

# *Cambridge*

## *A2 Level*

### *Biology*

*Code (9700)*

#### *Chapter 12 and Chapter 13*

#### *Biodiversity, classification and*

#### *conservation and Genetic*

#### *technology*



You will have noticed that some species are given both their common and scientific names. When a new species is discovered, it is given a scientific name using the **binomial system** that was developed by the Swedish scientist Linnaeus in the 18th century.

A **species** is a group of organisms with similar morphology and physiology, which can breed together to produce fertile offspring and are reproductively isolated from other species.

Over 1.5 million species of animals, a quarter of a million flowering plant species, and thousands of other plants are known. There are also fungi, bacteria, and single-celled organisms. However, there are still areas like oceans and tropical forests that have not been fully explored. Scientists often divide or group described species together, with estimates of the total number ranging up to 100 million. Species form communities that interact with each other and their environment.

### Ecosystems

An ecosystem is a community of organisms and their environment, including soil, dead leaves, water, air, rocks, and other factors. It is not entirely self-contained, as organisms from different ecosystems interact. Ecosystems can be categorized on different scales, from small ponds to large open oceans. Complex ecosystems like tropical rainforests and coral reefs, to simple ones like sandy deserts, are examples of ecosystems.

An **ecosystem** is a relatively self-contained, interacting community of organisms, and the environment in which they live and with which they interact.

Habitat refers to the place a species lives within an ecosystem, while a niche is an organism's role within the ecosystem. It describes its location, energy source, and interactions with its environment and other species. Organisms have special adaptations for obtaining resources from their surroundings. Similar niches may be occupied by the same species or different ones. A complete description of an organism's niche is difficult due to the numerous interactions within the ecosystem.

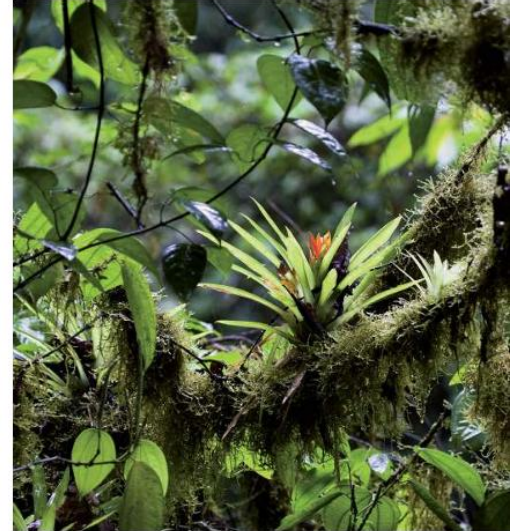
A **niche** is the role of an organism in an ecosystem.

### Biodiversity

Biodiversity can be defined as the degree of variation of life forms in an ecosystem. This is usually taken to include diversity at three levels:

- ■ the variation in ecosystems or habitats
- ■ the number of different species in the ecosystem and their relative abundance
- ■ the genetic variation within each species.

Some areas of the world have very high biodiversity. These areas have many endemic species – that is, species that are only found in these areas and nowhere else (Figure 18.4).



**Figure 18.2** Trees in tropical forests, as here in Costa Rica, are often covered with epiphytes – plants that grow on other plants. These provide far more habitats for small animals, such as beetles, than does the bare bark of trees in temperate forests.



**Figure 18.3** The niche of this great egret includes the freshwater ecosystem where it spends much of its time feeding. It also includes the nearby trees where it roosts and nests.



## Species diversity

The number of species in a community is known as species richness.

Species diversity is the ratio of species richness to the even distribution of organisms among different species. High diversity is crucial for ecosystem stability and resistance to change. Some ecosystems are dominated by one or two species, while others are rare. The tropics are important for biodiversity due to their low-elevation living conditions and high light intensity. For example, Central America has 1500 bird species, while Canada has 300.

## Genetic diversity

Genetic diversity refers to the variation in alleles within a species' genes. It can be assessed by determining the proportion of different alleles and the number per gene. This diversity is evident in cultivated plants and domesticated animals, as well as in natural populations. It helps populations adapt to biotic and abiotic factors, such as competition, disease resistance, and temperature changes.

## Assessing species diversity

### Collecting organisms and making species lists

Imagine you are in an ecosystem like that in Figure 18.6. The most obvious species are the large plants and maybe some of the larger animals, particularly bird species.

Biologists use identification keys to name organisms they find, such as drawings or photographs. The most common is a dichotomous key, which requires good observation skills. To identify species, start with a timed search and then take photographs. For small animals, use a pooter to collect them, which can be studied and identified using a hand lens before returning them to their habitat.

There are now two questions to ask: how are the different species spread throughout the ecosystem and how many individuals of each species are there? The answers to these two questions describe what we call **distribution and abundance**.

## Sampling

We can sometimes do this if the area is very small, or the species are very large. But it is only rarely possible. Instead, we take **samples** from the area we are interested in, and use these to make an estimate of the total numbers in the area.

Sampling can be **random or systematic**. If an area looks reasonably uniform, or if there is no clear pattern to the way species are distributed, then it is best to use random sampling.

### Random sampling using quadrats

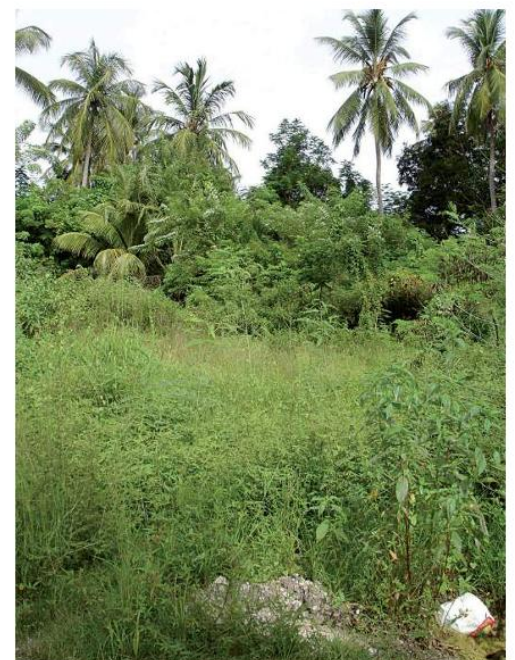
A quadrat is a square frame used to identify species and measure their abundance. To avoid bias, samples must be taken randomly from the area with



**Figure 18.4** New Zealand was isolated for many millions of years so that it has many endemic species and also some, like this tuatara, *Sphenodon* sp., that have become extinct elsewhere.



**Figure 18.5** These snails all belong to the same species, *Cepaea nemoralis*. The differences between them are the result of different alleles for shell colour and banding.



**Figure 18.6** How would you start to investigate and catalogue the biodiversity of an area like this?

the fewest species. Marking the area with measuring tapes and using a random number generator, such as a mobile app, ensures randomness. The random numbers provide the sampling points' coordinates.

You can use your results in two different ways: to calculate **species frequency and species density**.

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To count individual plants and animals, estimate the percentage cover of each species within a quadrat on a lawn. Use a 100 cm x 100 cm quadrat with wires running 10 cm intervals in each direction, dividing it into 100 smaller squares. Estimate the percentage of the area occupied by each species, as percentages may not add up to 100% due to bare ground or plants overlying each other. An alternative is using an abundance scale like the Braun-Blanquet scale.

Braun-Blanquet Cover Scale	
Description	Value
Very few plants, cover is less than 1%	+
Many plants, but cover is 1–5%	1
Very many plants or cover is 6–25%	2
Any number of plants; cover is 26–50%	3
Any number of plants; cover is 51–75%	4
Cover is greater than 75%	5

**Table 18.1** The Braun-Blanquet scale for recording vegetation within quadrats.

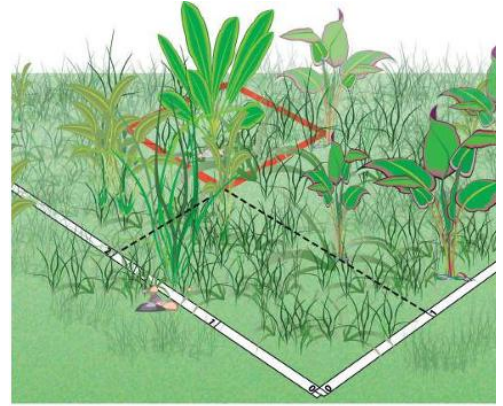
### Estimating numbers of mobile animals

Quadrats are not suitable for finding or counting mobile animals, so alternative methods are used. Small mammals can be caught in traps filled with hay and food, while insects and invertebrates can be captured using sweep netting. Pond nets are used for sampling aquatic organisms. Single birds can be counted easily, but flocks of birds are more difficult. The mark-release recapture technique is a good method for estimating the population size of mobile organisms. It involves catching and marking individuals, allowing them to mix with the rest of the population, and then calculating the proportion of marked to unmarked individuals.

### Simpson's Index of Diversity

When you have collected information about the abundance of the species in the area you are studying, you can use your results to calculate a value for the species diversity in that area. We can do this using Simpson's Index of Diversity,  $D$ . One formula for this is:

$$D = 1 - \left( \sum \left( \frac{n}{N} \right)^2 \right)$$



**Figure 18.8** In random sampling, quadrats are positioned randomly in an area marked off by measuring tapes. This reduces the chances of bias in sampling the ecosystem.



**Figure 18.9** Estimating percentage cover. This 1 m<sup>2</sup> quadrat is divided into 100 small squares to make it easier to make the estimation for each species.



**Figure 18.10** A red sea anemone that lives between the tides on rocky shores.

## Systematic sampling

The **line transect** technique is a method of sampling organisms along a straight line to investigate changes in physical conditions like altitude, soil moisture content, soil type, pH, exposure, or light intensity. This method records the identity of organisms that touch the line at set distances, providing qualitative data. The **belt transect** technique involves placing a quadrat at regular intervals along the line, recording the abundance of each species within the quadrat.

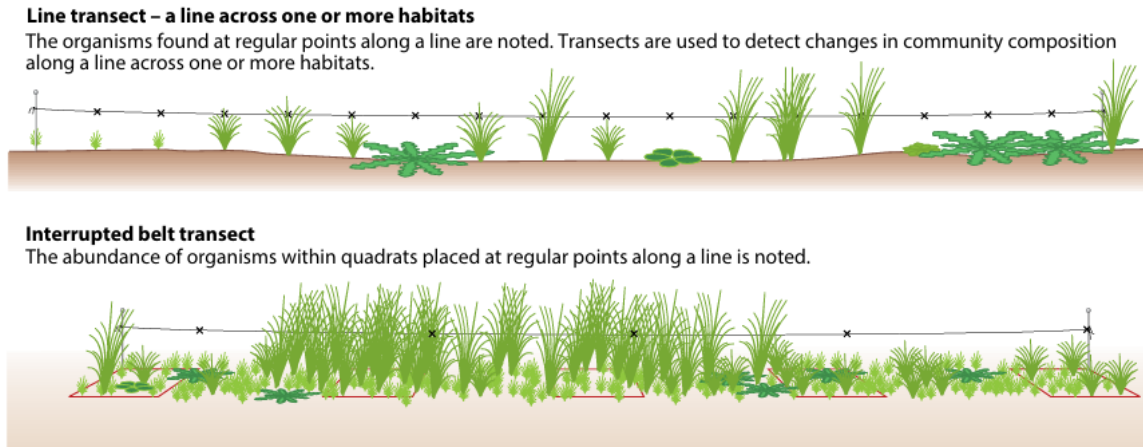


Figure 18.11 Systematic sampling using transects: a a line transect, and b an interrupted belt transect.

## Correlation

Observe plant species appearing together during random sampling or belt transects. Determine if there's an association between species distribution and abiotic factors like light, temperature, soil water content, or salinity. Plot scatter graphs or calculate a correlation coefficient to assess the strength of the relationship.

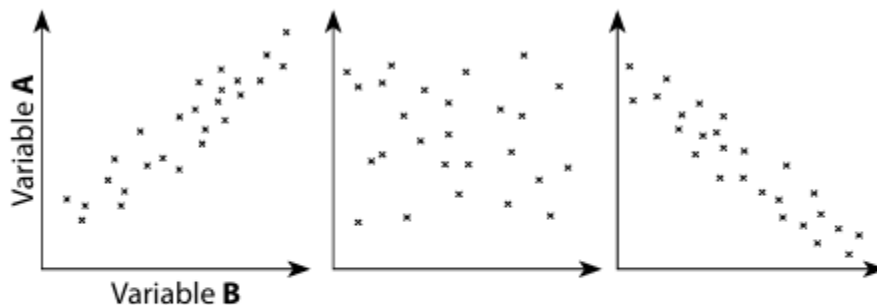


Figure 18.13 Three types of association: a a positive linear correlation, b no correlation, and c a negative linear correlation.

The strongest correlation you can have is when all the points lie on a straight line: there is a **linear correlation**.

**Pearson's correlation** coefficient can only be used where you can see that there might be a linear correlation (a and c in Figure 18.13) and when you have collected quantitative data as measurements (for example, length, height, depth, light intensity, mass) or counts (for example, number of plant species in quadrats).



If so, then you can calculate **Spearman's rank correlation coefficient**, which involves ranking the data recorded for each variable and assessing the difference between the ranks.

To find out if there is a relationship between the percentage cover of these two species, the first task is to make a **null hypothesis** that there is **no correlation** between the percentage cover of the two species.

The equation to calculate the Spearman rank correlation,  $r_s$ , is:

$$r_s = 1 - \left( \frac{6 \times \sum D^2}{n^3 - n} \right)$$

### Pearson's linear correlation

Pearson's correlation coefficient is used when data for two continuous variables shows a normal distribution. The test can be done using a spreadsheet and involves checking if the relationship between the two variables is linear. The strength of association between variables depends on the distance from the scatter of points. For correlation purposes, the data must show a normal distribution. A student measured the width of cracks in pine trees and found a correlation between tree size and crack width. She collected continuous data for tree circumference and crack width, and investigated the relationship using twelve randomly selected trees.

The student plotted these results on a scatter graph and found that they look as if there might be a linear correlation between them. She then used the following formula to calculate Pearson's correlation coefficient,  $r$ .

$$r = \frac{\sum xy - n\bar{x}\bar{y}}{ns_x s_y}$$

The student calculated the correlation coefficient as  $r = 0.79$ .

### Classification

Huge number of different kinds of organisms living on Earth, biologists have always wanted to arrange them into groups, a process called classification.

**Taxonomy** is the study and practice of classification, which involves placing organisms in a series of taxonomic units, or taxa (singular: taxon). In biological classification, these taxa form a **hierarchy**. Each kind of organism is assigned to its own **species**, and similar species are grouped into a **genus** (plural: genera). Similar genera are grouped into a **family**, families into an **order**, orders into a class, **classes** into a **phylum** (plural: phyla) and phyla into a **kingdom**. The domain is at the top of this hierarchical system. Table 18.4 shows how African bush elephants and hibiscus plants (Figure 18.14) are classified.

Number of tree	Circumference of tree / metres	Mean width of crack / mm
1	1.77	50
2	1.65	28
3	1.81	60
4	0.89	24
5	1.97	95
6	2.15	51
7	0.18	2
8	0.46	15
9	2.11	69
10	2.00	64
11	2.42	74
12	1.89	69

Table 18.3 Widths of cracks on pine trees in a plantation.

### Three domains

Biologists traditionally divided organisms into prokaryotes and eukaryotes based on cell structure. In the 1970s, prokaryotes were discovered in extreme environments, such as hot springs, and their genes coding for ribosome RNA were more similar to eukaryotes. This led to the introduction of a new taxon, the domain, to reflect the differences between these extremophiles and typical bacteria. Prokaryotes are divided into Bacteria and Archaea, while eukaryotes are placed in Eukarya. Archaea have more similarities with Eukarya than Bacteria, and it is believed that they separated early in life evolution.

### Domain Bacteria

Bacteria are prokaryotic as their cells have no nucleus. They are all small organisms that vary in size between that of the largest virus and the smallest single-celled eukaryote. The characteristic features of bacteria are:

- ■ cells with no nucleus
- ■ DNA exists as a circular 'chromosome' and does not have histone proteins associated with it
- ■ smaller circular molecules of DNA called plasmids are often present
- ■ no membrane-bound organelles (such as mitochondria, endoplasmic reticulum, Golgi body, chloroplasts) are present
- ■ ribosomes (70 S) are smaller than in eukaryotic cells
- ■ cell wall is always present and contains peptidoglycans (not cellulose)
- ■ cells divide by binary fission, not by mitosis
- ■ usually exist as single cells or small groups of cells.

Look at Figure 1.30 on page 21 to remind yourself of these features and to see how prokaryotic cell structure differs from that of eukaryotes. There are electron micrographs of two pathogenic species of bacteria in Chapter 10. Figure 18.16 shows the cyanobacterium *Nostoc*.



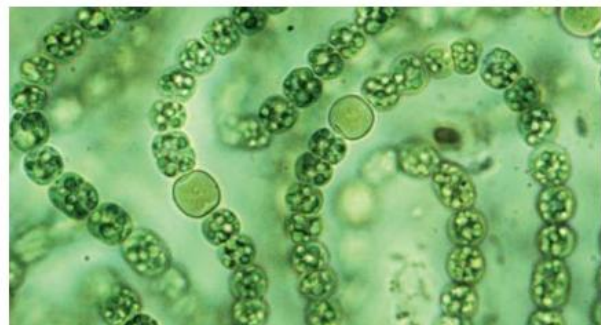
**Figure 18.15** A deep sea hydrothermal vent (black smoker) surrounded by giant tubeworms. The community relies on energy made available by bacteria and archaeans.

Taxon	African bush elephant	Hibiscus
domain	Eukarya	Eukarya
kingdom	Animalia	Plantae
phylum	Chordata	Angiosperms
class	Mammalia	Dicotyledonae
order	Proboscidea	Malvales
family	Elephantidae	Malvaceae
genus	<i>Loxodonta</i>	<i>Hibiscus</i>
species	<i>Loxodonta africana</i>	<i>Hibiscus rosa-sinensis</i>

**Table 18.4** The classification of African bush elephants and hibiscus plants.



**Figure 18.14** *Hibiscus rosa-sinensis* is a plant that has spread from Asia to much of the tropics and sub-tropics. Flower colour is a good example of the genetic diversity in this species.



**Figure 18.16** The filamentous cyanobacterium, *Nostoc*. This species fixes carbon dioxide in photosynthesis; it also fixes nitrogen by converting  $N_2$  into organic forms of nitrogen in the wider, lighter green cells in its filaments. ( $\times 600$ )

## Domain Archaea

Archaeans are also prokaryotic as their cells have no nucleus. Their range of size is similar to that of bacteria. Many inhabit extreme environments (Figure 18.17). The characteristic features of archaeans are:

- ■ cells with no membrane-bound organelles
- ■ DNA exists as a circular 'chromosome' and does have histone proteins associated with it
- ■ smaller circular molecules of DNA called plasmids are often present
- ■ ribosomes (70 S) are smaller than in eukaryotic cells, but they have features that are similar to those in eukaryotic ribosomes, not to bacterial ribosomes
- ■ cell wall always present, but does not contain peptidoglycans
- ■ cells divide by binary fission, not by mitosis
- ■ usually exist as single cells or small groups of cells.

## Domain Eukarya

All the organisms classified into this domain have cells with nuclei and membrane-bound organelles. Their characteristic features are:

- ■ cells with a nucleus and membrane-bound organelles
- ■ DNA in the nucleus arranged as linear chromosomes with histone proteins
- ■ ribosomes (80 S) in the cytosol are larger than in prokaryotes; chloroplasts and mitochondria have 70S ribosomes, like those in prokaryotes.
- ■ chloroplast and mitochondrial DNA is circular as in prokaryotes
- ■ a great diversity of forms: there are unicellular (Figure 18.18), colonial (Figure 18.19) and multicellular organisms
- ■ cell division is by mitosis
- ■ many different ways of reproducing – asexually and sexually.



**Figure 18.17** A scanning electron micrograph of the archaean *Pyrococcus furiosus* ( $\times 12\,500$ ). Although they look like bacteria, archaeans have differences in metabolism and genetics. The flagella seen here are also different. *P. furiosus* is only found in near-boiling water; if the temperature falls below  $70^{\circ}\text{C}$  it freezes and dies. It respire anaerobically using sulfur instead of oxygen as the final electron acceptor.



**Figure 18.18** *Stentor roseli*, a protist covered in many cilia which it uses for movement and for feeding. Although unicellular, it has considerable specialisation of regions within its body ( $\times 240$ ).



## Kingdom Protocista

The Protocista is made up of a very diverse range of eukaryotic organisms, which includes those that are often called protozoans ('simple animals') and algae, such as seaweeds. Any eukaryote that is not a fungus, plant or animal is classified as a protocista (Figures 18.18 and 18.19). The characteristic features of protocists are:

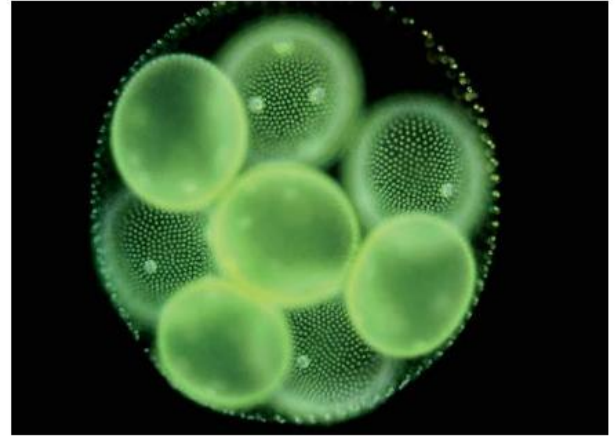
- ■ eukaryotic
- ■ mostly single-celled, or exist as groups of similar cells
- ■ some have animal-like cells (no cell wall) and are sometimes known as protozoa
- ■ others have plant-like cells (with cellulose cell walls and chloroplasts) and are sometimes known as algae.

## Kingdom Fungi

Fungi have some similarities with plants, but none of them is able to photosynthesise.

Characteristic features of fungi are:

- ■ eukaryotic
- ■ do not have chlorophyll and do not photosynthesise
- ■ heterotrophic nutrition – they use organic compounds made by other organisms as their source of energy and source of molecules for metabolism
- ■ reproduce by means of spores (Figure 18.20)
- ■ simple body form, which may be unicellular or made up of long threads called hyphae (with or without cross walls) (Figure 18.21); large fungi such as mushrooms produce large compacted masses of hyphae known as 'fruiting bodies' to release spores
- ■ cells have cell walls made of chitin or other substances, not cellulose
- ■ never have cilia or flagella.



**Figure 18.19** *Volvox globator* is a spherical colonial green protist. The body is composed of thousands of cells with flagella. These cells work together in a coordinated way but there is little specialisation of cells. Cells at one pole detect light so the colony swims towards the light. Cells at the other pole are specialised for reproduction. Inside there are new colonies that are just about to be released ( $\times 60$ ).



**Figure 18.20** A puffball fungus, *Lycoperdon* sp., releasing millions of microscopic spores. Their method of feeding on dead and decaying matter means that eventually the food is all used up. A few of these spores may land on a suitable food source and be able to grow.



**Figure 18.22** Tree ferns, *Cyathea* sp., growing in Whirinaki Conservation Park, New Zealand.

### Kingdom Plantae

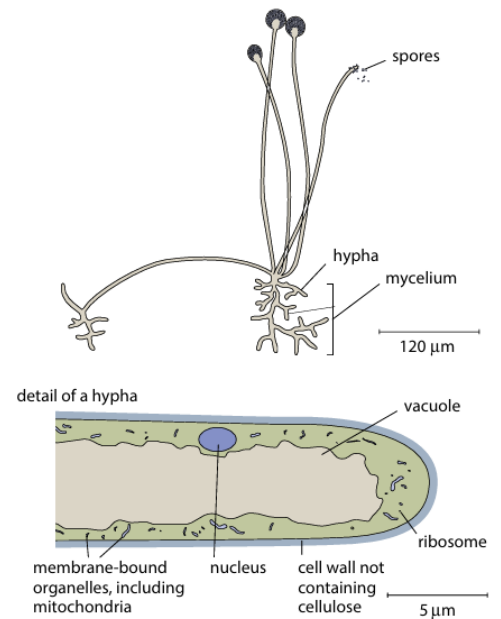
Plantae (plants) are all multicellular photosynthetic organisms (Figures 18.22 and 18.23). They have complex bodies that are often highly branched both above and below ground. Characteristic features of plants are:

- ■ multicellular eukaryotes with cells that are differentiated to form tissues and organs
- ■ few types of specialised cells
- ■ some cells have chloroplasts and photosynthesise (Figures 1.28 and 1.29, pages 20 and 21)
- ■ cells have large, often permanent vacuoles for support
- ■ autotrophic nutrition
- ■ cell walls are always present and are made of cellulose
- ■ cells may occasionally have flagella – for example, male gametes in ferns.

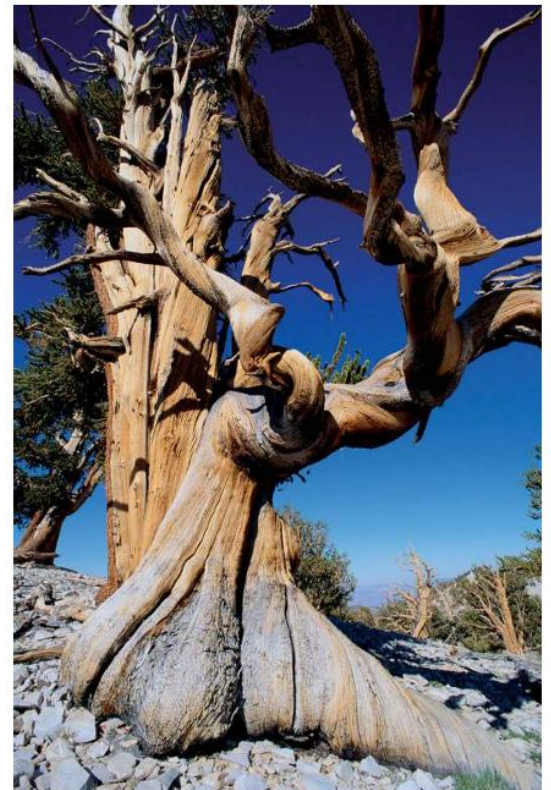
### Kingdom Animalia

The Animalia are multicellular organisms that are all heterotrophic with many ways of obtaining their food. There is a great diversity of forms within this kingdom (Figure 18.24). The nervous system is unique to the animal kingdom. Characteristic features of animals are:

- ■ multicellular eukaryotes with many different types of specialised cells



**Figure 18.21** The bread mould fungus, *Rhizopus nigricans*, and a detail of the end of one hypha.



**Figure 18.23** Bristlecone pines are some of the oldest trees on Earth, many estimated to be 2000 to 3000 years old. These grow at over 2700 metres in the Great Basin National Park in Nevada.



- ■ cells that are differentiated to form tissues and organs
- ■ cells do not have chloroplasts and cannot photosynthesise (although some, such as coral polyps have photosynthetic protocists living within their tissues)
- ■ cell vacuoles are small and temporary (for example, lysosomes and food vacuoles)
- ■ heterotrophic nutrition
- ■ cells do not have cell walls
- ■ communication is by the nervous system (Chapter 15)
- ■ cells sometimes have cilia or flagella

### Viruses

Viruses are microorganisms with acellular structures, visible only with electron microscopes. They have proteins and nucleic acids found in all cellular organisms, but they are not considered living organisms. When infected, they use the host cell's biochemical machinery to copy nucleic acids and proteins, often leading to cell destruction. The taxonomic system for classifying viruses is based on diseases they cause, the type of nucleic acid they contain (DNA or RNA), and whether the nucleic acid is single-stranded or double-stranded.

Nucleic acid	Number of strands	Example	Host organism	Disease
DNA	1	canine parvovirus type 2	dogs	canine parvovirus
	2	African cassava mosaic virus	cassava plants	mosaic disease
RNA	2	varicella zoster virus (VZV)	humans	chickenpox
		rotavirus	humans	gastroenteritis
	1	morbillivirus	humans	measles
		tobacco mosaic virus (TMV)	tobacco, tomato, pepper	mosaic disease
		human immunodeficiency virus	humans	HIV/AIDS

**Table 18.5** Viruses are classified into four groups using the type and structure of their nucleic acid as the criteria for grouping them.

### Threats to biodiversity

Biodiversity is under threat in many aquatic and terrestrial ecosystems as the human population continues to increase and we take more resources from the environment and produce increasing quantities of waste.

There are five major threats to biodiversity:

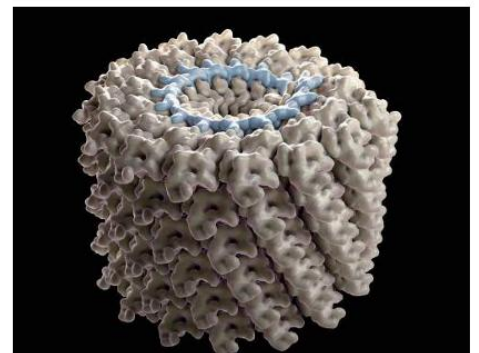
- ■ habitat loss and the degradation of the environment
- ■ climate change
- ■ excessive use of fertilisers and industrial and domestic forms of pollution
- ■ the overexploitation and unsustainable use of resources
- ■ the effects of invasive alien species on native species, especially endemics.



**Figure 18.24** The crown-of-thorns starfish, *Acanthaster planci*, feeds on coral in the Great Barrier Reef. It has been through several population explosions over recent years causing destruction of much of the coral on parts of the reef (page 443).



**Figure 18.26** Viruses are the ultimate in parasitism. This tobacco plant is showing signs of infection by the tobacco mosaic virus. TMV spreads rapidly through crops of plants, such as tobacco and tomato, severely reducing their growth.



**Figure 18.25** A model showing the structure of part of the tobacco mosaic virus, TMV. The blue strand is the single strand of RNA and the rest is composed of proteins. In a survey of plant pathologists in 2011, TMV was voted the most important plant virus.



The destruction of the natural environment leads to **habitat loss**.

Consequently, many species of plant and animal either lose their habitats completely or their habitats become divided into small areas; this is known as **habitat fragmentation**.

**Deforestation** has had a devastating effect on the biodiversity of some countries. Madagascar, famed for its unique plant and animal life, has lost almost all of its natural forest.

The response to the steep decrease in large, predatory species is to fish further down the food chain taking smaller fish that other animals, such as marine mammals and sea birds, depend upon. Fishing is just one example of the **overexploitation** of resources.

**Pollution** is a major threat to many ecosystems. In many countries, industrial and domestic waste is processed to reduce its impact on the environment.

Animals like dolphins and turtles die from discarded fishing nets and plastic bags. Unabsorbed fertilizers from farmland drain into rivers and seas, leading to algae growth, toxic substances, and unbalanced food webs. Acid rain from high sulfur fuel combustion destroys vegetation and aquatic ecosystems, reducing biodiversity. Many ecosystems are still at risk from acid rain, as few animals can survive in low pH waters.

Industrialisation and the extraction and combustion of fossil fuels have also led to an increase in the concentrations of carbon dioxide and methane in the atmosphere. These are both **greenhouse gases**. High emissions of methane are associated with cattle and rice farming and the breakdown, under anaerobic conditions, of organic waste in landfill sites. The build-up of greenhouse gases is leading to **climate change**.

**Why does biodiversity matter?**

#### Moral and ethical reasons

For many people, the loss of biodiversity is a simple moral or ethical issue: we share our planet with a huge range of other organisms and we have no right to drive them to extinction. Some people believe that humans have custody of the Earth and should therefore value and protect the organisms that share the planet with us.

#### Ecological reasons

Biodiversity is crucial for ecosystems as it helps balance them and prevent unbalanced changes due to threats like pollution. Humans rely on ecosystems for various purposes, such as using drugs from living organisms like fungi and bacteria. For example, antibiotics are isolated from plants like the Madagascan periwinkle and the Pacific yew tree. However, over-harvesting and fuel collection threaten these plants. Currently, there is interest in cataloguing plants used in traditional Chinese and Indian medicines to develop mass-produced drugs.



**Figure 18.28** A kelp forest in the Pacific Ocean off the coast of California. The giant seaweeds provide habitats for many species including sea urchins, fish and sea otters.



**Figure 18.27** A sea otter, *Enhydra lutris*.

### Aesthetic reasons

There is an aesthetic argument for maintaining biodiversity. Many people gain pleasure from studying or just appreciating the natural world, which continues to provide much inspiration for artists, photographers, poets, writers and other creative people

### Social and commercial reasons

Our crop plants lack genetic diversity due to selective breeding for uniform, high-yielding crops. Wild relatives of maize, such as *Oryza longistaminata*, grow in Mexico and can provide genetic resources for cultivated maize. However, these wild relatives are threatened by climate change, habitat destruction, and genetically modified crops. For example, *Oryza longistaminata*, a rice species in Mali, is resistant to bacterial blight but not suitable for cultivation. Potatoes, a potato crop, are vulnerable to diseases like potato blight, but the International Potato Center has used Andean potato species for resistance. Microorganisms, such as Taq polymerase, are also important for antibiotics and other useful products.

### Other services

Ecosystems provide services for us. Forests and peat bogs absorb carbon dioxide and may help to reduce the effect of increases in carbon dioxide in the atmosphere. Organic waste material added to waters is broken down by microorganisms. The transpiration of plants contributes to the water cycle providing us with drinking and irrigation water.

### Protecting endangered species

Endangered species are threatened with extinction and can be protected by maintaining their natural habitat and providing life support systems. Public awareness often focuses on individual species or groups, but conservation is crucial for whole ecosystems threatened by development, such as tropical rainforests and rare ecosystems like karst limestone.

### National parks

Most countries now set aside areas where wildlife and the environment have some form of protection, and where the activities of humans are limited. For example, conservation areas may be set up where there are strict limits on building, grazing farm animals, hunting or other activities that might adversely affect animals and plants that live there.

### Zoos

Zoos offer visitors the opportunity to study and observe endangered and vulnerable species, and have successfully implemented captive breeding programs to reintroduce animals to their natural habitats. However, inbreeding is a challenge in breeding small populations, as seen with the cheetah, a vulnerable species with low genetic diversity. Maintaining genetic diversity is crucial for species conservation, such as the cheetah.

Zoos play a crucial role in research, particularly in understanding breeding habits, habitat requirements, and genetic diversity. The Zoological Society of London (ZSL) has a significant research program. Captive breeding aims to reintroduce animals to their natural habitats, but this can be challenging due to various factors. For example, the Emperor Valley Zoo in Trinidad successfully reintroduced blue-and-gold macaws to the Nariva Swamp.



**Figure 18.29** Elephants in the Amboseli National Park, Kenya. Elephants throughout Africa are exposed to numerous threats, not least poaching. Biodiversity suffers if their numbers increase or decrease. There is a delicate balance to achieve and this needs careful management by park authorities.

However, some animals refuse to breed in captivity, and some, like the giant panda, struggle to adapt to their new environment. Despite a captive breeding program starting in 1963, no panda has been successfully returned to the wild.



**Figure 18.30** Golden lion tamarin, *Leontopithecus rosalia*, from the coastal forests of Brazil. As their habitat has been destroyed they have been rescued, bred in captivity and reintroduced to protected reserves.



**Figure 18.31** A conservation success story: the scimitar-horned oryx, *Oryx dammah*, saved from extinction and bred in captivity, is now protected in reserves in Senegal, Tunisia and Morocco.

### Assisted reproduction

Assisted reproduction is a solution to the problem of inbreeding. Zoos used to transport large mammals between them as part of their captive breeding programmes. Movement of large mammals is difficult and expensive and breeding did not always happen. A much cheaper option is to collect semen and keep it frozen in a **sperm bank**.

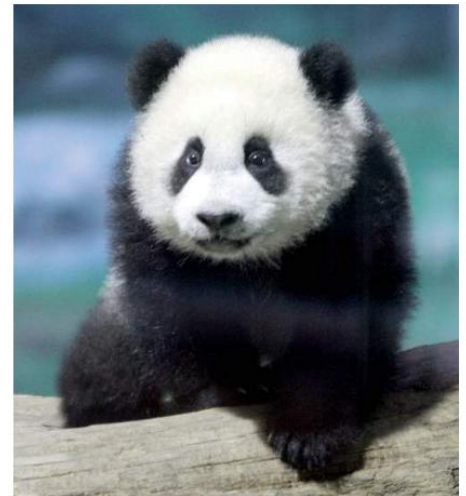
A '**frozen zoo**', such as the one at the San Diego Zoo, holds genetic resources in the form of sperm, eggs and embryos from many endangered and vulnerable species until they might be needed. Frozen zoos can hold much more genetic diversity than a normal zoo and the material can be kept for very long periods of time

### Problems of successful conservation

**Culling** is a controversial practice used to control population growth, particularly in elephants. Between 1966 and 1994, over 16,000 elephants were culled in Kruger National Park, South Africa. Alternative methods include birth control, such as sedating male wild mammals and cutting sperm ducts. Chemical contraceptives, like a vaccine targeting the zona pellucida, stimulate an immune response, blocking sperm from fertilizing eggs, with a 90% success rate in mammals.

### Botanic gardens

Botanic gardens play similar roles to zoos for endangered plants. Seeds or cuttings are collected from species in the wild and then used to build up a population of plants from which, one day, some plants may be reintroduced to their natural habitats.



**Figure 18.32** The giant panda cub, Yuan Zai, born in the Taipei Zoo in Taiwan on 6 July 2013. Is she destined to remain in captivity her whole life or will she be released into the wild?



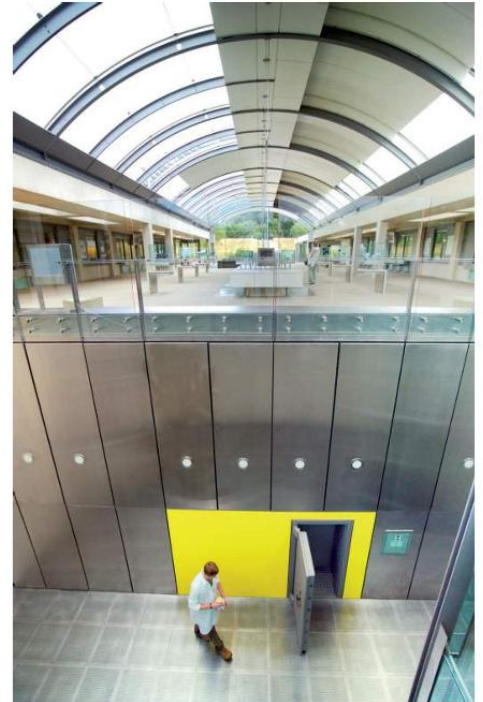
The roles of botanic gardens are to:

- ■ protect endangered plant species; the world's botanic gardens already cultivate around one-third of the world's known plant species, many of which are increasingly threatened in the wild by environmental degradation and climate change
- ■ research methods of reproduction and growth so that species cultivated in botanic gardens can be grown in appropriate conditions and be propagated
- ■ research conservation methods so plants can be introduced to new habitats if their original habitat has been destroyed
- ■ reintroduce species to habitats where they have become very rare or extinct
- ■ educate the public in the many roles of plants in ecosystems and their economic value

The Svalbard Global Seed Vault is a seed bank in Norway, located in the Arctic Circle. It holds over 770,000 seed samples from various crop varieties worldwide. The vault is open only in winter, and if seeds are lost due to environmental disasters, duplicate samples will remain available. The depositing seed bank owns its seeds, and research organizations must apply to the original seed bank for use. The storage of seeds is free of charge, with costs covered by the Norwegian government and the Global Crop Diversity Trust. Recalcitrant seeds, such as rubber, coconut palm, coffee, and cocoa, are difficult to store due to their large seeds and embryos. The vault helps maintain genetic diversity in crop plants.



**Figure 18.35** The future of cocoa, *Theobroma cacao*, is threatened by diseases, climate change, natural disasters, limited genetic diversity and the failure to manage plantations by replacing old trees. 30–40% of the world's production is lost to pests and disease.



**Figure 18.33** The Millennium Seed Bank, Wakehurst Place, UK. Seeds arriving at the seed bank are checked for pests and diseases, assessed for viability, dried, and then stored in airtight jars (Figure 18.34) and kept in the seed-storage vault at  $-20^{\circ}\text{C}$ .



**Figure 18.34** A botanist with one of the seed collections in a cold vault at the Millennium Seed Bank.

## Controlling alien species

Alien or invasive species have moved from one ecosystem to another, often introduced by humans through trade or unknowingly carried on ships. Some species have been introduced as biological control agents, such as the Indian mongoose *Herpestes auropunctatus*, which was introduced to Jamaica in 1872. However, it has since become a predator of other animals. The cane toad, introduced to Queensland, Australia, has become a pest due to its rapid breeding and spread across the country. Other alien species are escapees or animals introduced for sport, such as rabbits, Burmese pythons, and the red lionfish. These species have caused significant loss of biodiversity, making it difficult to conserve endangered species in these areas. Efforts to remove pythons have failed, and efforts to stop their population increasing have failed.

## International conservation organisations

### CITES

In 1973, 145 countries signed an agreement to control the trade in endangered species and any products from them, such as furs, skins and ivory. More countries have joined since. This agreement is called the Convention on International Trade in Endangered Species of Wild Flora and Fauna, CITES for short.



**Figure 18.37** Red lionfish, *Pterois volitans*, an alien species that escaped into the Caribbean and is causing havoc on coral reefs as it eats many reef animals and has no natural predator.

CITES assigns endangered and vulnerable species to one of three Appendices, reviewed by expert committees. The list is growing, but there's concern that listing doesn't always benefit a species. Illegal trade can increase product prices, making it worthwhile to break the law. Problems arise when species are announced in advance.



**Figure 18.36** Germinating coconut seeds ready to be planted in nursery plots. There are gene banks for coconut in Karnataka, India and in Papua New Guinea.



**Figure 18.38** Policemen in Malaysia examine dead sea turtles from a boat stopped on its way to the souvenir market. All sea turtles are listed in CITES [Appendix 1](#), and poaching remains a serious threat to sea turtle populations, along with fishing nets, pollution, and habitat destruction.



CITES Appendix	Criteria	Trading regulations	Animal examples	Plant examples
I	Species that are the most endangered and threatened with extinction	All trade in species or their products is banned	Orang-utans, <i>Pongo abelii</i> and <i>P. pygmaeus</i> (Borneo, Indonesia)	Kinabalu pitcher plant, <i>Nepenthes kinabaluensis</i> (Malaysia)
II	Species that are not threatened with extinction, but will be unless trade is closely controlled	Trade is only allowed if an export permit is granted by the countries concerned	Sir David's long-beaked echidna, <i>Zaglossus attenboroughi</i> (Papua, Indonesia)	All species in the genus <i>Nepenthes</i> ; Venus fly trap, <i>Dionaea muscipula</i>
III	Species included at the request of a country that regulates trade in the species and needs the cooperation of other countries to prevent unsustainable or illegal exploitation	Trade in these species is regulated; permits are required, but they are easier to obtain than for species in Appendix II	Mauritian pink pigeon, <i>Columba mayeri</i>	Spur tree from Nepal, <i>Tetracentron sinense</i>

Table 18.6 CITES Appendices I, II and III.

### Worldwide Fund for Nature

The Worldwide Fund for Nature (WWF) is one of the best known campaigning groups for wildlife. Established in 1961, WWF is the largest international non-governmental organisation (NGO) specialising in conservation. Its mission statement is 'to stop the degradation of the planet's natural environment and to build a future in which humans live in harmony with nature'

### Restoring degraded habitats

Conservation involves restoring areas that have been degraded by human activity or by natural catastrophes, such as flood, fire, hurricane, typhoon and earthquake. This can be done on a small scale when a farmer decides to plant trees on land that is no longer needed for food production or has become degraded by overuse.

Haiti is restoring forests after centuries of deforestation and land degradation, with 70% of the land unsuitable for agriculture and a shortage of firewood. NGOs and community groups are working on tree planting projects. The Eden Project in Cornwall, UK, educates people about plant biodiversity and conservation, highlighting the importance of conserving plants for their ecological and economic benefits.



Figure 18.39 Young people planting mangrove seedlings on the island of Bali in Indonesia. In the Philippines, a group of students led a community effort to replant 100 000 mangroves in seven months after learning how mangroves protect coastal areas from storms yet were being cut down for charcoal.



Figure 18.40 The Eden Project, near St Austell in Cornwall, UK. This is an example of reclamation, and also of education in the importance of sustainable development and the conservation of the world's plant life.



## Chapter 19: Genetic technology

### Genetic engineering

The aim of genetic engineering is to remove a gene (or genes) from one organism and transfer it into another so that the gene is expressed in its new host. The DNA that has been altered by this process and which now contains lengths of nucleotides from two different organisms is called recombinant DNA (rDNA).

**Recombinant DNA is DNA made by joining pieces from two or more different sources.**

An overview of gene transfer There are many different ways in which a GMO may be produced, but these steps are essential.

- 1 The gene that is required is identified. It may be cut from a chromosome, made from mRNA by reverse transcription or synthesised from nucleotides.
- 2 Multiple copies of the gene are made using the technique known as the polymerase chain reaction (PCR).
- 3 The gene is inserted into a vector which delivers the gene to the cells of the organism. Examples of vectors are plasmids, viruses and liposomes.
- 4 The vector takes the gene into the cells.
- 5 The cells that have the new gene are identified and cloned.

To perform these steps, the genetic engineer needs a 'toolkit' consisting of:

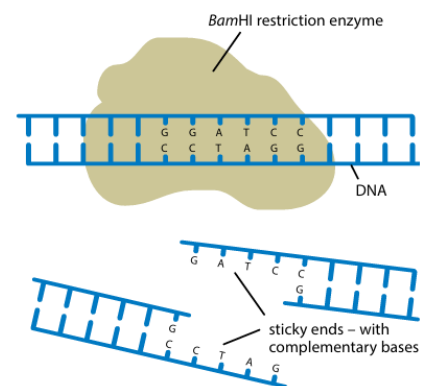
- ■ enzymes, such as restriction endonucleases, ligase and reverse transcriptase
- ■ vectors, including plasmids and viruses
- ■ genes coding for easily identifiable substances that can be used as markers.

### Tools for the gene technologist

#### Restriction enzymes

Restriction endonucleases are a class of enzymes from bacteria which recognise and break down the DNA of invading viruses known as bacteriophages (phages for short). Bacteria make enzymes that cut phage DNA into smaller pieces. These enzymes cut the sugar-phosphate backbone of DNA at specific places within the molecule. This is why they are known as endonucleases ('endo' means within). Their role in bacteria is to restrict a viral infection, hence the name restriction endonuclease or restriction enzyme.

Restriction enzymes cut specific sequences of bases on DNA, protecting it from attack by chemical markers or not having the target sites. These sites are specific sequences of bases, such as GGATCC on one strand and CCTAGG on the other. Restriction enzymes can cut straight across the sugar-phosphate backbone or staggered for sticky ends. To find the specific piece of DNA required, gel electrophoresis and gene probes are used, and polymerase chain reaction (PCR) can be used for multiple copies.



**Figure 19.2** The restriction enzyme, *Bam*HI, makes staggered cuts in DNA to give sticky ends.

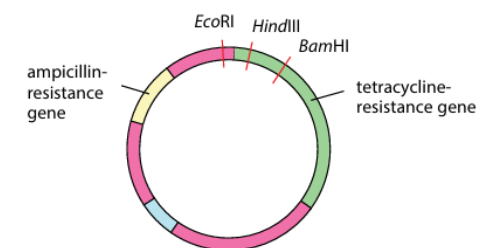
Restriction enzyme	Restriction site	Site of cut across DNA	Source of enzyme
<i>EcoRI</i>	5' –GAATTC– 3' 3' –CTTAAG– 5'	–G <span style="border: 1px solid red; padding: 0 2px;">AATTC</span> – –CTTAA <span style="border: 1px solid red; padding: 0 2px;">G</span> –	<i>Escherichia coli</i>
<i>BamHI</i>	5' –GGATCC– 3' 3' –CCTAGG– 5'	–G <span style="border: 1px solid red; padding: 0 2px;">GATCC</span> – –CCTAG <span style="border: 1px solid red; padding: 0 2px;">G</span> –	<i>Bacillus amyloliquefaciens</i>
<i>HindIII</i>	5' –AAGCTT– 3' 3' –TTCGAA– 5'	–A <span style="border: 1px solid red; padding: 0 2px;">AGCTT</span> – –TTCGA <span style="border: 1px solid red; padding: 0 2px;">A</span> –	<i>Haemophilus influenzae</i>
<i>HaeIII</i>	5' –GGCC– 3' 3' –CCGG– 5'	–GG <span style="border: 1px solid red; padding: 0 2px;">CC</span> – –CC <span style="border: 1px solid red; padding: 0 2px;">GG</span> –	<i>Haemophilus aegyptius</i>

**Table 19.1** Four restriction enzymes and their target sites.

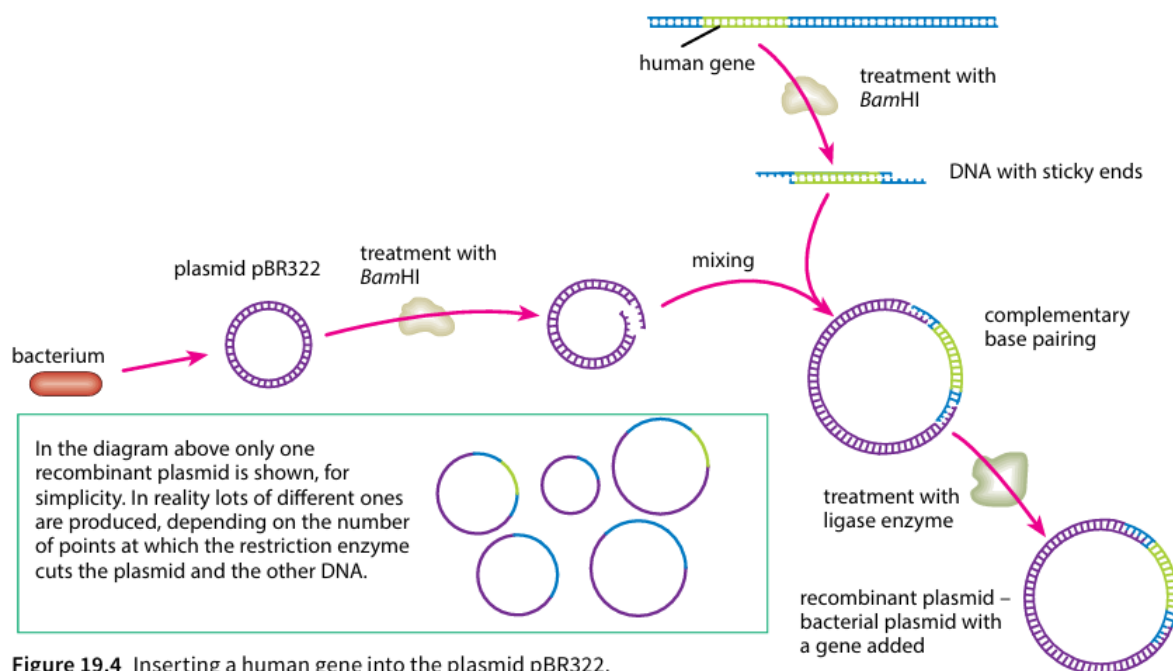
## Vectors

Inserting a gene into a plasmid vector In order to get a new gene into a recipient cell, a go between called a vector often has to be used. One type of vector is a plasmid (Figure 19.3). These are small, circular pieces of double-stranded DNA (page 114). Plasmids occur naturally in bacteria and often contain genes for antibiotic resistance.

The circular DNA of the plasmid is cut open using a restriction enzyme (Figure 19.4). The same enzyme as the one used to cut out the gene should be used, so that the sticky ends are complementary.



**Figure 19.3** Plasmid pBR322 was used in the production of human insulin.



**Figure 19.4** Inserting a human gene into the plasmid pBR322.

Bacterial plasmids can be modified to produce good vectors. Plasmids can also be made artificially. For example, the pUC group of plasmids have:

- ■ a low molecular mass, so they are readily taken up by bacteria
- ■ an origin of replication so they can be copied
- ■ several single target sites for different restriction enzymes in a short length of DNA called a polylinker
- ■ one or more marker genes, allowing identification of cells that have taken up the plasmid.

### Getting the plasmids into bacteria

The next step in the process is to get bacteria to take up the plasmids. The bacteria are treated by putting them into a solution with a high concentration of calcium ions, then cooled and given a heat shock to increase the chances of plasmids passing through the cell surface membrane.

### Identifying bacteria with recombinant DNA

It is important to identify which bacteria have been successfully transformed so that they can be used to make the gene product. This used to be done by spreading the bacteria on agar plates each containing an antibiotic.

### Insulin production

One form of diabetes mellitus is caused by the inability of the pancreas to produce insulin (Chapter 14). Before insulin from GM bacteria became available, people with this form of diabetes were treated with insulin extracted from the pancreases of pigs or cattle. In the 1970s, biotechnology companies began to work on the idea of inserting the gene for human insulin into a bacterium and then using this bacterium to make insulin.

They tried several different approaches, finally succeeding in the early 1980s. This form of human insulin became available in 1983. The procedure involved in the production of insulin is shown in Figure 19.5.

There were problems in locating and isolating the gene coding for human insulin from all of the rest of the DNA in a human cell. Instead of cutting out the gene from the DNA in the relevant chromosome, researchers extracted mRNA for insulin from pancreatic  $\beta$  cells, which are the only cells to express the insulin gene. These cells contain large quantities of mRNA for insulin as they are its only source in the body.

The mRNA was then incubated with the enzyme reverse transcriptase which comes from the group of viruses called retroviruses (Chapter 10, page 206). As its name suggests, this enzyme reverses transcription, using mRNA as a template to make single stranded DNA. These single-stranded DNA molecules were then converted to double-stranded DNA molecules using DNA polymerase to assemble nucleotides to make the complementary strand.



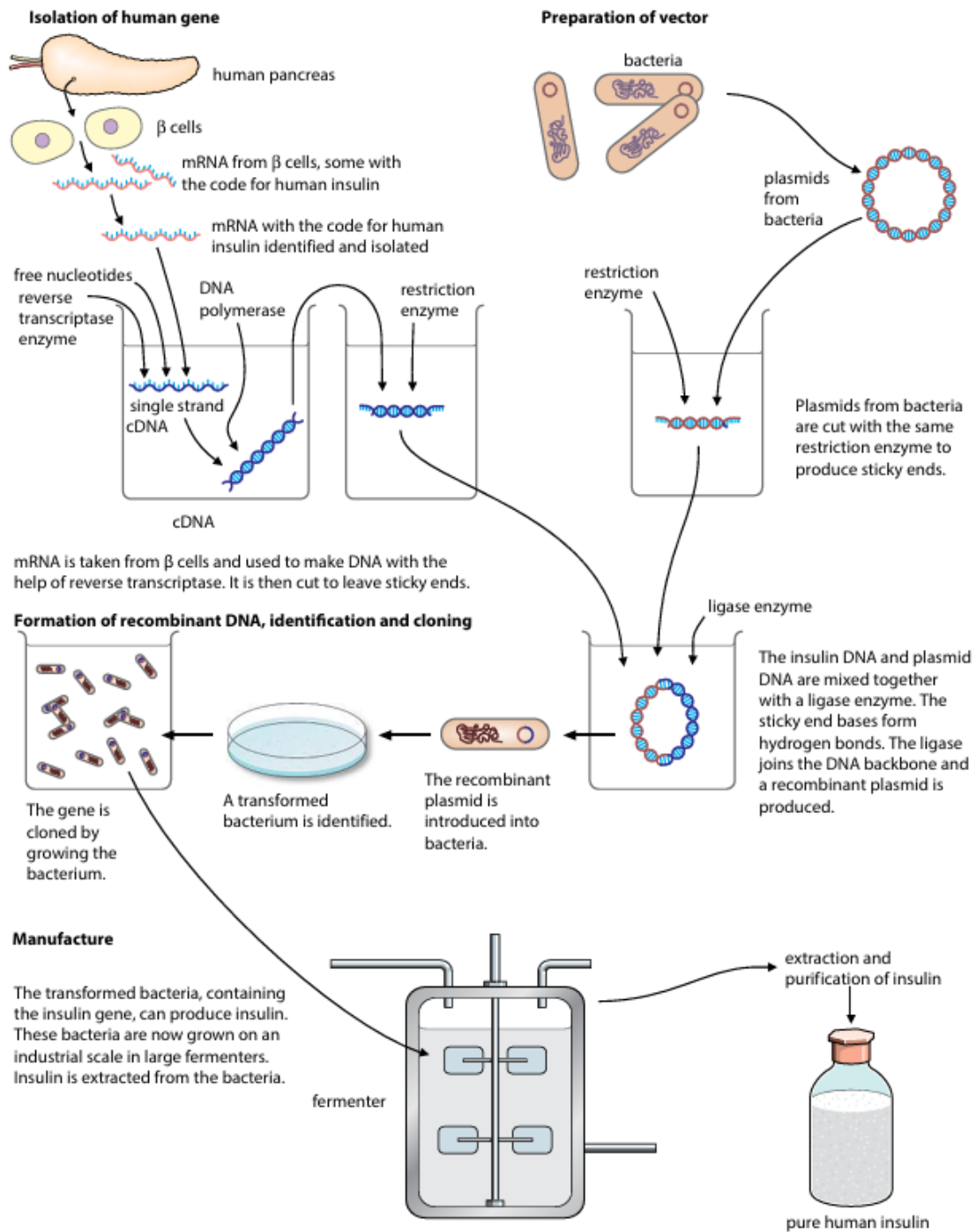


Figure 19.5 Producing insulin from genetically modified bacteria.

### Other genetic markers

Antibiotic resistance genes are being used as markers to identify bacteria that could spread to other bacteria, potentially leading to untreatable diseases. This is due to the risk of creating pathogenic bacteria. Instead, enzymes that produce fluorescent substances, such as GFP from jellyfish, can be used to identify the bacteria that have taken

up the plasmid. Another marker is the enzyme  $\beta$ -glucuronidase (GUS), which can transform cells into colored or fluorescent products when incubated with specific colorless or non-fluorescent substrates. This method is particularly useful in detecting the activity of inserted genes in plants.

### Promoters

Bacteria contain numerous genes that produce various proteins, but not all are activated simultaneously. They only produce proteins required in their growing conditions. The expression of genes is controlled by a promoter, a region of DNA where RNA polymerase binds. The insulin gene was inserted next to the  $\beta$ -galactosidase gene, and switched on when the bacterium needed to metabolize lactose. The promoter allows RNA polymerase to bind to DNA and recognizes the template strand. In eukaryotes, transcription factors are required to bind to the promoter region or RNA polymerase before transcription begins.

### Gel electrophoresis

Gel electrophoresis is a technique that is used to separate different molecules. It is used extensively in the analysis of proteins and DNA.

The movement of charged molecules within the gel in response to the electric field depends on a number of factors. The most important are:

- ■ net (overall) charge – negatively charged molecules move towards the anode (+) and positively charged molecules move towards the cathode (-); highly charged molecules move faster than those with less overall charge
- ■ size – smaller molecules move through the gel faster than larger molecules
- ■ composition of the gel – common gels are polyacrylamide for proteins and agarose for DNA; the size of the 'pores' within the gel determines the speed with which proteins and fragments of DNA move.

### Electrophoresis of proteins

The charge on proteins is dependent on the ionisation of the R groups on the amino acid residues. You will remember from Chapter 2 that some amino acids have R groups that can be positively charged ( $-\text{NH}_3^+$ ) and some have R groups that can be negatively charged ( $-\text{COO}^-$ ). Whether these R groups are charged or not depends on the pH.

### Electrophoresis of DNA

DNA fragments carry a small charge thanks to the negatively charged phosphate groups. In DNA electrophoresis, these fragments move through the gel towards the anode. The smaller the fragments, the faster they move. We will look at an example: the use of genetic profiling (fingerprinting) in forensic science. Figure 19.10 shows how genetic profiling is carried out.

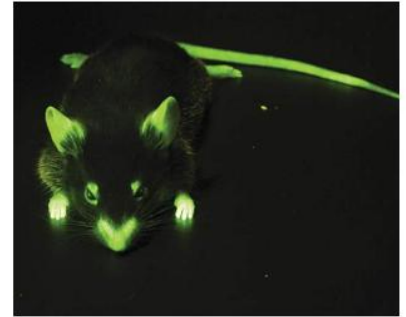


Figure 19.6 A transgenic mouse expressing a gene for a fluorescent protein.



Figure 19.7 Sundews are carnivorous plants that use sticky hairs to catch insects. On the left is a leaf of a transgenic sundew plant which is expressing the gene for GUS. The leaf has been placed in a solution of a colourless substance and the enzyme GUS has converted it into this dark blue colour. This indicates that the plant has been genetically modified successfully. On the right is a normal sundew leaf.

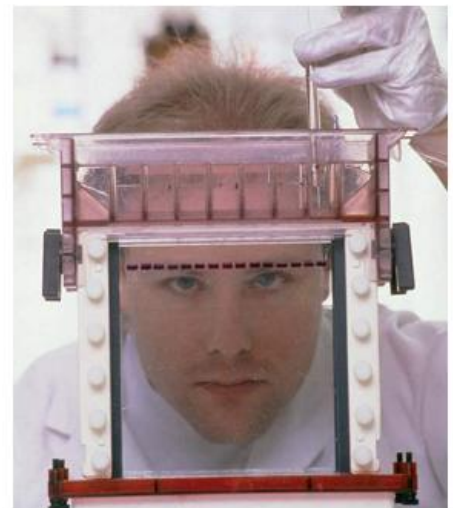
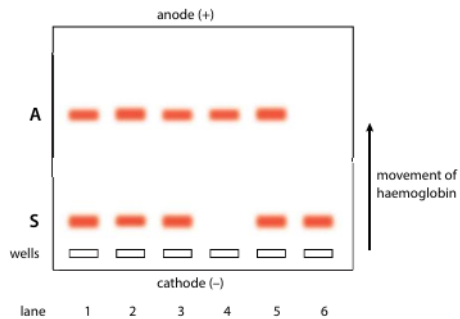


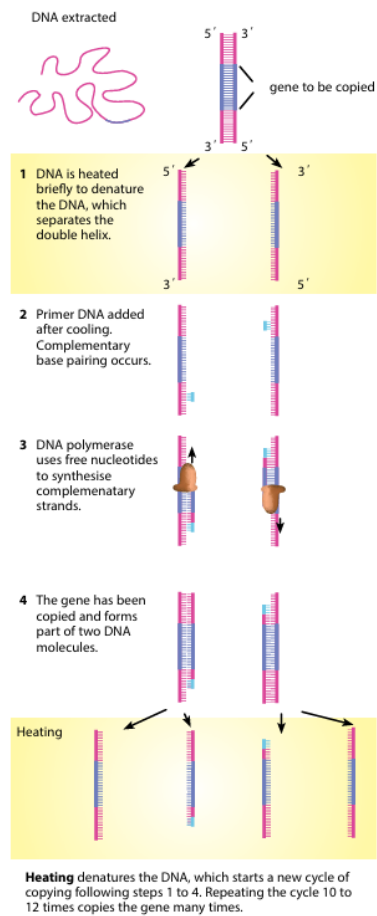
Figure 19.8 Gel electrophoresis of proteins. The gel was placed in the tank containing a suitable buffer solution. Protein samples stained red have been added to wells along the top of the gel. They are migrating downwards towards the anode.



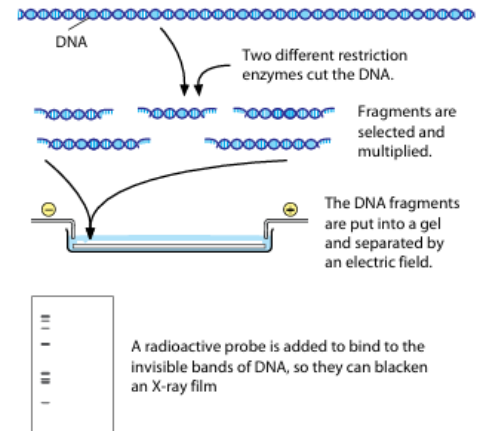
**Figure 19.9** Separation of haemoglobin by gel electrophoresis. This analysis was carried out on a family in which one child has sickle cell anaemia. Lane 1 contains haemoglobin standards, A = normal haemoglobin, S = sickle cell haemoglobin; lanes 2 and 3 are the haemoglobin samples from parents; lanes 4, 5 and 6 are haemoglobin samples from their children.

## Polymerase chain reaction

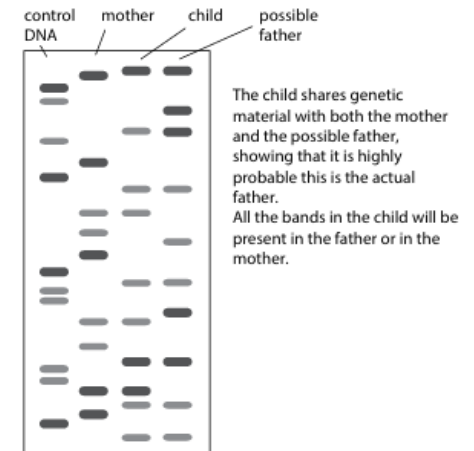
The polymerase chain reaction, generally known as PCR, is used in almost every application of gene technology. It is a method for rapid production of a very large number of copies of a particular fragment of DNA. Virtually unlimited quantities of a length of DNA can be produced from the smallest quantity of DNA (even one molecule).



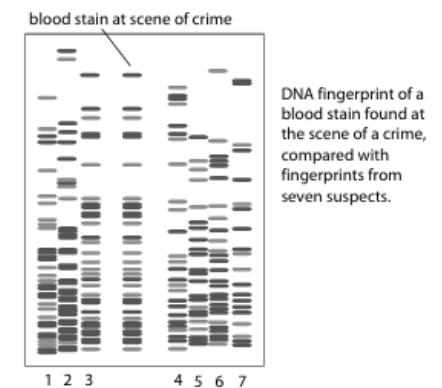
**Figure 19.13** The polymerase chain reaction.



**Figure 19.10** Genetic profiling.



**Figure 19.11** Using DNA profiling in paternity testing.



**Figure 19.12** Using DNA profiling in crime scene analysis.



The three stages in each round of copying need different temperatures.

- ■ Denaturing the double-stranded DNA molecules to make single-stranded ones requires a high temperature, around 95 °C.
- ■ Attaching the primers to the ends of the single stranded DNA molecules (known as annealing) requires a temperature of about 65 °C.
- ■ Building up complete new DNA strands using DNA polymerase (known as elongation) requires a temperature of around 72 °C. The DNA polymerases used for this process come from microorganisms that have evolved to live in hot environments.

### Microarrays

Microarrays are a valuable tool for studying genes in an organism's genome and identifying their expression within cells. They are based on a small piece of glass or plastic with short single-stranded DNA attached in a two-dimensional pattern. Researchers can search databases to find DNA probes for a wide range of genes. Automated processes apply probes to positions on the microarray, allowing for the analysis of genomic DNA. Microarrays can also be used to compare genes in two different species by collecting DNA fragments, denatured, and labeled with fluorescent tags. Hybridization occurs when DNA fragments are complementary to the probes, with green and red fluorescent spots indicating that one species has hybridized with the probes.

Microarrays detect genes expressed at specific times in cells, allowing for comparison of active genes. They convert mRNA into cDNA using reverse transcriptase, labeled with fluorescent tags, and hybridized with probes on the microarray. Spots on the microarray indicate genes being transcribed, and the intensity of light emitted indicates the activity level of each gene. This information is changing the way cancer treatments are treated, revealing the differences between cancer cells and non-cancerous ones.

### Bioinformatics

Research into the genes that are present in different organisms and the genes that are expressed at any one time in an organism's life generates huge quantities of data. As we have seen, one DNA chip alone may give 10 000 pieces of information about the presence and absence of genes in genomes or the activity of genes within cells.

Bioinformatics is a field that merges biological data with computer technology and statistics, creating databases that store gene sequences, complete genomes, amino acid sequences of proteins, and protein structures. These databases facilitate the collection and analysis of this vast amount of information, allowing access via the internet. Databases that specialize in specific types of information, such as DNA sequences and protein structures, are essential for this process. In 2014, these databases held over  $6 \times 10^{11}$  base pairs, equivalent to 200 human genome equivalents. Databases like Ensembl, UniProt, and BLAST help researchers compare primary biological sequence information and find similarities between sequences. Comparing genomes with other organisms, such as fruit flies, nematodes, and malarial parasites, allows researchers to explore the effects of genes and proteins on development.



Figure 19.14 A microarray, also known as a DNA chip.

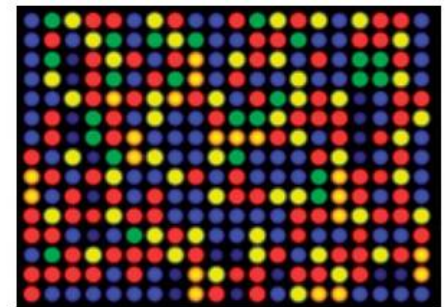


Figure 19.15 A DNA microarray as viewed with a laser scanner. The colours are analysed to show which genes or alleles are present.

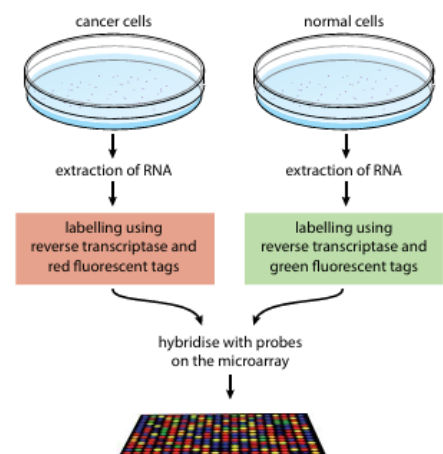


Figure 19.16 How to use a microarray to compare the mRNA molecules present in cancerous and non-cancerous cells. The results identify which genes in the cancerous cells that are not normally expressed are being transcribed.

## Genetic technology and medicine

Genetic technology allows products specific to humans to be made. We have already looked at the advantages of producing human insulin by recombinant DNA techniques (page 466). Other human proteins are produced by similar techniques – for example:

- ■ human growth hormone
- ■ thyroid stimulating hormone
- ■ factor VIII – a blood clotting protein.

Genetically modified hamster cells are used to produce factor VIII, a protein essential for blood clotting. The human gene for factor VIII is inserted into hamster kidney and ovary cells, which produce factor VIII. This protein is extracted and purified for treating haemophilia patients. The cabbage looper moth caterpillar also produces high yields of ADA, used to treat SCID.

Some proteins are even produced by transgenic animals. Sheep and goats have been genetically modified to produce human proteins in their milk:

- ■ human antithrombin is produced by goats – this protein is used to stop blood clotting
- ■ human alpha-antitrypsin is produced by sheep – this is used to treat people with emphysema

## Genetic screening

Genetic screening is the analysis of a person's DNA to check for a specific allele, which can be done in adults, fetuses, or embryos. For example, an adult woman with a family history of breast cancer may be screened for faulty alleles of genes Brca-1 and Brca-2, increasing her risk of developing breast cancer. In 1989, pre-implantation genetic diagnosis (PGD) was created.

## Gene therapy

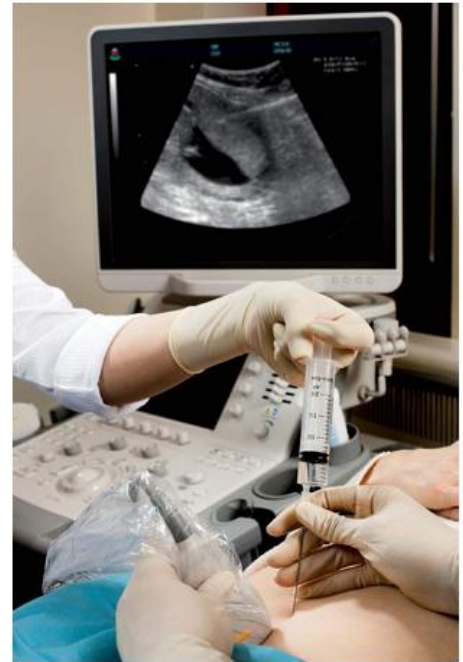
Gene technology and our rapidly increasing knowledge of the positions of particular genes on our chromosomes have given us the opportunity to identify many genes that are responsible for genetic disorders such as sickle **cell anaemia** and **cystic fibrosis**.

SCID is a genetic defect that prevents the immune system from producing the essential enzyme adenosine deaminase (ADA). To treat this, T-lymphocytes were removed and normal ADA gene alleles were introduced into them using a virus as a vector. However, this was not a permanent cure and regular transfusions were necessary. In 2000, four children with X-linked SCID developed leukemia due to using a retrovirus as a vector. Researchers have since used lentiviruses and the adeno-associated virus (AAV) as vectors, but these methods have limitations.

This work on vectors has led to increasingly successful gene therapies in the last few years, including the following.

- ■ The eyesight of young men with a form of hereditary blindness, Leber congenital amaurosis, in which retinal cells die off gradually from an early age, has been improved.
- ■ The normal allele of the  $\beta$ -globin gene has been successfully inserted into blood stem cells to correct the disorder,  $\beta$ -thalassaemia.

**Bioinformatics** is the collection, processing and analysis of biological information and data using computer software.



**Figure 19.17** In chorionic villus sampling, an ultrasound scanner is used to guide the needle to the placenta to remove a small sample of the fetal chorionic villi which are embedded in the placenta. A small sample of the fetal blood is removed for analysis.

■ Six people with haemophilia B (in which factor IX is missing) have at least seen their symptoms reduced.

■ ■ Five children were successfully treated for SCID in 2013. We will look in more detail at the genetic disorder, cystic fibrosis, to illustrate some of the problems facing gene therapy.

### Cystic fibrosis

Cystic fibrosis is a genetic disorder in which abnormally thick mucus is produced in the lungs and other parts of the body.

A person with cystic fibrosis is very prone to bacterial infections in the lungs because it is difficult for the mucus to be removed, allowing bacteria to breed in it.

This reduces the water potential below that of the cytoplasm of the cells. So water moves out of the cells by osmosis, down the water potential gradient. It mixes with the mucus there, making it thin enough for easy removal by the sweeping movements of cilia (Figure 19.19). However, in someone with cystic fibrosis, much less water moves out of the cells, so the mucus on their surfaces stays thick and sticky. The cilia, or even coughing, can't remove it all.

### The CFTR gene

The CFTR gene is found on chromosome 7 and consists of about 250 000 bases. Mutations in this gene have produced several different defective alleles. The commonest of these is the result of a deletion of three bases. The CFTR protein made using the code on this allele is therefore missing one amino acid. The machinery in the cell recognises that this is not the right protein and does not place it in the cell surface membrane.

### Somatic and germ cell gene therapy

Gene therapy involves introducing a 'correct' allele into a person's cells as a treatment for a genetic disease. So far, all attempts to do this in humans have involved placing the allele in body cells, otherwise known as somatic cells.

### Genetic technology and agriculture

#### Genetically modified plants

Proteins for use in medicine can be produced from genetically modified plants, so avoiding any problem of contamination by animal proteins. Examples include vaccines, albumin and the proteins found in breast milk that are used to treat diarrhoea in infants. However, the vast bulk of genetically modified plants grown around the world are crop plants modified to be resistant to herbicides, such as glufosinate and glyphosate, or crops that are resistant to insect pests.

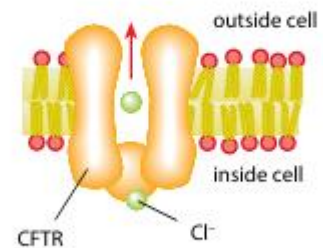
#### Herbicide-resistant crops

Oil seed rape, *Brassica napus*, is used in vegetable oil production, biodiesel fuel, lubricants, and animal food. A hybrid called canola is bred in Canada to produce low concentrations of undesirable substances. Gene technology has been used to produce herbicide-resistant strains, allowing fields to be sprayed after germination. Glyphosate,



**Figure 19.18** A person with cystic fibrosis is often treated with 'percussion therapy' – pummeling against the back to loosen the thick mucus so that it can be coughed up.

In a normal cell, the loss of chloride ions pulls water with it by osmosis. This keeps the surface moist and well lubricated.



**Figure 19.19** The CFTR protein forms channels for chloride ions in the cell surface membrane.



an herbicide, inhibits an enzyme involved in amino acid synthesis, causing plant death. Tobacco has also been made resistant to herbicides like sulfonylurea and dinitroaniline, using genes from other plant species.

The most likely detrimental effects on the environment of growing a herbicide-resistant crop are that:

- ■ the genetically modified plant will become an agricultural weed
- ■ pollen will transfer the gene to wild relatives, producing hybrid offspring that are invasive weeds
- ■ herbicide-resistant weeds will evolve because so much of the same herbicide is used.

### Insect-resistant crops

Another important agricultural development is that of genetically modified plants protected against attack by insect pests.

Insect-resistant tobacco also exists, and is protected against the tobacco bud worm, but as yet it has not been grown commercially.

The most likely detrimental effects on the environment of growing an insect-resistant crop are:

- ■ the evolution of resistance by the insect pests
- ■ a damaging effect on other species of insects
- ■ the transfer of the added gene to other species of plant.

Bt toxin, a toxin lethal to insects but harmless to other animals, has been taken from *Bacillus thuringiensis*. Crop plants with the Bt toxin gene produce insecticides, but insect populations can evolve resistance. In the USA, corn borers are resistant to Bt toxin, leading growers to plant up to 50% of their maize as non-genetically modified maize in "refuges." Bt toxin's pollen expresses the gene and disperses at least 60 meters by wind. Milkweed, a food source for monarch butterflies, is found in maize-growing areas. An experiment showed caterpillar survival after four days of pollen from Bt maize was 56%. However, further studies show this does not reflect the situation in the field. Bt maize may also pollinate its wild parent species, teosinte, transferring genes to it.

### Golden Rice

Rice is a staple food in many parts of the world. Where people are poor and rice forms the major part of their diet, deficiency of vitamin A is a common and serious problem. Vitamin A deficiency can cause blindness. The World Health Organization estimates that as many as 500 000 children go blind each year as a result of vitamin A deficiency. Even more importantly, lack of vitamin A can cause an immune deficiency syndrome, and this is a significant cause of mortality in some parts of the world, particularly in children.

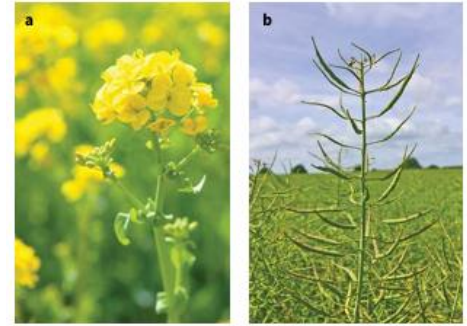


Figure 19.20 Oil seed rape a in flower and b in seed.



Figure 19.21 a Corn borer, b boll weevil.



**Figure 19.23** Normal rice on the left; Golden Rice on the right.

Genetically modified rice is being bred into other varieties to produce varieties with the same yield, pest resistance, and eating qualities as the original varieties. The International Rice Research Institute (IRRI) has collaborated with Bangladesh to create a pro-vitamin A enhanced ('Golden') variety of Bangladesh's most popular rice variety. Research in China has shown that Golden Rice may be useful as a source of vitamin A to overcome vitamin A deficiency in rice-consuming populations. Despite the research, Golden Rice is not yet available to farmers and consumers due to national authorities approval. With funding from the Bill and Melinda Gates Foundation, Golden Rice seed will be made available in developing countries at no greater cost than white rice seed.

### Genetically modified animals

Genetically modified animals for food production are much rarer than crop plants. An example is the GM Atlantic salmon, developed in the USA and Canada (Figure 19.24). A growth-hormone regulating gene from a Pacific Chinook salmon and a promoter from another species of fish, an ocean pout, were injected into a fertilised egg of an Atlantic salmon. By producing growth hormone throughout the year, the salmon are able to grow all year, instead of just in spring and summer.

### Social implications of using genetically modified organisms in food production

Some genetically modified plants are grown in strict containment in glasshouses, but a totally different set of problems emerges when genetically engineered organisms such as crop plants and organisms for the biological control of pests are intended for use in the general environment.

However, most objections are raised against the growth of herbicide-resistant or insect-resistant crops. The concerns about these genetically modified crops are as follows.

- ■ The modified crop plants may become agricultural weeds or invade natural habitats.
- ■ The introduced gene(s) may be transferred by pollen to wild relatives whose hybrid offspring may become more invasive.
- ■ The introduced gene(s) may be transferred by pollen to unmodified plants growing on a farm with organic certification.

Genes for the production of carotene were extracted from maize and the bacterium *Pantoea ananatis*.

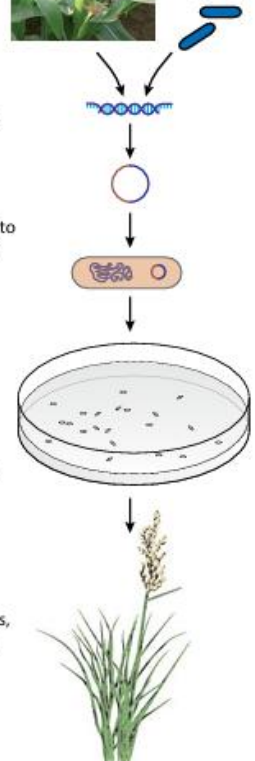


These genes, together with promoters, were inserted into plasmids.

The plasmids were inserted into bacteria called *Agrobacterium tumefaciens*.

These bacteria naturally infect plants and so could introduce the genetically modified plasmid into rice cells. They were mixed with rice embryos in Petri dishes, some of which were infected by the bacteria carrying the carotene genes.

The rice embryos, now containing the carotene genes, were grown into adult plants. They produced seeds containing carotene in their endosperm.



**Figure 19.22** Pro-vitamin A enhanced rice was engineered using genes from the maize and a bacterium.

- ■ The modified plants may be a direct hazard to humans, domestic animals or other beneficial animals, by being toxic or producing allergies.
- ■ The herbicide that can now be used on the crop will leave toxic residues in the crop.
- ■ Genetically modified seeds are expensive, as is herbicide, and their cost may remove any advantage of growing a resistant crop.
- ■ Growers mostly need to buy seed each season, keeping costs high, unlike for traditional varieties, where the grower kept seed from one crop to sow for the next.



**Figure 19.24** A GM salmon and non-GM salmon of the same age. Note that the GM fish do not grow larger than non-GM salmon, but attain their maximum size more quickly.

- In parts of the world where a lot of genetically modified crops are grown, there is a danger of losing traditional varieties with their desirable background genes for particular localities and their possibly unknown traits that might be useful in a world where the climate is changing. This requires a programme of growing and harvesting traditional varieties and setting up a seed bank to preserve them.

Despite concerns, millions of hectares of genetically modified crops and trees are growing worldwide. In the USA, half of cotton, maize, and soya crops were genetically modified in 2011. China, Brazil, and India are also using these crops. However, Europe has strict controls, and protesters have organized. There is little evidence of genes escaping into the wild, superweeds reducing crop growth, or toxic or allergenic foods. The effect on human societies may be small but positive.

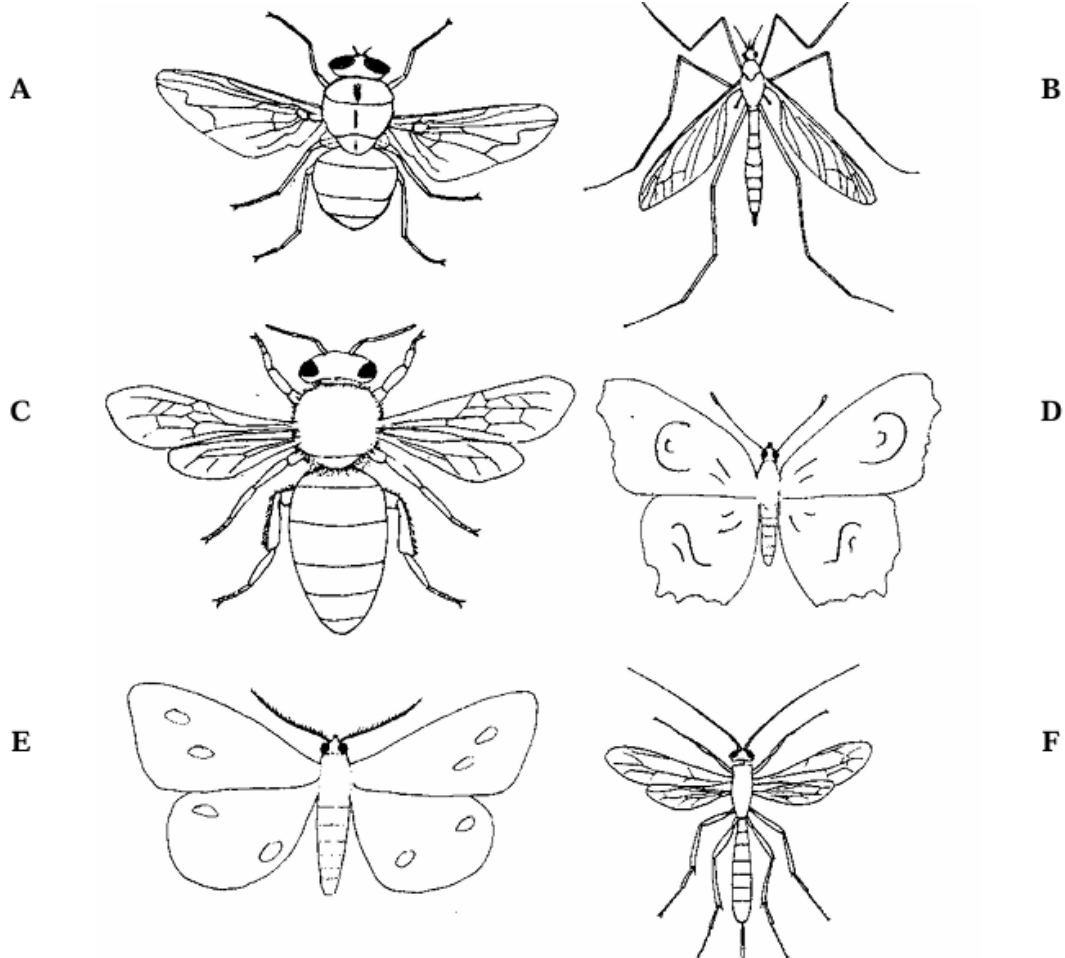


**Figure 19.25** This genetically modified maize, growing in Shropshire in the UK, is protected by an electric fence.



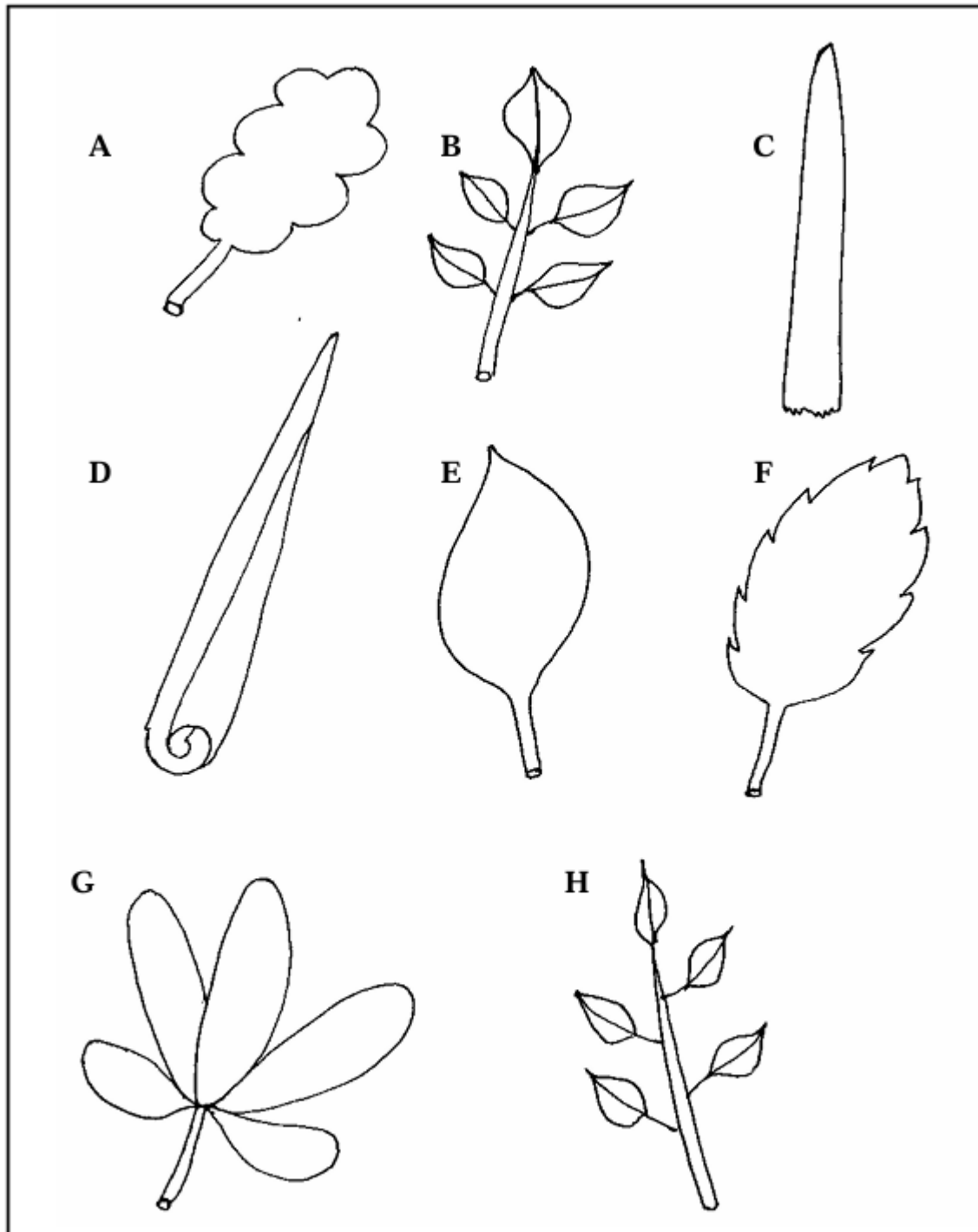
## Revision questions

1. The drawings illustrate the structure of six insects.

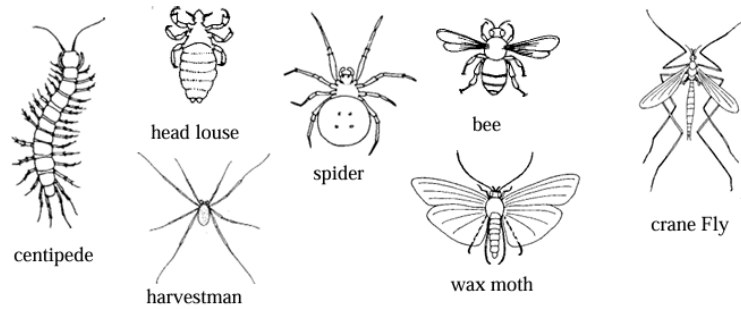


Make a simple dichotomous key which could be used to identify the six insects. Use only the following features in your key: number of abdominal segments, wing number, wing size and shape, body shape, antennae, leg length. It may not be necessary to use all these features in your key.

2. Leaves consist of a leaf base which joins the stem near an axillary bud, a petiole and a leaf blade or lamina. Leaves may be simple or compound, when they are divided into leaflets. Devise a dichotomous key which would distinguish the following leaves. Only use features that are visible in the drawings in your key.



3. The diagrams below are of seven different Arthropods.



- (a) (i) State a typical feature of Arthropods which is visible in all the organisms above.

..... [1]

- (ii) The phylum Arthropoda contains several classes, four of which are shown in the table below. Complete the table by naming the organisms above in the correct columns.

Insecta	Crustacea	Arachnida	Chilopoda (Myriapoda)

[ 3]

- (a) Devise a simple dichotomous key which would distinguish between the seven organisms. Only use features that are visible in the drawings.

- 4.

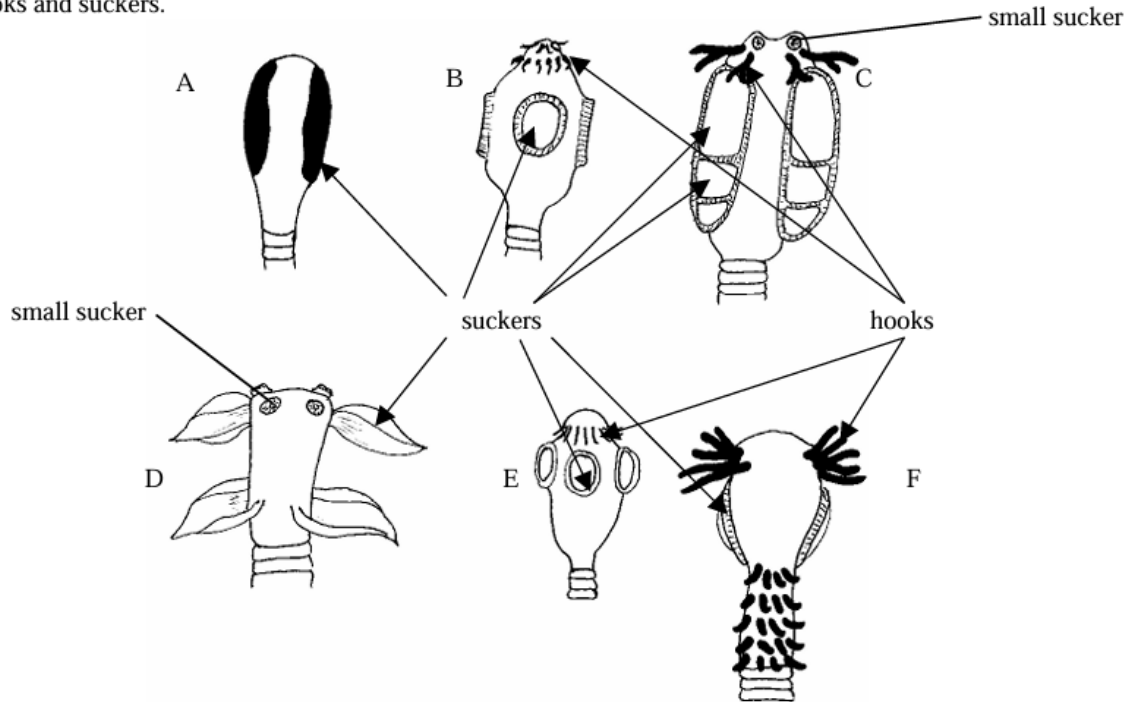
The table below refers to viruses, bacteria, algae and protozoa. If the statement is correct for the organism place a tick (✓) in the appropriate box and if a statement is incorrect, place a cross (X) in the appropriate box.

Feature	Viruses	Bacteria	Algae	Protozoa
Cannot reproduce independently				
Are heterotrophic				
Can cause diseases				
Contain DNA or RNA but not both				
Can photosynthesise				



5.

The drawings below illustrate the heads (scolices) of six tapeworms which show differing arrangements of hooks and suckers.



(a) What are the functions of the suckers and hooks?

(b) Devise a dichotomous key which would distinguish the six species of tapeworms shown above. Only use features which you can see in the drawings

6. (a) List two features shown by a bacterium such as *Escherichia coli* and a cyanobacterium, such as *Nostoc*, which place them both in the kingdom Prokaryotae.

(b) List two features shown by *Mucor* (pin mould) and a mushroom which place them both in the kingdom Fungi.

(c) List two features shown by a moss and a pine tree which place them both in the kingdom Plantae

(d) Suggest two features which distinguish monocotyledonous plants from dicotyledonous plants.

(e) Why are fishes, frogs and elephants classed together in the phylum Chordata?

(f) Why are dolphins, bats, weasels, seals and humans placed together in the class Mammalia?

7.

Read through the following account of genetic engineering and then fill in the spaces with the most appropriate word or words.

During the process of hormone manufacture by genetic engineering, human RNA is extracted and converted to single stranded DNA by treatment with ..... This is then treated with ..... to produce double stranded (double helix) DNA. Plasmid DNA is also extracted from suitable bacteria for use as a ..... The human and plasmid DNAs are then treated separately with ..... which cuts them into fragments which have the same complementary ..... The fragments of human and plasmid DNA are then mixed with ..... This joins the two types of DNA together as ..... DNA which will hopefully contain the gene required for hormone synthesis. The plasmids are then mixed with host bacterial cells, such as cells of ..... The presence of the chemical ..... aids the plasmid uptake by the bacteria. The bacteria can then undergo large scale culture and should produce suitable quantities of the required hormones. Hormones made in this way are.....and

8. Recombinant DNA products can be made either from genetically modified cloned cells such as bacteria or from genetically modified mammalian cells in tissue culture.

(a) Name three recombinant DNA products that are manufactured for medical use

(b) Suggest three advantages of producing genetically modified products from cloned bacterial cells rather than from tissue cultures of mammalian cells.

(c) Recombinant DNA products can also be produced in transgenic animals. For instance, alpha-1 antitrypsin can be produced by transgenic sheep. Suggest an advantage of producing a recombinant DNA product from a sheep rather than from a bacterial culture.