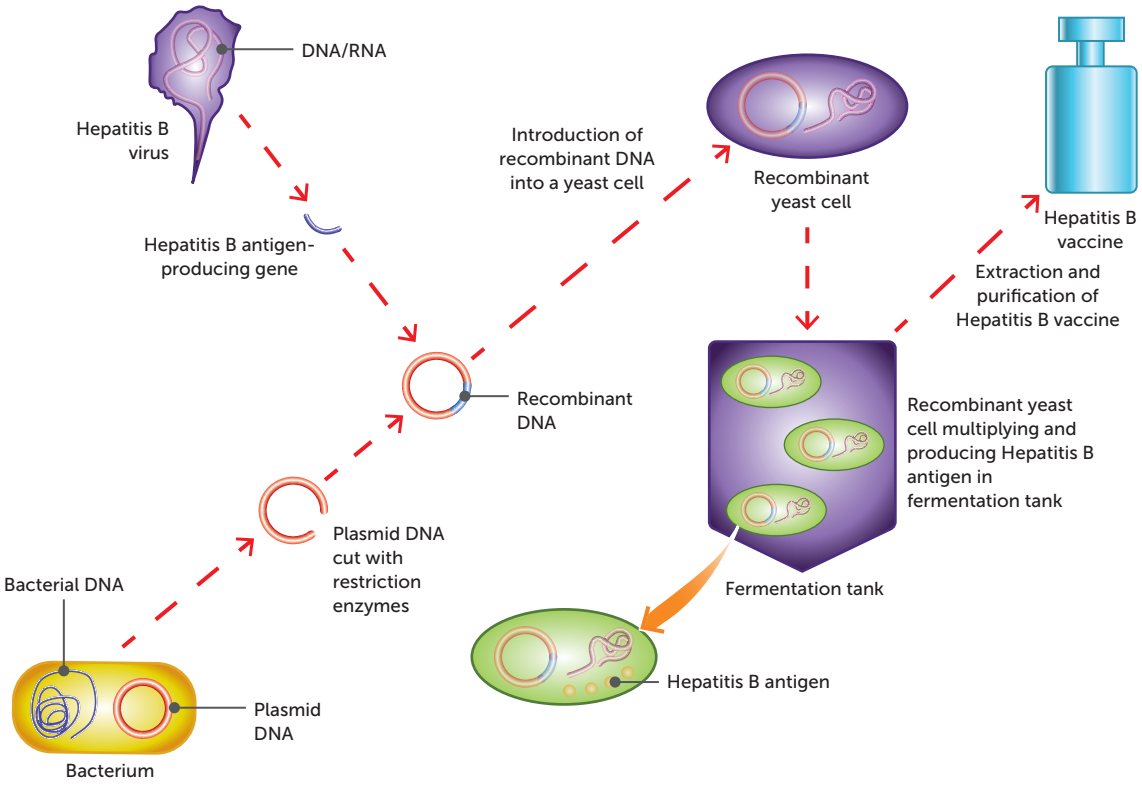


FIGURE 8.11
Recombinant yeast cells are used to produce a vaccine for hepatitis B

Currently, the vaccine for hepatitis B is produced using recombinant technology. The gene for a surface antigen on the virus is isolated and added to a plasmid. The plasmid is introduced into a yeast cell. When the yeast cell divides, the new cells also contain the plasmid with the gene for the antigen. This gene allows the yeast cells to produce the antigen protein, which can be collected and purified.

Another vaccine produced by recombinant technology is for the human papilloma virus (HPV). It is produced in a very similar process, using recombinant yeast or insect cells. These cells produce proteins that are found in the coat of the virus and are collected to be used in the vaccine.

Another area of research is **DNA vaccines**. With recombinant vaccines, the antigen is produced and then introduced in the vaccine. Yet, with DNA vaccines the DNA for the antigen is introduced in the vaccine instead of the antigen itself. The DNA is incorporated into the host's cells, which will produce the antigen. The thought is that the antigen will then be expressed by the host cells, in a similar way to what happens during a viral infection.

Development of recombinant DNA vaccines does have disadvantages. It is very expensive, as the genes for the desired antigens must be located, cloned and expressed efficiently in a new vector. In addition to this financial deterrent to innovation, those involved in vaccine research must also be conservative. Because vaccines are used on large numbers of healthy people, many of whom are children, the safety of the product is paramount. Therefore, if a conventional vaccine is known to be safe, there is little incentive to develop a new one using genetic engineering.

Key concept

Recombinant DNA technology can be used to synthesise hormones and vaccines.

9780170449168

Questions 8.1

RECALL KNOWLEDGE

1 Define:
- **a** recombinant DNA technology
- **b** genetically modified organism
- **c** transgenic organism
- **d** vector
- **e** palindromic
- **f** plasmid
- **g** bacteriophage.

2 Draw a labelled diagram to show the structure of DNA.

3 What base is complementary to:
- **a** adenine?
- **b** cytosine?
- **c** guanine?
- **d** thymine?

4 Describe the function of:
- **a** restriction enzymes
- **b** DNA ligase.

5 What vector is used in the production of insulin by recombinant DNA technology?

6 Explain how recombinant DNA technology is used to produce a vaccine for hepatitis B.

APPLY KNOWLEDGE

7 Use a Venn diagram or table to compare and contrast artificial breeding with genetic engineering.

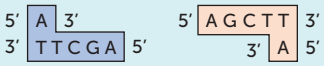
8 Explain why it is possible for an organism of one species to use a gene from another species to produce a protein.

9 Explain the importance of complementary bases with respect to inserting a fragment of DNA into a vector.

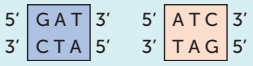
10 What restriction enzyme is the third one isolated from the d strain of *Haemophilus influenzae*?

11 Classify each of the following as blunt ends or sticky ends.

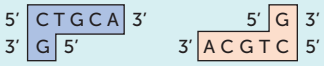
a



b



c



8.2 SYNTHETIC HORMONES

In Chapters 5 and 6, you looked at the important role that hormones play in homeostasis. Unfortunately, some disorders lead to the body being unable to produce certain hormones, which will have significant consequences on the body. A few examples of these disorders are Type 1 diabetes mellitus, hyperthyroidism and hypothyroidism.

Diabetes mellitus

Diabetes, or more correctly **diabetes mellitus**, is a hormonal problem that seriously disrupts homeostasis. A person with diabetes has an abnormally high blood glucose level, a condition called **hyperglycaemia**. As you learnt in Chapter 5, a balance between the hormones insulin and glucagon usually keeps the blood glucose at the correct level for normal body functioning. However, this is not possible in someone with diabetes.

The main role of insulin is to lower the levels of glucose in the blood by stimulating cells to take in glucose, and by liver and muscle cells converting glucose into glycogen. If a person produces insufficient insulin, or if their cells are resistant to the effects of insulin, the amount of glucose in the blood remains high and they excrete large quantities in the urine.

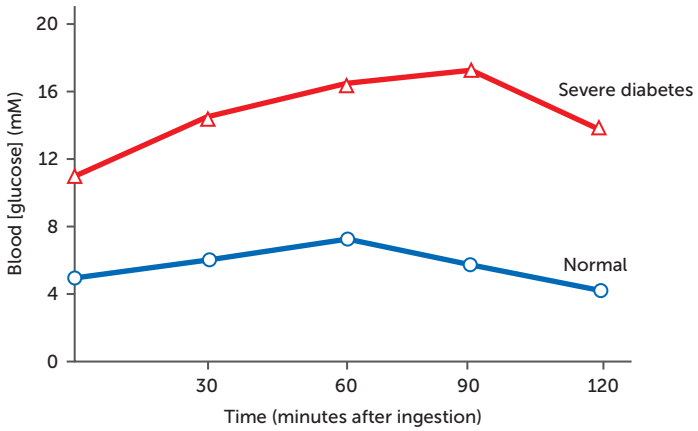


FIGURE 8.12 Glucose levels following ingesting glucose

There are two forms of diabetes: type 1 and type 2.

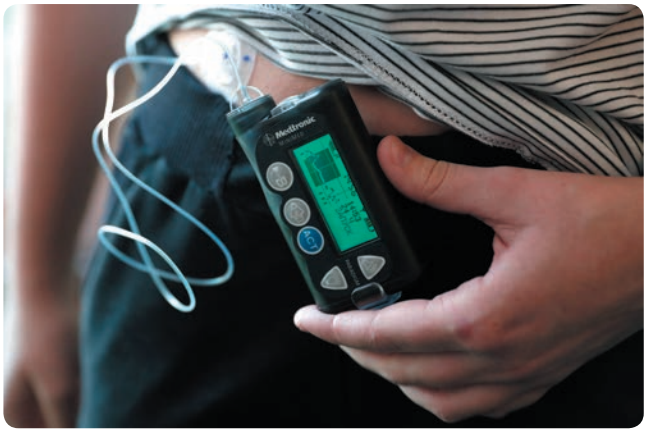
Type 1 diabetes

Type 1 diabetes, sometimes called **insulin-dependent diabetes**, usually begins in childhood, and therefore used to be called juvenile diabetes. In Australia, 10–15% of diabetes patients suffer from type 1. It occurs because a fault in the patient's immune system causes the destruction of beta cells in the islets of Langerhans of the pancreas. Beta cells produce insulin; therefore, a person with type 1 diabetes does not produce insulin. In most cases, the person's cells respond to insulin in the normal way, so the disease can be managed by giving the person insulin.

Insulin cannot be taken in tablet form because it is digested in the alimentary canal. Hence, the only treatment is regular injections of insulin or the use of a programmable pump that provides a continuous supply of insulin under the skin. Insulin injections do not cure type 1 diabetes; they simply fulfil a role to ensure the body is able to function. The patient must have regular injections to stay alive, but even with injected insulin the long-term effects are likely to be kidney failure, heart attack, stroke, amputations, blindness or nerve damage.

Science Photo Library/Michael Donne

FIGURE 8.13 A person suffering from type 1 diabetes is injecting herself with insulin using a NovoPen®, a device that measures the correct insulin dose from a portable cartridge



Alamy Stock Photo/ITAR-TASS News Agency

FIGURE 8.14 An insulin pump. The pump delivers a constant, small dose of insulin with a larger dose before meals or whenever the blood glucose level needs correcting

Type 2 diabetes

Type 2 diabetes is also known as non-insulin-dependent or **adult-onset diabetes**. It usually develops in people over the age of about 45 years, although increasing numbers of younger people are now being diagnosed. Unlike people with type 1 diabetes, type 2 patients are able to produce insulin, but their cells do not respond to it.

Type 2 diabetes is a lifestyle disease; it is more common in people who are not physically active and are overweight or obese. The incidence of type 2 diabetes in Australia and other affluent countries is increasing rapidly due to the large number of people who do not adopt a healthy lifestyle. There are so many Australians developing type 2 diabetes that it has become a health crisis.

Lifestyle factors that increase the risk of developing type 2 diabetes include:

- lack of physical activity
- being overweight or obese
- a diet that is regularly high in fat, sugar and salt, and low in fibre
- high blood pressure
- high blood cholesterol
- smoking.

Type 2 diabetes develops gradually and often there are no symptoms, or they are not noticed. It is estimated that about half of those Australians who have type 2 diabetes have not yet been diagnosed.

9780170449168

Because the cells do not respond to insulin, they do not take up glucose from the blood. Therefore, a blood test taken after fasting (not eating) will detect abnormally high levels of glucose.

There is no cure for type 2 diabetes, but the earlier a diagnosis is made, the better the chances of successful management of the condition. If it remains undiagnosed or untreated, there is an increasing risk of complications such as heart disease, stroke, kidney disease, eye problems, nerve damage, and skin and foot problems.

The treatment of type 2 diabetes involves a management program that aims to keep blood glucose levels within the normal range. Management includes a careful diet, regular physical activity, maintaining a healthy weight, monitoring blood glucose, and sometimes medication if blood glucose cannot be controlled by other measures.

Type 2 diabetes is preventable. The chances of suffering from the disease can be reduced by adopting a healthy lifestyle.

Diabetes
The Diabetes Australia website provides more information on diabetes, as well as links to many other useful sites including state-based organisations.

Insulin animation
This site contains an animation that explains how insulin is produced.

Treating diabetes

Type 1 diabetes, and sometimes type 2 diabetes, is treated by injections of insulin. The insulin for treatment of diabetics used to be obtained from the pancreases of cows and pigs. This made supplies of insulin expensive and limited. The extracts had to be purified, and patients sometimes suffered allergic reactions or infections from the animal-derived insulin.

In the 1980s, genetically engineered human insulin began to be produced. The gene for human insulin was inserted into DNA of the bacterium *Escherichia coli*. The bacteria were cultured, and the transgene allowed bacterial cells to produce the protein of human insulin. This was then extracted and used to treat people.

Activity 8.3
Investigating the regulation of blood sugar

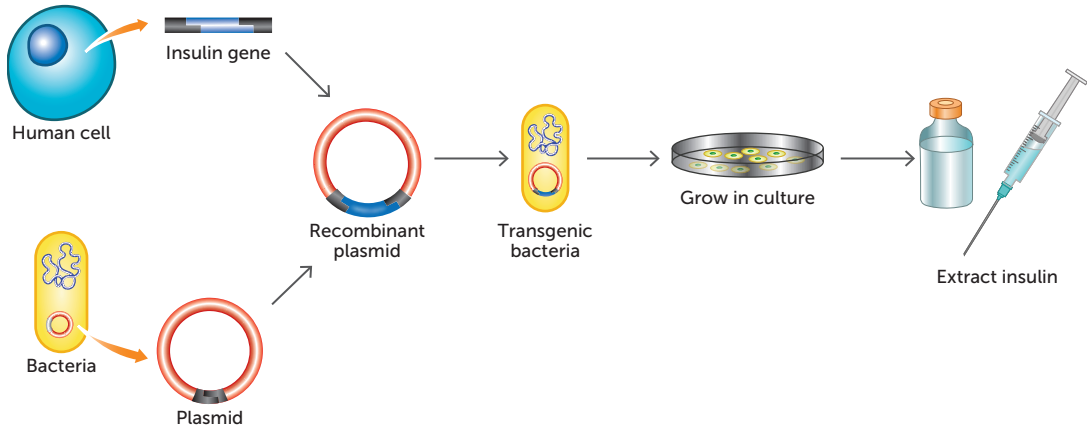


FIGURE 8.15
Recombinant DNA allows bacteria to produce human insulin

8.1 Disruptions to homeostasis

This insulin, produced from recombinant DNA, was marketed as Humulin®. Yeast is now used in a similar way to make insulin, and so almost all the insulin used throughout the world is now biosynthetic recombinant 'human' insulin rather than animal insulin.

Alamy Stock Photo/Science Photo Library

FIGURE 8.16
Scientists working in a facility for the production of recombinant human insulin from the fermentation of yeast cells in these large vats

Thyroid disorders

The thyroid gland is located in the neck and secretes two hormones: **thyroxine (T4)** and **tri-iodothyronine (T3)**. Both have the same effect, but the most important form is thyroxine. Thyroxine affects nearly every tissue in the body by stimulating carbohydrate, protein and fat metabolism. Thus, the secretion of thyroxine from the thyroid regulates basal metabolic rate.

Some of the energy released from the chemical reactions stimulated by thyroxine is in the form of heat, which is important in maintaining body temperature. Thyroid hormones are therefore also important in the long-term homeostasis of body temperature by the gradual change in metabolic rate that occurs from summer to winter.

Secretion of thyroxine is controlled by thyroid-stimulating hormone (TSH). TSH is secreted by the anterior lobe of the pituitary, but its release is controlled by the hypothalamus in the brain.

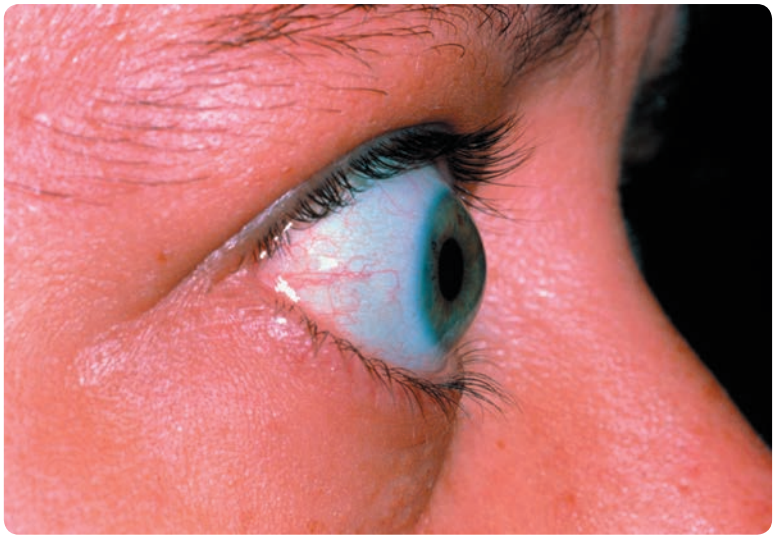
An excess of or a deficiency in thyroxine can cause disorders. This may be due to a problem in the thyroid itself, but in some cases it can be due to an imbalance in TSH.

Hyperthyroidism

Too much thyroxine, called **hyperthyroidism**, occurs when the thyroid gland produces too much hormone. The most common type of hyperthyroidism is known as **Graves' disease**. It is an enlargement of the thyroid caused by an immune system reaction. Although not inherited, there does seem to be a genetic predisposition for the condition. Because the cells are overstimulated, and the metabolic rate is increased, the symptoms of hyperthyroidism are rapid heartbeat, weight loss, increased appetite, fatigue, sweating, anxiety and, in the case of Graves' disease, protruding eyeballs, known as exophthalmia.

During the production of thyroxine and tri-iodothyronine, iodine is absorbed from the bloodstream, concentrated in cells in the thyroid and then incorporated into the molecules to produce the hormones. Therefore, hyperthyroidism can be treated with drugs that block the thyroid gland's use of iodine. Another method is to give the patient a drink containing radioactive iodine. The radioactive iodine molecules are taken up by the thyroid cells, which are then killed by the radioactivity. Cells elsewhere in the body do not absorb iodine and are unaffected. The radioactive iodine is eventually excreted in the urine. A third method is to use surgery to remove some, or all of, the gland. Less thyroid gland will result in less hormone being produced.

When cells in the thyroid gland are destroyed, there is the risk that the individual may develop hypothyroidism and require treatment with synthetic thyroxine. This is discussed below.



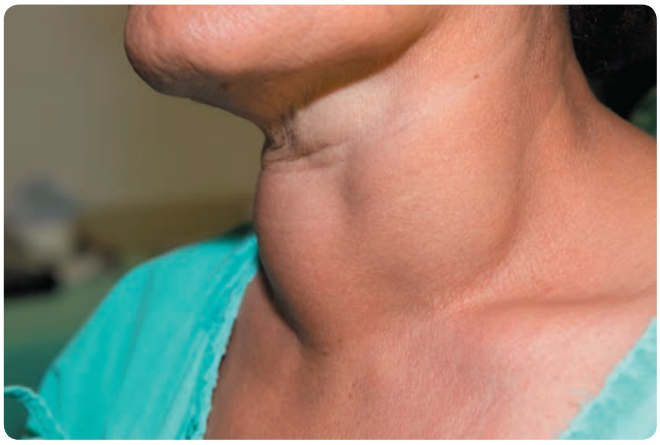
Alamy Stock Photo/Science Photo Library

FIGURE 8.17 Exophthalmia, protruding eyeballs, is a symptom of Graves' disease, the most common result of an overactive thyroid gland

9780170449168

Hypothyroidism

Too little thyroxine, **hypothyroidism**, is much more common than hyperthyroidism. About 6–10% of Australian women, and a smaller proportion of men, may be affected. Hypothyroidism occurs either through problems with the thyroid, pituitary gland or hypothalamus. Symptoms of hypothyroidism arise due to a decrease in metabolism and may include slow heart rate, unexplained weight gain, fatigue or a feeling of lack of energy, intolerance to cold, swelling of the face, and goitre.



Shutterstock.com/Karan Bunjean

FIGURE 8.18 Goitre is the enlargement of the thyroid gland

One thyroid gland problem is due to lack of iodine. A thyroxine molecule contains four iodine atoms (hence T4) and a tri-iodothyronine molecule contains three atoms of iodine (T3). Thus, a deficiency of iodine in the diet can prevent the thyroid gland from making enough hormones. The thyroid gland may then become enlarged in an effort to increase hormone production. Enlargement of the thyroid is known as **goitre**.

www.dlibrary.com.au / Saxa

FIGURE 8.19 Using iodised table salt during cooking and with meals can reduce the risk of suffering from hypothyroidism due to iodine deficiency

Many people may suffer from iodine deficiency without it being severe enough to produce visible swelling of the neck. In Australia, about 46% of people are affected, so iodine deficiency is now a public health problem. To try to ensure that people get sufficient iodine, the federal government introduced compulsory addition of iodine into most breads in October 2009. All bread, except organic bread and bread mixes for baking at home, must now be made with iodised salt.

Although a severe deficiency of iodine can cause hypothyroidism, the most common cause is an attack on the thyroid gland by the patient's immune system. This is known as Hashimoto's disease. Another cause is surgery for cancer of the thyroid that involves removal of all, or a large part, of the gland.

If the cause of hypothyroidism is a lack of iodine, it is easily treated by the inclusion of extra iodine in the diet. For treatment of other causes, tablets containing thyroid hormone are prescribed. There is no cure and the hormone tablets must be taken for the rest of the person's life. The dose of thyroid hormone must be carefully monitored because too little will not relieve the symptoms of hypothyroidism but too much will result in hyperthyroidism.

Hypothyroid patients used to be treated with tablets made from the dried and powdered thyroid glands of animals, mainly pigs. The tablets contain both thyroid hormones T3 and T4 but not necessarily in the same proportions as produced by the human thyroid. They also contain traces of other hormones. Although these 'natural' tablets are still available, today most patients are treated with hormones made synthetically by a chemical process.

Thyroxine was first isolated in 1914 and was synthesised for the first time in 1927. Levothyroxine is a manufactured form of thyroxine, and is now considered so safe and effective that it is listed on the World Health Organization's List of Essential Medicines. It is available in both oral and injectable forms under many different brand names. In Australia, levothyroxine is sold as Oroxine, Eutroxsig or Eltroxin. Levothyroxine is the most commonly prescribed drug for thyroid hormone replacement.

Thyroid gland
The Thyroid Australia website provides more information about the thyroid and links to relevant websites.

Activity 8.4
Investigating thyroid hormone

Key concept

Synthetic hormones produced by either recombinant DNA or chemical manufacturing can be used to treat disorders such as diabetes mellitus and hypothyroidism.

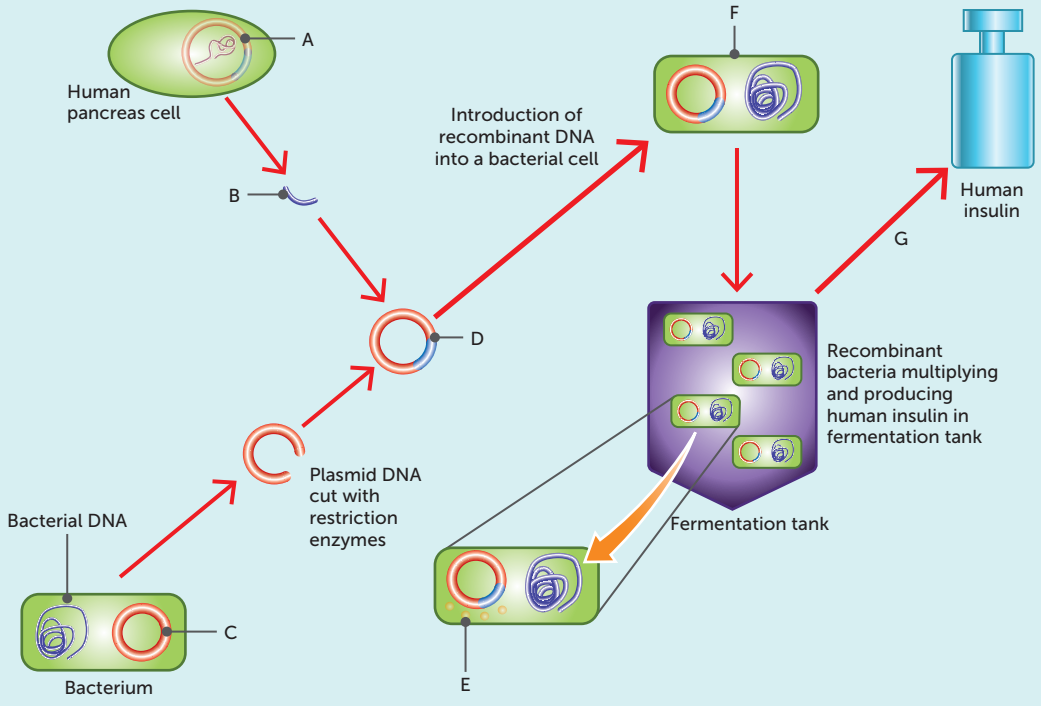
Questions 8.2

RECALL KNOWLEDGE

1 Define 'hyperglycaemia' and explain why a lack of insulin results in this condition.
2 What causes type 1 diabetes?
3 List the symptoms of hyperthyroidism.
4 a Is goitre a symptom of hypothyroidism or hyperthyroidism?
  b Explain why goitre occurs.
5 Explain why iodine supplements are used to treat some forms of hypothyroidism.
6 Explain why levothyroxine is considered the preferred treatment for hypothyroidism.

APPLY KNOWLEDGE

7 Explain why insulin in not an effective treatment for type 2 diabetes.
8 Insulin pumps are programmed to deliver a surge of insulin after meals and a small, steady rate of insulin at other times. Explain why this is preferred over traditional injections.
9 Suggest why type 2 diabetes is more common in adults.
10 Label parts A–G on the diagram below to show the steps involved in producing insulin.



11 Thyroxine hormone replacement is used to treat hypothyroidism. Explain why some patients with hyperthyroidism may need to receive hormone replacement after initial treatments.

8.3 OTHER TECHNOLOGIES

Gene therapy and cell replacement therapies are also used to treat disorders.

Gene therapy

Gene therapy aims to treat or cure genetic abnormalities by identifying faulty genes and inserting healthy ones. It is a way of using the genes themselves as the treatment. In many ways, it is the most obvious application of the **Human Genome Project**, which has revealed the location of around 4000 potentially faulty genes. Currently, gene therapy research is concentrating on single-gene disorders such as cystic fibrosis, Huntington's disease, muscular dystrophy and sickle-cell anaemia. It is also being investigated for curing type 1 diabetes. Unlike most conventional medicines, which treat the symptoms of a disease, gene therapy has the potential to correct the underlying cause.

Gene therapy was first introduced in 1970. However, it is still only at a research level. Some of the areas of possibility are:

- replacing a mutated gene with a healthy copy
- fixing or inactivating mutated genes
- inserting a new gene that will fight the disease
- making the immune system recognise diseased cells.

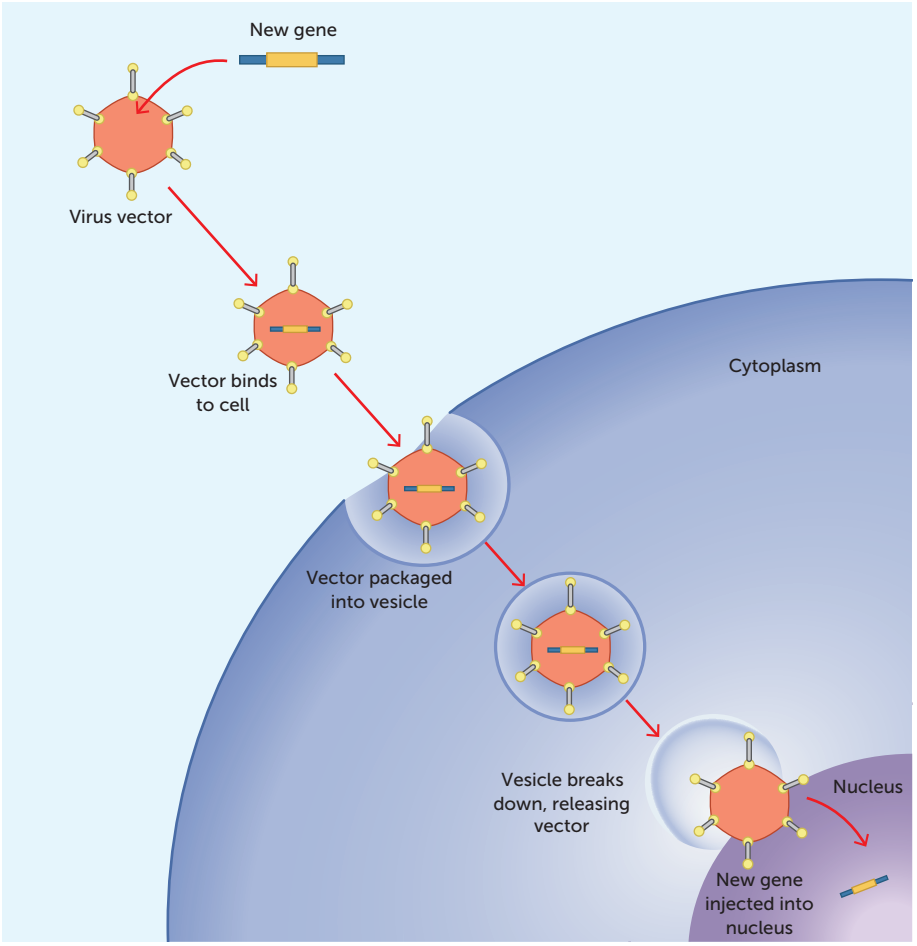


FIGURE 8.20 A gene being introduced into a cell during gene therapy

The concept of gene therapy is that a vector can be used to deliver desired DNA into a cell. This DNA can be incorporated into the cell's nucleus and undergo transcription and translation to produce the desired protein.

Key concept

Gene therapy aims to correct the cause of the problem by correcting the faulty gene. This can be achieved by replacing, correcting or inactivating the gene, or by inserting a new gene to correct the disease or to encourage the immune system to destroy the diseased cell.

Sickle-cell anaemia
This website provides information about recent progress with gene therapy for sickle-cell anaemia.

9780170449168

Type 1 diabetes

Earlier in this chapter, you saw how type 1 diabetes is caused by an autoimmune disease that destroys the beta cells in islets of Langerhans in the pancreas. This means that the body cannot produce insulin and, therefore, blood glucose levels remain high after a meal.

Traditional treatment of diabetes focuses on introducing the insulin that the body cannot make. However, gene therapy is now looking at methods of making it possible for the body to produce insulin again.

Gene therapy
This article discusses how gene therapy could be used to cure type 1 diabetes.

One possibility is reprogramming other cells to produce insulin. In order to achieve this, the gene for insulin is introduced into a vector. The vector is then used to 'infect' the desired cells, such as the alpha cells in the islets of Langerhans. These cells incorporate the new DNA into their nucleus and are able to use protein synthesis to produce insulin.

Key concept

Type 1 diabetes could possibly be treated with gene therapy by using vectors to introduce the gene for insulin into alpha cells so that they can produce the hormone and allow the body to function normally.

Cystic fibrosis

Cystic fibrosis (CF) is the most common life-threatening genetic disorder among Australians of European descent. It mainly affects the lungs and pancreas, but sometimes the liver and reproductive organs. CF is characterised by thick sticky mucus secreted by the mucous glands. In the lungs, this mucus may clog the tiny air passages and trap bacteria, making a person with CF susceptible to infection. Repeated infections and continual blockage of the airways may cause irreversible lung damage and shorten life expectancy. The pancreas is also affected, preventing secretion of enzymes required for digestion. Therefore, people with CF frequently have problems with nutrition and need to take care with their diet.

CF results when an individual inherits the recessive allele for the condition from each parent. In most Australian states, a blood sample is usually taken from a baby's heel within two to three days after birth. If the blood test reveals that a child has the disease, a special low-fat, high-carbohydrate and high-protein diet is advised.

The identification of the Cystic Fibrosis Transmembrane Regulator (CFTR) gene in 1989 was a major step forward in developing a treatment for CF. Mutations in this single gene result in the disease, and since its discovery more than 900 mutations have been identified. In 1991, scientists successfully corrected faulty CFTR genes in cultured cells by adding normal copies of the gene to the culture. This was the first step towards gene therapy for CF.

CF was a logical choice for treatment using gene therapy. It is a single-gene disorder, and the most severely affected organ, the lung, is relatively easy to access to provide treatment. In addition, the disease is slow to progress, with the lungs of a newborn being virtually normal. This would enable gene therapy to begin before significant lung damage started to occur. The first experimental gene therapy treatment was given to a patient with CF in 1993. Researchers modified a common cold virus to act as the vector to carry normal genes to the CFTR cells in the airways of the lung. This first study was mainly concerned with the safety issues of the treatment. The amount of gene transfer was probably too small to have any real therapeutic benefit and any benefit was short-lived. Trials with alternative methods of gene transfer are continuing.

Huntington's disease

Another single-gene disorder is **Huntington's disease**, and researchers believe that gene therapy could be used to slow down or prevent its development. It is caused by a mutation in a single gene on chromosome 4 called IT15. The symptoms of this incurable genetic disorder seldom appear before the age of 40. The mutated form of a protein called huntingtin results in nerve cells in the brain

being damaged, causing physical, mental and emotional changes. The disease is characterised by occasional unintentional flailing movements of the arms and legs, and difficulty in making voluntary movements of the limbs. The affected person also suffers from progressive dementia, the loss of ability to think clearly.

Research in the United States on mice has indicated that gene therapy for Huntington's disease could be effective in humans. In other research, French scientists experimented with a modified virus to deliver a corrective gene into brain cells that boosts a natural shield against the effects of the defective huntingtin protein. This research has been conducted on rats and primates, and the positive results have encouraged movement towards a clinical trial on humans.

Key concept

Genetic disorders such as cystic fibrosis and Huntington's disease could possibly be treated with gene therapy by introducing the correct gene into the affected cells so that they can function normally.

Cell replacement therapy and tissue engineering

Stem cells are undifferentiated cells that are capable of repeated mitotic divisions for long periods of time and, given the right conditions, can differentiate into specialised cells. These characteristics make them ideal for producing replacement tissues. In *Human Perspectives ATAR Units 1 & 2*, the types of stem cells and their sources were discussed.

Any disorder involving loss of, or injury to, normal cells is a potential candidate for stem **cell replacement therapy**. However, cell replacement therapy for the nervous system has generated the most interest, due to the debilitating nature and widespread occurrence of neurodegenerative disorders such as Parkinson's and Alzheimer's diseases. The most attractive method for restoring brain function in, say, Parkinson's disease is the replacement of dying neurons with healthy neuronal tissue. Pilot studies using embryonic stem cells have been carried out in humans with some success. The transplanted cells not only survived but also grew and established connections with adjacent neurons. However, the use of human embryonic stem cells is controversial and raises a number of ethical questions. Researchers into Parkinson's disease are currently exploring other sources of cells to help restore patients' brain function.

Stem cells are increasingly being used for tissue engineering. The primary objective of **tissue engineering** is to restore healthy tissues or organs for patients and thus eliminate the need for tissue or organ transplants, or artificial implants. Early research in this area used cells from the intended recipient but, in many cases, such as genetic diseases, this was not practicable. In other situations, the organ from which cells were to be harvested was diseased, and so not enough normal cells were present to enable a successful culture. The use of stem cells overcomes both problems.

Tissue engineering
This website provides an interesting account of how tissue engineering is being used to manufacture artificial skin.

Tissue engineering requires an abundant supply of disease-free cells of specific types. These cells then need to be induced to grow on a **scaffold** of natural or synthetic material to produce a three-dimensional tissue. Tissue engineering scaffolds serve as a template for tissue growth, and need to have high pore sizes that enable the cells to grow while at the same time allowing the diffusion of nutrients throughout the whole structure. They frequently need to be biodegradable so that they can be absorbed by the surrounding tissues without having to be removed surgically. This needs to be carefully established, as the rate at which the scaffold degrades needs to match, as far as possible, the rate of tissue formation. That is, while the new cells are manufacturing their own natural matrix structure around themselves, the scaffold is providing a support structure that will eventually break down, leaving newly formed tissue.

Once a scaffold has been devised, suitable stem cells need to be cultured. These cells are seeded on to the scaffold, which then enables further cell growth and proliferation. This cell-covered scaffold is then implanted into the patient at the site where new tissue is required. As the new cells continue to grow and divide, the material making up the scaffold begins to degrade or, in some cases, to be absorbed. Such tissue engineering techniques are being used to develop a wide range of tissues, including bone, skin, cartilage and adipose tissues.

Key concept

Stem cells can be utilised in cell replacement therapy and tissue engineering due to their ability to multiply and differentiate.

Questions 8.3

RECALL KNOWLEDGE

1. Define 'gene therapy', and give an example of its possible use.
2. List three ways that gene therapy could possibly correct faulty genes.
3. Explain the role of a vector in gene therapy.
4. Explain why it is important to diagnose cystic fibrosis at infancy.
5. Huntington's disease results in the death of brain cells. Explain how scientists believe gene therapy could work to treat this disease.
6. What are stem cells, and why are they suitable for cell replacement therapy?
7. Why is it possible that cell replacement therapy could be used to treat patients with Alzheimer's or Parkinson's disease?

APPLY KNOWLEDGE

8. Suggest why gene therapy is more difficult than recombinant DNA technology to develop safely and effectively.
9. Discuss why gene therapy for type 1 diabetes is focused on introducing the gene into alpha cells.
10. Discuss why research into cell replacement therapy has focused on neurological conditions.
11. Tissue engineering utilises a scaffold for the tissues to grow on. Explain why this is necessary, and why it needs to be biodegradable.
12. 'Gene therapy has suffered from skepticism from both [the] scientific community and [the] pharmaceutical industry. In addition to the risk of insertional mutagenesis/tumorigenesis, the widespread clinical application of gene therapy is hampered due to the inefficient systemic delivery. However, in recent years, new approaches, including stem cell-based gene therapy, have boosted the potential comeback of gene therapy' (Ye, Z and Mahato, R, 'Combining Stem Cells and Genes for Effective Therapeutics', NCBI Online, 2009: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3207237/ [Accessed 30 August 2020].
    - **a** Suggest how stem cell replacement therapy can be combined with gene therapy.
    - **b** Discuss why tumour growth is a possible risk with this technology.
    - **c** Suggest a disorder for which this combined therapy may be a viable treatment, and explain how it could work.

9780170449168

CHAPTER 8 ACTIVITIES

ACTIVITY 8.1 Investigating restriction enzymes

Recombinant DNA technology, or genetic engineering as it is frequently called, involves the introduction into cells of fragments of DNA that are foreign to the organism. To do so, the strands of DNA under investigation need to be cut into useful fragments. The 'scissors' that cut the DNA are called restriction enzymes. The fragments can then be inserted into a suitable vector and joined with DNA ligase.

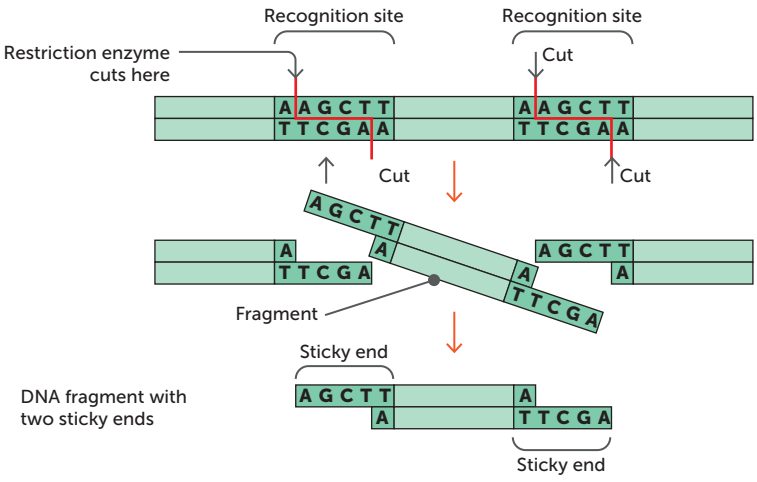
In this activity, we will investigate how a sequence of DNA can be cut into suitable fragments using an appropriate restriction enzyme.

What to do

Answer the questions below, referring to the relevant parts of this chapter where necessary.

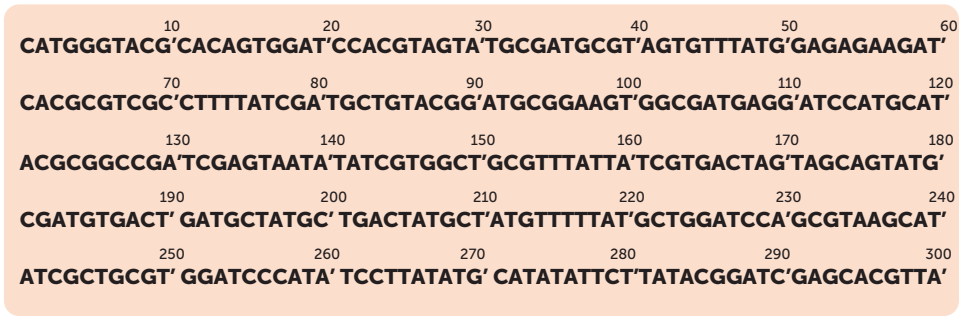
1 Explain the following terms by describing their role in recombinant DNA technology.
   - **a** Restriction enzymes
   - **b** Recognition sites
   - **c** Blunt ends
   - **d** Sticky ends

2 Use Table 8.1 (page 203) to identify the restriction enzyme that is being used in the following figure and the organism from which it was first isolated.



A restriction enzyme cuts a double-stranded DNA molecule at the recognition site

3 Imagine that you are a genetic engineer and need to cut the DNA sequence shown on the following page. Using the five restriction enzymes listed in Table 8.1, study the sequence carefully and circle every recognition site that could be cut by each of the enzymes in turn. You may wish to use pens or pencils of four different colours. How many fragments of DNA have you created for each enzyme?



A single strand of DNA

4 A process called ligation is used to reassemble the fragments. Name the enzyme involved in this process.
5 Explain why the process of ligation can be viewed as the reverse of the restriction enzyme procedure.
6 Use a short summarising statement to explain why the discovery of restriction enzymes and DNA ligase has been so important for the advancement of genetic engineering.

SOUTHERN Biological Developed by Southern Biological

ACTIVITY 8.2 Investigating bacterial transformation

DNA can mutate spontaneously or after an error is made in DNA replication. Biotechnologists have developed methods of controlled DNA mutation, such as intentionally mutating cell DNA to alter how the cell behaves. However, it is also possible to transfer DNA from one organism into another. This method, called genetic transformation, uses an engineered molecule of DNA to transfer a gene or genes from one organism to another so that the organism is capable of producing the protein encoded by the transformed gene.

Aim

To perform bacterial transformations using the green fluorescent protein plasmid pGreen
Time requirement: 50 minutes

You will need

Escherichia coli (E. coli) MM294 starter plate; 10 µL pGreen plasmid; 2 Luria broth agar plates; 2 Luria broth with ampicillin agar plates; 500 µL Luria broth, sterile; 500 µL calcium chloride ($CaCl_2$), 50 mM, sterile; 2 transformation tubes, sterile; 8 plastic pipettes, 1 mL, sterile; 3 inoculation loops, sterile, disposable; 4 inoculation spreaders, sterile, disposable; 2–20 µL variable micropipette; sterile tips for 2–20 µl micropipette; water bath; thermometer (if necessary); ice bath; fine-point marker pen; stopwatch; microtube rack; sticky tape (to seal plates); incubator; ethanol (or bleach); disposable gloves

Risks

WHAT ARE THE RISKS IN THIS INVESTIGATION?	HOW CAN YOU MANAGE THESE RISKS TO STAY SAFE?
Some bacteria may cause disease, so assume them to be pathogenic. (Note: *E. coli* MM294 is a harmless school-safe biological.)	Wear lab coats, safety glasses and gloves; wash hands thoroughly at end. Decontaminate benches before and after activity. Flood spills with bleach.
Micro-organisms will grow on the agar plates.	Do not open plates once they are securely taped. Dispose of plates appropriately after autoclaving.
Disinfectants or bleach may leave a corrosive residue.	After wiping the bench clean with bleach, ensure the residue is wiped off; ensure lab coat sleeves are rolled down and gloves are worn.

What to do

Note: To use aseptic technique, wipe your bench down with ethanol (or bleach) and keep your work near the Bunsen burner to waft potential contaminants away from your materials.

Preparing the transformation solution

1. Label one transformation tube '+ Plasmid' and the other '– Plasmid'. Keep the tubes cold by placing them upright in the ice bath. Keep tubes capped at all times except when in use.
2. Add 250 µL (0.25 mL) of ice-cold calcium chloride $CaCl_2$ solution to each transformation tube, using a sterile plastic pipette. Maintain the temperature by placing the tubes back into the ice bath.

Suspending the bacteria

1. Transfer a single colony of *E. coli* from the starter plate to the ice-cold $CaCl_2$ solution in the '+ Plasmid' transformation tube using a sterile inoculation loop. To dislodge the *E. coli* cells from the loop, spin the loop rapidly in the solution. Check whether your *E. coli* has transferred successfully – it should be visible in your tube.
2. Suspend the *E. coli* in the $CaCl_2$ solution by drawing the solution in and out of a sterile pipette by squeezing and releasing the bulb several times. You should see the solution begin to become milky white as cell mass is suspended. To check there are no lumps or particles in the tube, hold it up to the light; then return the tube to the ice.
3. Repeat the same steps to transfer a single colony of *E. coli* from the starter plate to the ice-cold $CaCl_2$ solution in the '– Plasmid' transformation tube.

Adding the plasmid

1. The technician/teacher will bring the plasmid to your workstation. Transfer 10 µL (0.01 mL) of plasmid solution to the transformation tube labelled '+ Plasmid' using a micropipette. Add the plasmid directly to the liquid in the tube without allowing it to touch the sides.
2. Immediately return the tube to the ice bath and mix the plasmid into the bacterial suspension by placing a sterile inoculation loop into the liquid and rapidly spinning it with your fingers. Cap the tube when done. Incubate the two tubes for 15 minutes on ice.
3. Label the four plates as follows:
   - LB + Plasmid
   - LB – Plasmid
   - LB/Amp + Plasmid
   - LB/Amp – Plasmid

Heat shock

1. Once the 15-minute incubation is finished, take the two tubes from the ice bath and transfer to the warm-water bath (42°C) and hold them for 90 seconds with your gloved hands, keeping the tube caps from being fully submerged in the water. Gently agitate the tubes while they are warming up in the water. Immediately move the tubes back to the ice bath when the time is up.
2. Allow the tubes to rest in the ice bath for at least 1 minute before continuing.

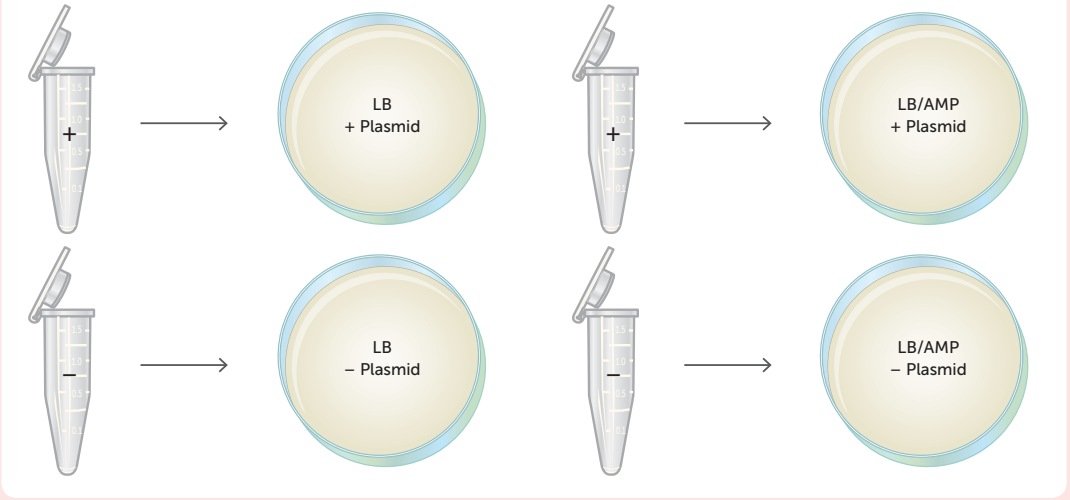
Recovery

1. Add 250 µL (0.25 mL) of Luria broth to each tube using a sterile plastic pipette. Hold the capped tubes at the top and gently tap/flick the base with your finger to mix the liquids.
2. Allow tubes to recover for 10 minutes in a microtube rack at room temperature.

Plate inoculation

1. Transfer two drops of liquid from the '+ Plasmid' tube to the 'LB + Plasmid' plate using a sterile plastic pipette. Quickly spread the liquid evenly over the plate surface using a sterile spreader.
2. Repeat for the 'LB/Amp + Plasmid' plate.
3. Transfer two drops of liquid from the '– Plasmid' tube to the 'LB – Plasmid' plate using a sterile plastic pipette. Quickly spread the liquid evenly over the plate surface using a sterile spreader.
4. Repeat for the 'LB/Amp – Plasmid' plate.

9780170449168



5 Secure the lid of each Petri dish to its base using sticky tape. Leave plates to rest on the bench for 5 minutes and then place them upside down (agar on top) in a 33°C incubator for 24–36 hours. You can inspect growth after this time. You should see either a bacterial lawn (colonies of bacteria covering all or most of the plate), single colonies (spots), or no growth on the individual plates. Take the plates into a dark room to observe evidence of fluorescence in the transformed colonies. Use of a UV light may enhance the fluorescence.

6 To count the number of individual colonies, mark the lid of the Petri dish above each colony with a marker as you count it. Mark any plates with cell growth too dense to count as individual colonies, as a lawn. Record your results.

Studying your results

1 Record the results of your experiment by copying the table below.

Bacteria colony results

PLATE	RESULT
– Plasmid on LB agar	
+ Plasmid on LB agar	
– Plasmid on LB/Amp agar	
+ Plasmid on LB/Amp agar	

2 What growth and phenotypes can you observe?

3 Describe what you see on your plates when you look at your plates under UV light.

Discussion

1 Explain what a plasmid is.

2 Why is the plasmid–bacteria solution placed on ice for 5 minutes?

3 Which plate forms the control in this experiment? Explain.

4 Explain the function of the LB broth. What is the purpose of incubating the cells at room temperature?

5 Explain how the DNA plasmid is put into bacteria. What is the advantage of being able to do this? Consider what the plasmid DNA allows the bacteria to do.

6 Explain how we are able to identify that the plasmid DNA is in the bacteria.

9780170449168

ACTIVITY 8.3 Investigating the regulation of blood sugar

1 Two men (A and B) were subject to a glucose tolerance test. Each was given 100 g of glucose at the start of the experiment. The table below shows their blood glucose concentration during the period of the experiment.

TIME SINCE START (HOURS)	MAN A(mg/100 mL)	MAN B (mg/100 mL)
0	80	170
0.5	100	250
1	160	310
1.5	130	300
2	80	280
3	60	210
4	55	180
5	90	160

a Plot the data for the two men as a graph.
b One of the men had a diseased pancreas. Which man was it? Give reasons to support your answer.
c What is the name of the disease from which the man was suffering?

2 Research workers who were investigating blood glucose regulation injected hormones singly, or as mixtures, into the vein of a dog for five hours. They then measured any increase in blood glucose above the normal level. Their results are shown in this table.

HORMONE INJECTED	RISE IN BLOOD GLUCOSE LEVEL (mg/100 mL)
Adrenaline	30
Glucagon	10
Cortisol	3
Adrenaline and glucagon	58
Adrenaline and cortisol	58
Glucagon and cortisol	35
Adrenaline, glucagon and cortisol	140

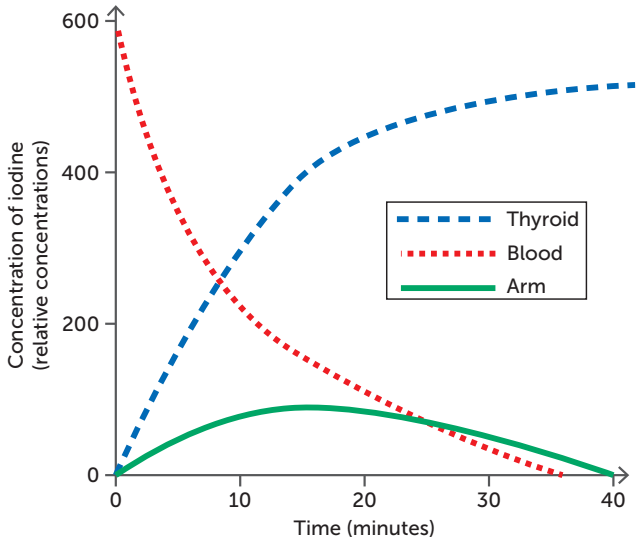
a Which parts of the endocrine system are responsible for releasing each of the hormones listed in the table?
b A technician suggested giving the hormones to the dog by adding them to its drinking water. Would this method be effective? Explain.
c Compare the effects of these hormones when acting:
i singly
ii together.
d What do the data indicate about the response of body tissues to the hormone mixtures?
e In light of your answers to parts **c** and **d**, explain how stress could raise blood glucose levels. What would be the advantage of this response?
f Describe at least three major criticisms of the design of the experiment on the dog.

9780170449168

ACTIVITY 8.4 Investigating thyroid hormone

A scientist injected radioactive iodine into a blood vessel in a person's arm. He then measured the concentration of iodine in the arm, in the blood and in the thyroid gland for the next 40 minutes.

The following graph shows the concentrations of iodine measured by the scientist.



Graph showing iodine concentrations in the thyroid and blood over time, after injection into an arm

1. Suggest a hypothesis the scientist may have been testing.
2. Why was the scientist using iodine, rather than some other substance, to investigate the thyroid gland?
3. Why was radioactive iodine used?
4. What do you think the scientist was trying to demonstrate with this experiment?
5. Using the graph, explain what happened to the iodine in the 40 minutes after it was injected into the arm.
6. An important part of the investigation was to measure the concentration of iodine in the person's arm. Why was this necessary?
7. Would it have made any difference to the investigation if the iodine had been injected into the person's leg?

9780170449168

CHAPTER 8 SUMMARY

- Biotechnology uses cellular processes to make products that are used by humans. It includes newer processes such as recombinant DNA, gene therapy and cell replacement therapy.
- DNA is a molecule made up of nucleotides joined in two strands twisted to make a double helix. Each nucleotide is composed of a sugar, phosphate and nitrogenous base.
- Nitrogenous bases are complementary, and only bond to the complementary pair. Adenine is complementary to thymine, while cytosine is complementary to guanine.
- Recombinant DNA technology is also known as genetic engineering. It involves artificially modifying DNA and it produces genetically modified organisms (GMOs).
- Transgenic organisms are an example of GMOs where DNA from one species is introduced into another species.
- Restriction enzymes are isolated from bacteria. They recognise a certain base sequence, the recognition site, and separate the nucleotides at a certain position. As this occurs on both strands, the DNA is opened with either a straight cut producing blunt ends or a staggered cut with sticky ends.
- Restriction enzymes are named by the bacterium that they are isolated from.
- DNA ligase is an enzyme that joins the phosphate of one nucleotide to the sugar of another. This joins two segments of DNA together.
- Vectors are used to transfer DNA from one organism to another. Restriction enzymes are used to cut the DNA at the same sequence on the vector and the DNA to be inserted. DNA ligase is then used to join the two pieces of DNA together.
- Plasmids are circular pieces of DNA that are often found in bacteria. Bacteriophages (phages) are viruses that infect bacteria. Plasmids and phages are commonly used as vectors.
- Recombinant DNA is used to produce hormones, including insulin, and vaccines such as the hepatitis B and HPV vaccines.
- DNA vaccines are currently being researched. These introduce the DNA for the antigen into the host cells. The host cell can produce the antigen and initiate an immune response.
- Diabetes mellitus leads to hyperglycaemia, as insulin is unable to reduce the blood glucose levels.
- Type 1 diabetes occurs when the immune system destroys beta cells, which means that they cannot produce insulin. It is managed by insulin injections. This insulin can be synthesised by recombinant yeast cells which have had the gene for insulin introduced by a plasmid vector.
- Type 2 diabetes usually develops in adults as a result of lifestyle choices such as obesity and lack of exercise. It is due to the cells being unable to respond to insulin. It is managed by using diet and exercise to keep blood glucose levels within the normal range.
- The thyroid gland produces thyroxine and tri-iodothyronine, which regulate metabolism.
- Hyperthyroidism is when there is too much thyroxine produced, which increases the rate of metabolism. It is treated by blocking the uptake of iodine, using radioactive iodine to destroy thyroid cells, or surgically removing some of the thyroid gland.
  The destruction of thyroid cells can lead to hypothyroidism.
- Hypothyroidism is when there is not enough thyroxine and so the rate of metabolism is too slow. A lack of iodine can lead to hypothyroidism; therefore, iodine supplements can be used as a treatment.
- Another cause of hypothyroidism is an autoimmune disease that destroys the

9780170449168

thyroid gland. In this case, a synthetic thyroxine called levothyroxine is used as a treatment.

- Gene therapy aims to work by inserting genes to perform the function of abnormal genes. It is currently in the research stage, but is hoped to be used for disorders such as cystic fibrosis, Huntington's disease and type 1 diabetes.
- Gene therapy uses a vector to introduce DNA into the host cells. Research is being conducted into introducing the gene for insulin into alpha cells so that they take on the role of beta cells in the body of people with type 1 diabetes.
- Cystic fibrosis and Huntington's disease are due to a faulty gene. Gene therapy research is looking into using a vector to introduce normal genes into cells to allow them to function normally.
- Stem cells can be used in cell replacement therapy as they have the ability to multiply and differentiate. In particular, this raises the possibility of replacing dying neurons in patients with Alzheimer's or Parkinson's disease.
- Tissue engineering can be used to replace damaged tissue. It is achieved by using a scaffold with stem cells. The scaffold degenerates as the cells multiply, producing new tissue.

9780170449168

CHAPTER 8 GLOSSARY

Adult-onset diabetes A common form of diabetes that usually occurs in people over the age of 45 who are overweight; it can usually be controlled by diet; also known as type 2 diabetes

Artificial selection An ancient form of genetic engineering where humans select desired traits and choose parents based on these traits

Bacteriophage A virus that infects bacteria

Biotechnology The use of biological processes to produce useful products

Blunt end The end produced by a straight cut of a sequence of nucleotide bases

Cell replacement therapy The replacement of damaged cells with healthy ones

Cystic fibrosis (CF) A disorder controlled by a recessive allele carried on an autosome that is incurable but can be detected during foetal development; mucus-secreting glands, particularly in the lungs and pancreas, become fibrous and produce abnormally thick mucus, resulting in, among other things, chest infections

Diabetes *see* diabetes mellitus

Diabetes mellitus A group of diseases, all of which result in an abnormally high level of glucose in the blood and excretion of glucose in the urine; common name is diabetes

DNA ligase An enzyme capable of combining two small components of single-strand DNA into one single structure

DNA vaccine A vaccine that stimulates an immune response by introducing antigen DNA, which causes the host cells to produce the antigen

Endonuclease An enzyme that breaks a nucleic acid within the strand by separating two nucleotides

Gene therapy The treatment of disease by replacing, manipulating or supplementing non-functional genes in cells and tissues

Genetic engineering *see* recombinant DNA technology

Genetically modified organism (GMO) An organism produced by genetic engineering

Goitre A swelling of the neck caused by an enlargement of the thyroid gland

Graves' disease A medical condition in which overactivity of the thyroid gland results in the secretion of excess amounts of the thyroid hormones

Human Genome Project A project with the aim of mapping the base pairs and identifying the genes in human DNA

Huntington's disease An inherited disease that causes the death of brain cells and results in changes in mood, a lack of coordination and an unsteady gait

Hyperglycaemia An abnormally high level of sugar in the blood; frequently found in people with diabetes mellitus

Hyperthyroidism Overactivity of the thyroid gland resulting in abnormally high levels of thyroid hormones in the blood

Hypothyroidism Underactivity of the thyroid gland resulting in low levels of thyroid hormones in the blood

Insulin-dependent diabetes A form of diabetes that develops rapidly, usually before the age of 20; caused by a decline in insulin-producing cells of the pancreas; treated by injections of insulin at regular intervals; also known as type 1 diabetes

Ligation The process of joining short strands of DNA during replication

Palindromic A sequence that reads the same backwards and forwards

Phage *see* bacteriophage

Plasmid In a bacterial cell, small circular strands of DNA distinct from the main bacterial genome; composed of only a few genes and able to replicate independently within cells

Recognition sequence The sequence of bases in the recognition site

Recognition site A specific sequence of nucleotides at which an enzyme cuts a strand of DNA

9780170449168

Recombinant DNA Synthetic DNA; made by inserting genes from one source into a DNA molecule from a different source

Recombinant DNA technology The procedures used to produce recombinant DNA; involve introducing DNA into a cell from a different type of organism or DNA that has been modified in some way

Recombinant vaccine A vaccine produced through recombinant DNA technology

Restriction enzyme An enzyme that cuts strands of DNA at a specific sequence of nucleotides

Scaffold A structure used in tissue engineering as a template for tissue growth

Selective breeding *see* artificial selection

Staggered cut A cut produced when a restriction enzyme creates fragments of DNA with unpaired nucleotides that overhang at the break in the strands; called sticky ends

Sticky end The overhanging end produced by a staggered cut of a sequence of nucleotide bases; sometimes called cohesive end

Straight cut A cut produced when a restriction enzyme makes a clean break across the two strands of DNA so that the ends terminate in a base pair; called blunt ends

Thyroxine (T4) A hormone secreted by the thyroid gland that regulates metabolism, growth and development

Tissue engineering The rebuilding of damaged tissue by the use of biology, medicine and engineering

Transgenic organism An organism that has had DNA from another species introduced into it artificially

Tri-iodothyronine (T3) A hormone secreted by the thyroid gland; contains iodine and is the most powerful of the thyroid hormones; affects many body processes, including body temperature, growth and heart rate

Type 1 diabetes *see* insulin-dependent diabetes; also see diabetes mellitus

Type 2 diabetes *see* adult-onset diabetes; also see diabetes mellitus

Vector A bacterial plasmid, viral phage or other such agent used to transfer genetic material from one cell to another

9780170449168

CHAPTER 8 REVIEW QUESTIONS

Recall

1 a What is recombinant DNA technology?
  b List three possible applications of recombinant DNA technology.
2 a What are restriction enzymes?
  b How are recognition sites related to restriction enzymes?
  c List examples of restriction enzymes. For each, give their bacterial origin.
  d Differentiate between 'sticky' and 'blunt' ends in relation to restriction enzymes.
3 What is DNA ligase, and what is it used for?
4 a What are vectors, and how are they used in recombinant DNA technology?
  b List two different types of vectors that are used in this technology.
5 Which of the two types of diabetes can frequently be treated by modifying the patient's behaviour? Explain the nature of the behaviour modification that is necessary for effective treatment.
6 a What is gene therapy?
  b How is gene therapy likely to advance the treatment of type 1 diabetes, cystic fibrosis and Huntington's disease?
7 a Define 'cell replacement therapy'.
  b How could cell replacement therapy aid the treatment of diseases such as Parkinson's and Alzheimer's?
8 List two diseases that are being prevented with recombinant vaccines.

Explain

9 Explain, with examples, how a transgenic organism is considered a GMO.
10 Explain the differences between type 1 and type 2 diabetes.
11 Explain how the treatment of type 1 diabetes has been assisted by recombinant DNA technology.
12 Explain why scaffolds are used in tissue engineering.
13 Explain the difference between hyperthyroidism and hypothyroidism.
14 Explain how a dietary deficiency can cause hypothyroidism.
15 Annotate a diagram to explain how biosynthetic insulin is produced.
16 Drugs used to treat thyroid deficiency are produced synthetically. What advantages are there in using synthetic drugs rather than those obtained naturally?

Apply

17 Name the third restriction enzyme isolated from *Haemophilus aegyptius*.
18 The diagram below shows the base sequence for a section of DNA.

GGTCAAGCTTACTCGGATCCAGCTGAATTC
CCAGTTCGAATGAGCCTAGGTCGACTTAAG

Use Table 8.1 (page 203) to identify the recognition site for each of the following restriction enzymes, and hence show the cuts that would be made and state whether they produce blunt ends or sticky ends.
  a BamHI
  b EcoRI
  c HindIII
  d PvuII
19 Compare and contrast synthetic hormones and biosynthetic hormones.
20 The most commonly used test to see whether thyroid function is adequate is a blood test for thyroid-stimulating hormone (TSH).
  a How would a blood test for TSH show whether the thyroid is functioning normally?
  b A test for TSH in the blood can also be used to determine whether a person's diet has sufficient iodine. How would such a test be able to show whether iodine levels are adequate?

9780170449168

21 Explain how gene therapy is different from cell therapy.

22 Imagine that you are a doctor. One of your patients is overweight and complains of feeling constantly hungry and thirsty. You suspect the patient may have type 2 diabetes.

- **a** What tests would you do to find out whether the person is suffering from type 2 diabetes?
- **b** If type 2 diabetes is positively diagnosed, what treatment would you recommend for the patient?

23 Goitre, enlargement of the thyroid gland, can be associated with both over-production and under-production of thyroid hormone. Explain how this is possible.

24 Graves' disease is caused by an abnormality of the immune system. The immune system produces an antibody that behaves in the same way as TSH. Explain how this would lead to hyperthyroidism.

Extend

25 When the Human Genome Project was launched in 1990 it was expected to take until 2005 for complete mapping to be achieved. However, the results of the project were published in 2001, four years ahead of schedule. Find out what enabled the project to advance much faster than originally anticipated.

26 Pregnant women need up to three times more insulin than normal. If the body is unable to produce that much insulin, a condition called gestational diabetes develops. Find out how gestational diabetes could affect the developing foetus.

27 The use of blood products sourced from living donors and human growth hormone from cadavers resulted in products that were devised to improve quality of life, but which also had life-threatening side effects. Using the Internet, find out the types of diseases that were involved with these contaminated products and how they affected the recipients of those products. How has recombinant DNA technology overcome these life-threatening side effects?

28 Uncontrolled diabetes may result in unconsciousness or diabetic coma. Conduct research to find out:

- **a** the three different types of diabetic coma and the cause of each
- **b** the relationship between each type of coma and the two types of diabetes
- **c** the first aid and treatment for diabetic coma.

29 One researcher in the United States stated:

> Tissue engineering holds out promise of truly healing the heart after congestive heart failure.… Through tissue engineering we could actually restore the function of the heart by replacing large portions of the damaged heart muscle by a bioartificial one.

This same researcher has been working for a long time on developing the ideal scaffolding to support the injected cells and the architecture of the heart. Use an Internet search engine to find out the type of scaffolding material that is being used in such research and the success that has been achieved to date.

30 The impact of biotechnology on our daily lives is growing. Much is being said and written about developments in the use of stem cells to aid the treatment of disease. Hold a class debate to canvas both sides of the question: 'Should the Australian federal government support stem cell research?' Remember to keep an open mind and to respect the opinions of others.

9780170449168

31 Population projections by the Australian Bureau of Statistics indicate that by the year 2051 the proportion of the total Australian population that is aged 65 years or more will almost double. Discuss how the impact of this shift in the age structure of the population will affect diseases of ageing such as Parkinson's and Alzheimer's, with particular reference to the stress it will create for health systems and resources.

32 Recombinant DNA technology has resulted in the manufacture of far more human growth hormone than was available in the past. It is now being used to overcome some of the cosmetic effects of ageing. Find out:

- **a** when human growth hormone first became available for use with adults
- **b** what evidence there is of beneficial results from anti-ageing use of this hormone
- **c** if there are risks involved in the use of the hormone in this way
- **d** if there are other benefits to adults in the use of this hormone.

9780170449168