19. Genetic Technology

19.1 Principles of Genetic Technology

Paper 4

Question Paper

1 The gene *BRCA1* is expressed in breast tissue and in several other tissues of the body. *BRCA1* codes for a tumour suppressor protein. This protein is involved in either repairing damaged DNA, or in triggering the death of a cell if DNA cannot be repaired.

Some mutations in *BRCA1* are associated with an increased risk of developing breast cancer.

(b) DNA sequencing is one method used to test for mutations in *BRCA1*. Before DNA sequencing occurs, the DNA sample goes through the polymerase chain reaction (PCR).

Suggest why PCR is used for testing for mutations in *BRCA1*.

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2 White-clawed crayfish*, Austropotamobius pallipes*, live in rivers and lakes in Europe.
In the 1850s, the North American signal crayfish, *Pacifastacus leniusculus*, was introduced to Europe. The introduced species carried a pathogen that causes a disease known as crayfish plague. This disease kills *A. pallipes*.
Since 1850, the population size of *A. pallipes* has reduced in many areas of Europe due to the spread of crayfish plague.

(b) It is difficult to locate crayfish because they live underwater.
After an outbreak of crayfish plague in one country in Europe, researchers used an environmental DNA technique to find the locations in a river where populations of *A. pallipes* were still surviving.
Water samples were taken from locations along the river. Each water sample was filtered to obtain any cells or DNA that had been released by organisms into the water. The polymerase chain reaction (PCR) was carried out on this DNA using primers specific to *A. pallipes* DNA.

(i) Outline **and** explain the steps that occur in PCR.

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(ii) Suggest how the researchers were able to discover suitable sequences for the primers.

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(c) The PCR technique used in this research is quantitative. A fluorescent dye binds to the DNA and the fluorescence is monitored throughout the process, showing the quantity of DNA present.

Fig. 4.1 shows standard curves for four known concentrations of DNA, **A**, **B**, **C** and **D**, and the result obtained for *A. pallipes* DNA at one location along the river (dotted line).

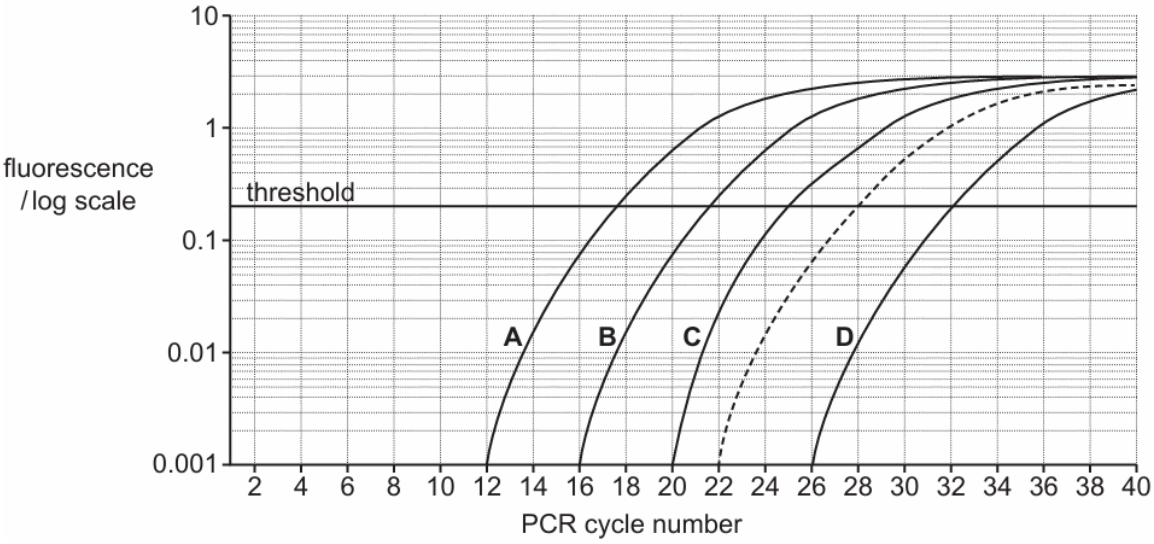


Fig. 4.1

(i) State the letter of the curve with the highest starting concentration of DNA.

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(ii) The quantity of *A. pallipes* DNA reaches a threshold (marked as a horizontal line on Fig. 4.1) at 28 cycles. The DNA in curve **C** reaches the threshold after 25 cycles. In one cycle of PCR the concentration of DNA doubles.

Calculate the relative difference in the starting concentrations of *A. pallipes* DNA and the DNA in curve **C**.

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(d) Fluorescent molecules have other uses in gene technology.

Fig. 4.2 shows an agar plate with normal bacteria and genetically modified bacteria. The genetically modified bacteria make a green fluorescent protein.

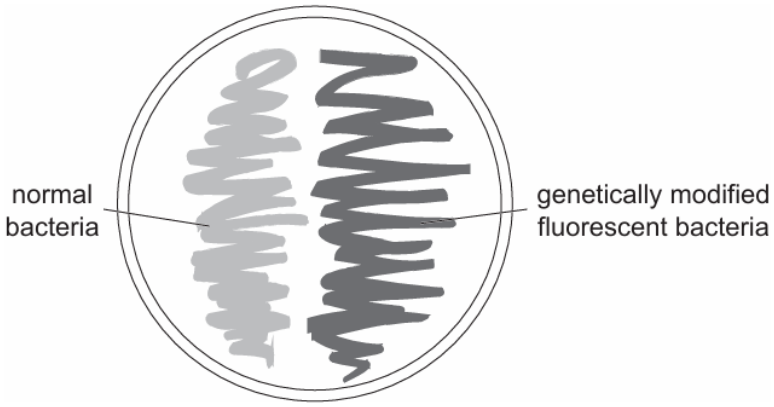


Fig. 4.2

(i) Identify the component that is taken up by normal bacteria to produce the genetically modified bacteria in Fig. 4.2 **and** state what this component contains to allow the bacteria to make green fluorescent protein.

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(ii) Suggest why the gene for green fluorescent protein is sometimes transferred in addition to the desired gene in genetic engineering.

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3 The treatment of many diseases has been improved by the use of genetic technology in medicine.

(a) Explain what is meant by genetic engineering.

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(c) LCA10 is a different form of LCA caused by a recessive mutation in the *CEP290* gene. This gene codes for the protein CEP290, which is involved in the correct functioning of photoreceptor cells in the retina.

The mutation in *CEP290* causes an error to be made when the primary transcript is spliced to form messenger RNA (mRNA). The abnormal mRNA that is formed has an extra sequence of RNA nucleotides, known as exon X, between exon 26 and exon 27. Exon X contains a STOP codon.

Fig. 3.1 compares the effect of the mutation in *CEP290* with the normal gene expression.

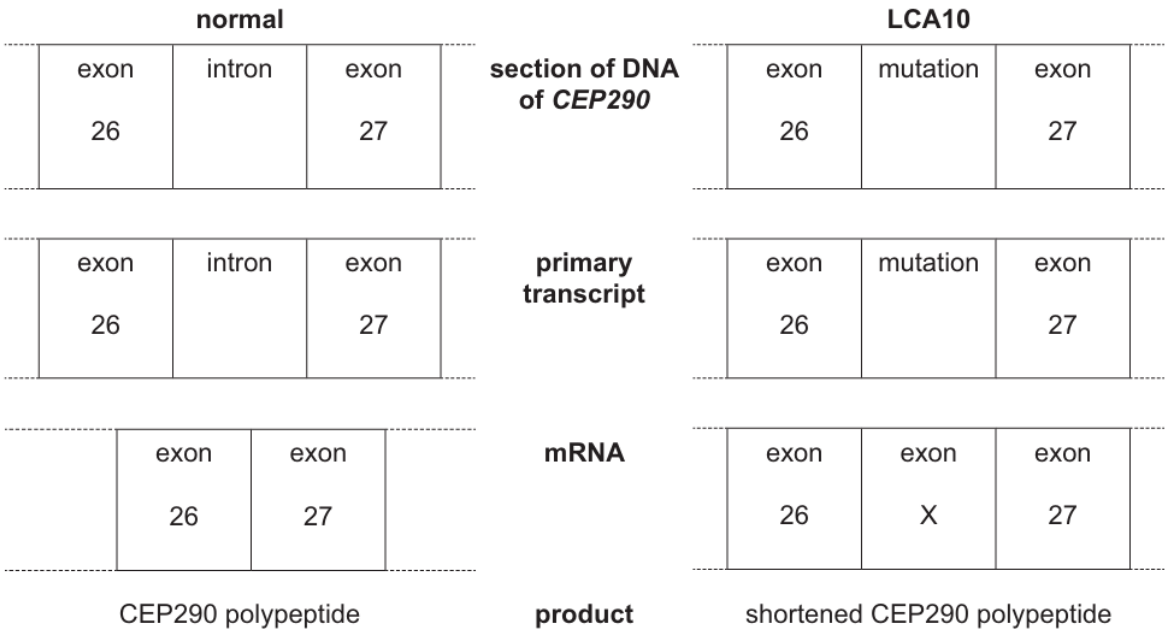


Fig. 3.1

In 2022, research was carried out into possible treatment of LCA10 using genetic technology.

(ii) Another method being investigated to treat LCA10 is to use a gene editing tool known as the CRISPR/Cas9 system.

The CRISPR/Cas9 system uses a short length of RNA called guide RNA. Guide RNA is complementary to the target DNA and is linked to a nuclease enzyme called Cas9. Cas9 breaks phosphodiester bonds in DNA.

The cell repair mechanisms repair the cut in DNA after the modification has taken place.

- A vector delivers Cas9 and two specific guide RNAs to the photoreceptor cells.
- They act on the section of DNA which contains the mutation.
- Exon X is no longer added to the mRNA.

Explain how this method used to treat LCA10 is an example of gene editing.

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4 Scientists use many different techniques in genetic engineering.

(a) Sometimes the gene for genetic engineering cannot be extracted from the donor organism. Instead, the gene is synthesised using one of two different methods.

Outline the **two** methods for synthesising a gene for use in genetic engineering.

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(b) DNA ligase and DNA polymerase are two enzymes that are used in genetic engineering.

Complete Table 8.1 to show the roles of DNA ligase and DNA polymerase in genetic engineering.

Use a tick (✓) if the enzyme has the role or a cross (✗) if the enzyme does **not** have the role.

Table 8.1

role in genetic engineering	DNA ligase	DNA polymerase
joins two sections of sugar phosphate backbone in DNA		
adds a gene to a plasmid		
adds free activated DNA nucleotides to a polynucleotide		

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(c) The polymerase chain reaction (PCR) is used to make many copies of a gene.

Three temperatures are used in a PCR cycle.

State the three temperatures that are used, **and** outline what happens at each temperature during a PCR cycle.

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5 Genetic technology uses many different enzymes and techniques.

(b) Originally, the method used to obtain a restriction endonuclease was to:
- grow large numbers of the specific prokaryotic cells that are the source of the enzyme
- break open the cells and extract and purify the restriction endonuclease.

This original method produced only a small quantity of restriction endonuclease and was **not** economical.

The newer method for large-scale production is to:
- obtain the gene coding for a specific restriction endonuclease
- introduce the gene into *Escherichia coli*, with a promoter that allows the gene to be expressed continuously.

The newer method increases the quantity of specific restriction endonuclease produced.

Suggest **and** explain the steps needed to carry out the newer method for large-scale production of a specific restriction endonuclease.

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(c) Describe the advantages of databases for the study and use of restriction endonucleases.

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(d) Electrophoresis is a technique used in genetic technology.

Paper chromatography is a technique used to investigate the photosynthetic pigments found in chloroplasts.

Compare the similarities and differences between electrophoresis and chromatography.

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6 **(a)** Recombinant human proteins can be used to treat disease.

(i) Define the term recombinant DNA.

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(b) Insulin is composed of two polypeptide chains, the A chain and the B chain, that are linked by disulfide bonds.

Variations in amino acid sequence occur:

- in the insulin molecules of different animals
- in new versions of human insulin that have been engineered to control blood glucose concentration more effectively than normal recombinant human insulin. These new versions of human insulin are called analogues.

Table 4.1 shows the amino acid positions where variation occurs in different animal and human analogue insulin molecules. The dashes indicate a missing amino acid.

Table 4.1

amino acid position	type of insulin					
	human	**cow**	**pig**	**cat**	**short-acting analogue**	**long-acting analogue**
A8	threonine	alanine	threonine	alanine	threonine	threonine
A10	isoleucine	valine	isoleucine	valine	isoleucine	isoleucine
A18	asparagine	–	asparagine	histidine	asparagine	asparagine
A21	asparagine	asparagine	asparagine	asparagine	asparagine	glycine
B3	asparagine	asparagine	asparagine	asparagine	asparagine	–
B28	proline	proline	proline	proline	lysine	proline
B29	lysine	lysine	lysine	lysine	proline	lysine
B30	threonine	alanine	alanine	alanine	threonine	threonine
B31	–	–	–	–	–	arginine
B32	–	–	–	–	–	arginine

(ii) Suggest ways in which analogue insulin molecules can be produced by genetic engineering techniques.

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(c) Information about amino acid and nucleotide sequences is stored in computer databases.

Outline the advantages of using databases of nucleotide sequences to investigate evolutionary relationships between species.

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7 **(a)** A marker gene can be used when a gene of interest is introduced into a plant by genetic engineering.

Describe **and** explain how the use of a marker gene coding for a fluorescent product can show that the introduced gene of interest is being expressed in plants.

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(c) DNA microarray analysis is a technique used in genetic technology that involves fluorescence. A DNA microarray has single-stranded probes attached to its surface. These probes hybridise to the fluorescently tagged single-stranded DNA that is added.

(i) Explain why DNA hybridisation occurs between the probe DNA and the added DNA.

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(ii) A DNA microarray analysis can be used to identify the level of expression of some genes.

Describe **and** explain how the level of expression of some genes can be identified using the DNA microarray analysis technique.

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8 The potato plant, *Solanum tuberosum*, is an important food crop. Crop yield is reduced if the leaves of the plant are eaten by the larvae (immature stages) of the Colorado beetle, *Leptinotarsa decemlineata*.

Crop scientists used recombinant DNA technology to create two genetically modified (GM) varieties of potato plant. These plants produce proteins that are poisonous to insects.

- GM potato variety **A** contains two new genes, *SN* and *Bt*.
- GM potato variety **B** contains two new genes, *SN* and *OCII*.

The new varieties were tested by having a constant number of Colorado beetle larvae introduced to the plants at time 0 hours. The number of larvae that were alive after 24, 48 and 72 hours was recorded. The percentage of the larvae that had died in each time interval was calculated. This was repeated for potato plants that had not been genetically modified (non-GM).

Table 4.1 shows the percentage of Colorado beetle larvae that had died on the GM potato plant varieties and on non-GM potato plants.

Table 4.1

type of potato plant	percentage of Colorado beetle larvae that had died		
	24 h	48 h	72 h
GM potato variety **A**	50	93	100
GM potato variety **B**	37	70	93
non-GM potato	0	0	0

(a) (i) Suggest what is meant by recombinant DNA technology.

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9 Environmental conditions such as light intensity affect plant physiology.

(b) An experiment investigated how light intensity affected gene expression in kale, *Brassica oleracea sabellica*.

- Two groups of kale plants were grown, with one group in high light intensity and one group in low light intensity. All other conditions were standardised.
- After the same period of time, messenger RNA (mRNA) was extracted from each group of kale plants.
- The mRNA was used to produce cDNA.
- The cDNA was hybridised with probes for 89 621 kale genes on a microarray.
- The microarrays showed which genes were switched on (expressed) in each set of conditions.

The results showed that expression of 18% of the genes was affected by light intensity.

- 14% of the genes were switched on only in high light intensity.
- 4% were switched on only in low light intensity.

(i) State the name of the enzyme that produces cDNA from an mRNA template.

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10 Adenosine deaminase (ADA) deficiency is an immune system disorder caused by a recessive autosomal mutation.

Severe combined immunodeficiency caused by a lack of ADA is called ADA-SCID.

(a) Genetic engineering is used to make a recombinant human protein to treat people with ADA-SCID.

Outline the principles of genetic engineering.

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(b) In 2016 gene therapy to cure ADA-SCID was approved in Europe. The gene therapy involves three main steps.

- Blood (haematopoietic) stem cells are taken from the bone marrow of the person with ADA-SCID.
- The functional gene and its promoter are inserted into the blood stem cells.
- A single infusion (injection) of the gene-corrected cells is given to the patient.

(ii) Explain why a promoter has to be transferred as well as the desired gene.

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(c) The gene therapy technique used to cure ADA-SCID is **not** suitable for treating the genetic disease called Huntington's disease. A newer technique called gene editing could potentially be used instead to cure Huntington's disease.

Explain why gene editing is more suitable as a potential cure for Huntington's disease.

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11 In 1973 a technique for genetic engineering was used for the first time. Recombinant DNA was made using a plasmid and this was successfully transferred into an organism.

In 2012 a new technique for genetic engineering, called gene editing, was developed.

(a) Table 4.1 lists some statements about the two genetic engineering techniques.

Complete Table 4.1 to compare the original genetic engineering technique using a plasmid vector with the newer technique of gene editing. For each row, place a tick (✓) in the correct column if the statement applies and leave a blank if the statement does not apply.

Table 4.1

statement	genetic engineering using a plasmid	gene editing
It can produce a transgenic organism.		
It can modify the characteristics of an organism.		
It can delete unwanted DNA.		
It uses an enzyme that cuts DNA.		
It can use RNA to precisely locate the target gene.		

[5]

(b) Orange trees, *Citrus sinensis*, produce fruits that are an important food crop. The functional leaf area of orange trees may be reduced by the growth of citrus canker bacteria. These bacteria cause citrus canker disease.

Scientists used gene editing to develop two types of orange tree with different mutations (changes to the DNA). The mutant orange tree leaves showed resistance to citrus canker disease.

Fig. 4.1 shows the area of leaf with citrus canker disease in wild type (not gene edited) and gene edited orange tree leaves after they have been exposed to citrus canker bacteria.

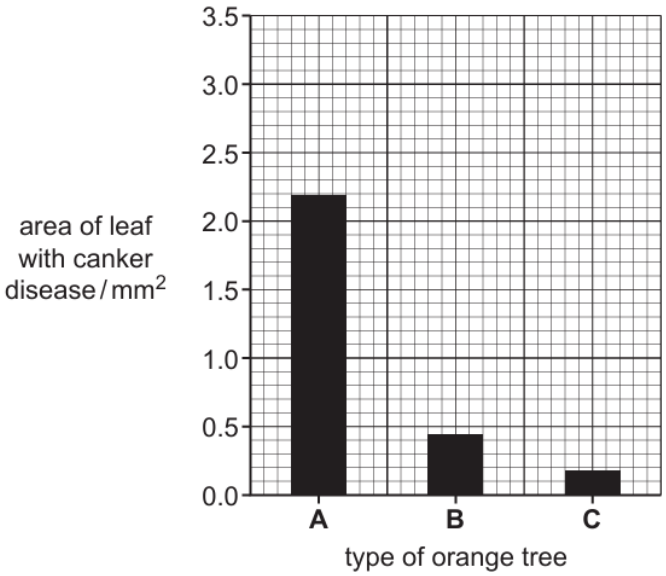


Fig. 4.1

(i) Identify the letter that represents the wild type orange trees on Fig. 4.1.

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12 In 1973, a technique for genetic engineering was used for the first time. Recombinant DNA was made using a plasmid and this was successfully transferred into an organism.

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Table 4.1

statement	genetic engineering using a plasmid	gene editing
It may cause the organism to produce a different protein.		
It may cause a single base pair in a gene to be changed.		
The success of the technique can be evaluated using marker genes.		
It may use the CRISPR system.		
It uses DNA ligase.		

[5]

13 Bison are a type of large wild cattle. Ancestors of modern bison appeared in Asia 2.5 million years ago. This ancestral bison species increased its range into Europe and North America.

While the ancestral species is now extinct, its descendants include *Bison bonasus*, the European bison, and *Bison bison*, the American bison.

Fig. 5.1 shows an American bison.



Fig. 5.1

(d) Outline how practical techniques could be used to test the hypothesis that farmed cattle are closely related to European bison.

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14 **(a)** Genetic engineering involves the manipulation of naturally occurring enzymes and processes.

List the enzymes used in genetic engineering (genetic modification) **and** outline their roles in natural processes.

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15 **(a)** Genome-wide association studies find links between single nucleotide polymorphisms (SNPs) and phenotypic features such as human diseases. SNPs are points on the DNA that vary in the population because of DNA base substitutions.

A genome-wide association study investigates the effect of genetic variation on a disease. A large number of people with the disease and a large number of healthy control individuals provide DNA. Microarray chips are used to identify the genotype of each individual at many SNPs.

The Wellcome Trust Case Control Consortium (WTCCC) study was an important genome-wide association study.

- The study used a microarray chip that identified each person's genotype at 500 000 different SNPs.
- The study looked for links between SNPs and 7 different diseases.
- For each disease, 2000 people with the disease were tested.
- Their results were compared with the results of 3000 healthy control individuals.

(i) Outline how microarrays are used in the analysis of genomes.

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(ii) Explain why bioinformatics was important to the WTCCC study.

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(b) Fig. 4.1 summarises results for three diseases in the WTCCC study. The 22 human autosomes and the X chromosome (chromosome 23) are shown.

Chromosome locations with SNPs that are associated with a disease at a statistically significant level (greater than 5 arbitrary units) are shown in black.

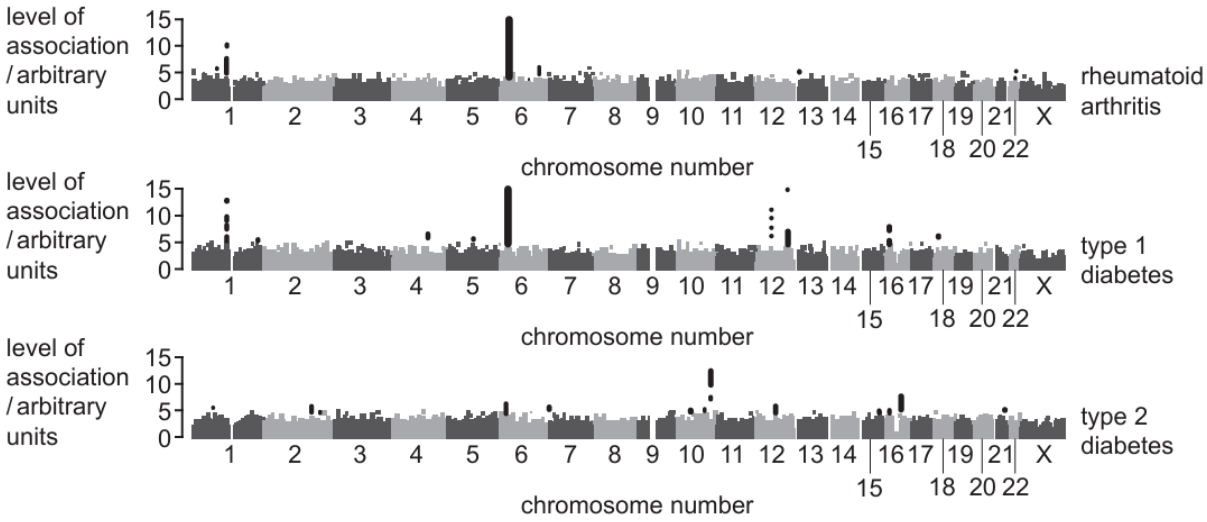


Fig. 4.1

(i) Identify the chromosomes that contain SNPs that have a high level of association with **both** rheumatoid arthritis and Type 1 diabetes.

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(ii) With reference to Fig. 4.1, compare the genetic basis of the three diseases.

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(c) Individuals can choose to have their DNA analysed on a microarray chip to predict their risk of developing different diseases.

Outline the social **and** ethical considerations of this type of DNA analysis.

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16 A subspecies is a genetically distinct population within a species that has some phenotypic differences from the rest of the species, but is not yet reproductively isolated.

Nine subspecies of the tiger, *Panthera tigris*, have been identified. Six of these subspecies are found on mainland Asia. Three of the subspecies originate from the Sunda Islands. These islands include Bali, Java and the large island of Sumatra.

Fig. 3.1 shows these three islands.

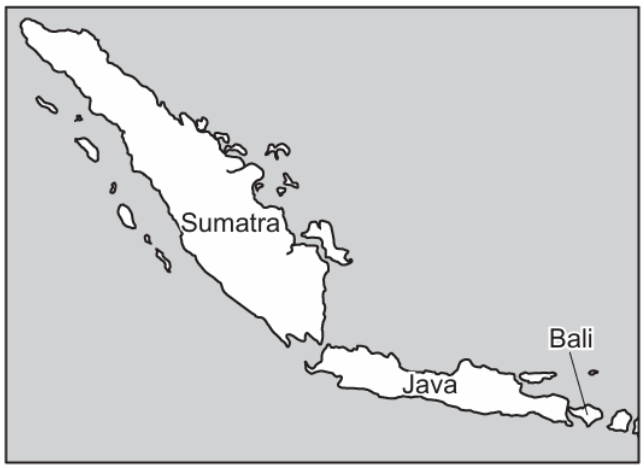


Fig. 3.1

- The Bali tiger, *Panthera tigris balica* (*P. t. balica*), became extinct in the 20th Century. The Bali tiger was found only on the island of Bali.
- The Javan tiger, *P. t. sondaica*, became extinct in the 20th Century. The Javan tiger was found only on the island of Java.
- The Sumatran tiger, *P. t. sumatrae*, lives only on Sumatra and is the closest living relative of Bali and Javan tigers.

20 000 years ago land bridges temporarily connected the Sunda Islands.

A recent study carried out a genetic analysis of the nine subspecies of tiger. Specific sections of mitochondrial DNA (mtDNA) that are useful in studies of evolution were amplified using PCR and compared to assess their evolutionary history.

- The source of DNA for the extinct subspecies came from museum specimens.
- mtDNA was extracted and polymerase chain reaction (PCR) carried out using primers based on specific sections of tiger mtDNA.
- The mtDNA sections for the three island subspecies were genetically distinct from the other six mainland subspecies.
- The mtDNA sections for the three island subspecies were all found to be very similar.

(b) Explain why specific primers were used for the tiger mtDNA sections.

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17 Yeast cells respond to changes in glucose concentration in their environment by using transcription factors to switch off genes.

When glucose is **present**:

- Mig1 transcription factors bind to the promoters of five genes
- Mig1 binding to the promoters stops (represses) transcription of these genes.

The genes that are repressed by Mig1 code for five enzymes that allow yeast cells to metabolise the sugar galactose when glucose is absent.

(b) Mig1 binds to promoter sites with these features:

- 17 base pairs long
- includes a region of five repeating adenine-thymine pairs
- includes a region of six repeating cytosine-guanine pairs.

Promoter sites to which Mig1 binds are known as Mig1-binding promoter sites.

Bioinformatic techniques were used to analyse the yeast genome to look for sections of DNA that match these features. The information obtained for four chromosomes is shown in Table 5.2.

Table 5.2

yeast chromosome	chromosome size / base pairs	number of Mig1-binding promoter sites per chromosome
A	230 018	1
B	813 184	10
C	316 620	2
D	1 531 933	14

(i) Explain why bioinformatic techniques were used to obtain the information in Table 5.2.

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(ii) Identify, with a reason, the yeast cell chromosome that is **most likely** to include genes that code for enzymes that metabolise galactose.

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(iii) Mig1 binds to 27 promoters on these four chromosomes. Yeast cells also have other chromosomes where Mig1 binds to additional promoters.

Five different enzymes, coded by five genes, must be made for yeast cells to metabolise galactose.

Suggest reasons why an individual diploid yeast cell has a larger number of Mig1-binding promoter sites than the expected number of ten.

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18 **(a)** In 1984, the geneticist Alec Jeffreys invented a DNA testing technique, known as DNA profiling, that produces a DNA banding pattern on a gel. The DNA banding pattern (profile) is unique to each individual.

DNA profiling can be used in police forensic work to catch criminals.

Since 1987, police in many countries have collected and stored DNA from crime scenes to create DNA profiles, which they try to match with the DNA profiles of criminal suspects.

(i) DNA at a crime scene may be obtained from hairs and traces of blood, semen and saliva.

Explain why PCR may be needed before DNA from a crime scene can be profiled.

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(ii) Explain why electrophoresis produces a DNA banding pattern on a gel.

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(b) GEDmatch is described as 'an open data personal genomics website'. It can be used by people who want to upload their DNA data to trace their ancestors and other relatives.

In 2018, police in the USA solved a large number of serious crimes. Some of these crimes had been unsolved for over thirty years. The police used GEDmatch to profile DNA taken from crime scenes and to look for matching DNA profiles. In many cases the police found partial matches to the relatives of criminals. This allowed the criminals to be identified and then charged on the basis of a complete DNA profile match.

(i) Suggest why the police strategy of comparing crime scene DNA with the GEDmatch database was so successful.

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(ii) Explain why GEDmatch is an example of bioinformatics.

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19 The patty pan squash plant, *Cucurbita pepo*, produces edible fruits that vary in colour.

(b) In 1994, two new varieties of squash were grown in the USA. Both varieties showed resistance to two viral diseases of squash plants.

- The variety 'Tigress' was developed by selective breeding.
- The variety 'Freedom II' was developed by adding genes for viral coat proteins to the squash genome.

(i) Identify, with reasons, the variety of squash that can be described as recombinant.

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20 Cats are members of the Felidae family. Two genera of Felidae are *Leopardus* and *Panthera*.

(b) Illegal trade threatens the survival of endangered species. Forensic tests can identify animal species from the DNA of their body parts, for example bones.

Cat species differ in the number of repeats of bases C and A (CA repeats) within one particular region of DNA, known as Ple46.

Table 3.1 shows the number of repeats in Ple46 for three endangered cat species.

Table 3.1

cat species	number of CA repeats in Ple46
Asiatic lion	22
Bengal tiger	7–8
leopard	14–15

(i) Gel electrophoresis is used to estimate the length of Ple46 in a sample of DNA to check whether the sample comes from one of the endangered cat species in Table 3.1.

Outline how gel electrophoresis is carried out to confirm whether the sample comes from one of these endangered cat species.

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(ii) Customs officials are responsible for checking whether animal body parts come from species on the CITES list of endangered species. Officials can now use a small DNA barcoding kit linked to a computer database to identify a species.

Suggest **two** advantages of using this method to identify a species.

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21 Sickle cell anaemia is a non-infectious chronic disease. If not treated, sickle cell anaemia can be painful and life-threatening.

Sickle cell anaemia is caused by a base substitution mutation in the gene coding for the β-globin polypeptide of haemoglobin. This leads to a change in the primary structure of the polypeptide, as valine is present instead of glutamine. This results in abnormal sickle-shaped red blood cells, which stick together in blood vessels.

Symptoms of sickle cell anaemia include painful attacks when red blood cells block capillaries in tissues and organs.

(b) Sickle cell anaemia is an autosomal recessive inherited disorder:

- allele Hb^A codes for the normal β-globin polypeptide
- allele Hb^S codes for the sickle-cell polypeptide.

People who are heterozygous (Hb^A Hb^S) have sickle cell trait (SCT). For a child to inherit sickle cell anaemia (Hb^S Hb^S), both parents must have SCT.

A genetic screening program is available for sickle cell anaemia and SCT:

- when a mother is screened and found to have SCT, the father is then screened
- if the mother becomes pregnant, the fetus is screened for both sickle cell anaemia and for SCT
- the test is done either by amniocentesis or by chorionic villus sampling, both of which carry a small risk of the pregnancy failing.

(ii) To test for the presence of Hb^S, DNA is extracted and the polymerase chain reaction (PCR) is carried out with two specific primers.

One mutation to produce Hb^S is a base substitution in the sixth codon of the β-globin gene. The normal codon GAG changes to GTG. The normal-specific primer detects GAG whereas the mutant-specific primer detects GTG.

Explain:
- why primers are used in PCR
- how the use of two specific primers allows the amplification of the normal, sickle cell anaemia and SCT genotypes.

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(iii) Gel electrophoresis is carried out on the products of the PCRs.

Fig. 4.1 includes the results for two individuals, **A** and **B**, tested for the sickle cell allele.

- Each lane has an 860 base pair (bp) band to indicate the test is valid.
- Lane 1 is a control lane with a 207bp band for an individual with known normal phenotype.
- Lane 2 is a control lane with a 207bp band for an individual with known sickle cell anaemia phenotype.
- Lanes 1, 3 and 5 contain DNA from the PCR that used normal-specific primer.
- Lanes 2, 4 and 6 contain DNA from the PCR that used mutant-specific primer.

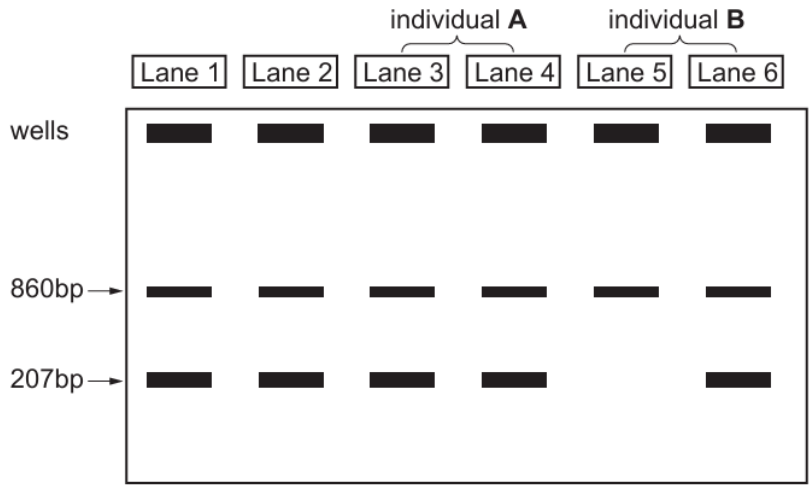


Fig. 4.1

Deduce the genotypes and phenotypes of individuals **A** and **B** in Fig. 4.1.

A .....

B .....

[2]

22 There are a number of mutations affecting the production of fetal haemoglobin, HbF, and normal adult haemoglobin, HbA.

- The Hb^A allele codes for the normal β-globin polypeptide of haemoglobin.
- The Hb^S allele, caused by a base substitution mutation, codes for an abnormal β-globin polypeptide.
- The base substitution results in the amino acid glutamine, which has a polar R group, to be replaced by valine, which has a non-polar R group, in the polypeptide.

The abnormal haemoglobin molecules (HbS) form fibres in low partial pressures of oxygen (pO_2). The fibres cause red blood cells to become sickle shaped and the cells can block blood capillaries.

Individuals with adult haemoglobin molecules that are all abnormal (HbS) have sickle cell anaemia. This is a painful chronic condition that can be life-threatening.

(c) Gel electrophoresis can be carried out to test individuals for the different versions of haemoglobin: HbA, HbS and HbF.

- A buffer with alkaline pH is used to make all haemoglobin molecules negatively charged.
- HbS molecules have an additional positive charge compared to HbA.

(i) Describe **and** explain how gel electrophoresis is used to diagnose sickle cell anaemia.

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(ii) Four individuals had their haemoglobin analysed by gel electrophoresis. One of the individuals was heterozygous for the Hb^A and Hb^S alleles and had a condition known as sickle cell trait (SCT).

Some of the results are shown in Fig. 4.1. In Fig. 4.1, lane 1 and lane 5 are complete.

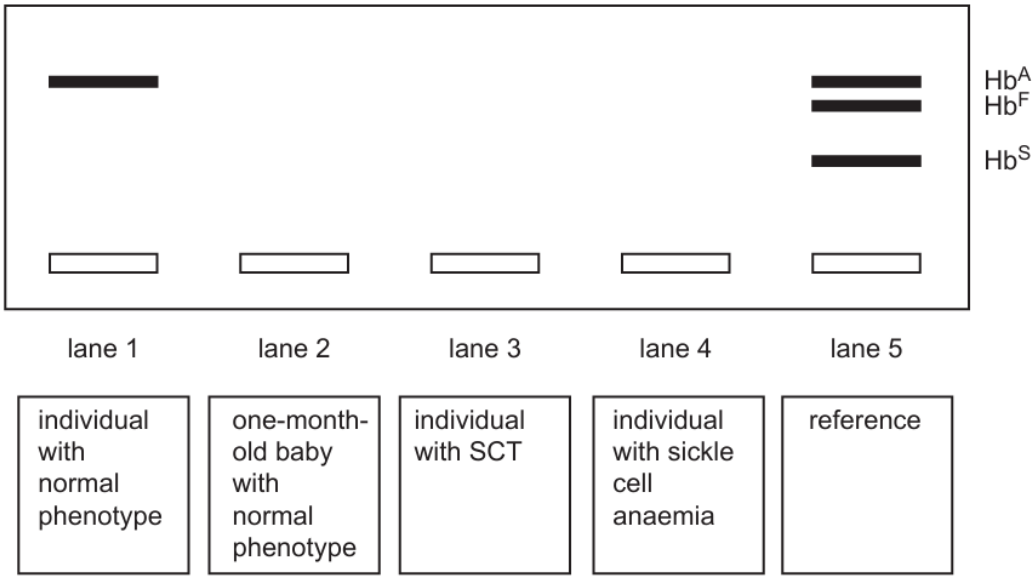


Fig. 4.1

Predict the results for the individuals analysed, by adding bands to lanes 2, 3 and 4 on Fig. 4.1. [2]

23 Therapeutic proteins are used to treat disease. The first purified therapeutic protein used was insulin, in 1922. The insulin was extracted from animal pancreases. Since 1982 most insulin has been made by recombinant DNA technology.

Calcitonin is a small protein hormone consisting of 32 amino acids. One of its functions is to inhibit the activity of cells, called osteoclasts, that break down bone tissue.

Calcitonin has been used as a therapeutic protein to treat osteoporosis. In osteoporosis too much breakdown of bone tissue in older people leads to reduced bone density and a risk of breaking bones.

(b) Fig. 3.1 shows the amino acid sequences of human calcitonin and calcitonin from the salmon fish, *Salmo salar*.

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
human	Cys	Gly	Asn	Leu	Ser	Thr	Cys	Met	Leu	Gly	Thr	Tyr	Thr	Gln	Asp	Phe
salmon	Cys	Ser	Asn	Leu	Ser	Thr	Cys	Val	Leu	Gly	Lys	Leu	Ser	Gln	Glu	Leu

position	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
human	Asn	Lys	Phe	His	Thr	Phe	Pro	Gln	Thr	Ala	Ile	Gly	Val	Gly	Ala	Pro
salmon	His	Lys	Leu	Gln	Thr	Tyr	Pro	Arg	Thr	Asn	Thr	Gly	Ser	Gly	Thr	Pro

Fig. 3.1

(i) The two amino acid sequences shown in Fig. 3.1 can be compared. The number of amino acids that occur at the same position in both sequences can be counted and expressed as a percentage of the total number of amino acids present in one sequence. This is called the percentage sequence similarity.

Use Fig. 3.1 to calculate the percentage sequence similarity of human and salmon calcitonin.

Show your working.

..... % [2]

(ii) Compared to human calcitonin, salmon calcitonin is more biologically active. It remains active in the human body for longer and binds to calcitonin receptors more readily.

Bioinformatics was used to identify this more biologically active form of calcitonin to treat osteoporosis.

Explain how bioinformatics helped identify salmon calcitonin as a suitable form of calcitonin to treat human osteoporosis.

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(c) Salmon calcitonin to treat osteoporosis is made by genetically engineered *Escherichia coli* bacteria. To produce these bacteria, a plasmid was cut and joined to the new gene to form a recombinant plasmid. The recombinant plasmid was then introduced into the bacterial cells.

(i) Name an enzyme that can:

- cut plasmid DNA

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- join the salmon calcitonin gene with plasmid DNA.

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(ii) Identify **and** explain two properties of plasmids that allow them to be used as vectors in gene cloning.

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(d) The gene coding for salmon calcitonin is introduced into bacteria in a specially designed plasmid called an expression vector. An expression vector must contain a prokaryotic promoter, such as the *lac* promoter.

Explain why differences in the control of gene expression in prokaryotes and eukaryotes mean that expression vector plasmids must contain a prokaryotic promoter.

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24 Steelhead trout, *Oncorhynchus mykiss*, are fish that live in streams in North America.

To increase the number of steelhead trout, captive breeding has occurred since 1992. Fish eggs and sperm are mixed and the young fish grow in large tanks of aerated water for the first year of their lives. Most are then released into the wild, however a few male and female fish are kept to become the parents of the next generation of captive-bred fish.

Each tank may hold up to 50 000 fish. The young captive fish are fed processed food. Some young fish are unable to survive these conditions and a proportion die. Death is usually the result of poor wound-healing after accidents due to overcrowding and due to the spread of diseases.

(c) (i) Describe how microarray analysis can detect differences in the expression of many genes when comparing two samples, such as the offspring of wild and captive-bred fish.

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25 Soybean, *Glycine max*, is an important food crop for human consumption and for feeding to animals.

Two varieties of soybean are Vinton 81 and GTS 40-3-2.

Vinton 81 has been developed in the traditional way by selective breeding (artificial selection) and GTS 40-3-2 is an example of a genetically modified (GM) organism.

(a) GTS 40-3-2 is described as herbicide-resistant, as it can withstand the application of glyphosate herbicide. Glyphosate is sprayed on the crop to kill weeds.

Outline how genetic engineering gave GTS 40-3-2 the trait of herbicide resistance.

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26 Maize, *Zea mays*, is an important food crop for human consumption and for feeding to animals.

Two varieties of maize are MON810 and Justina. Justina has been developed in the traditional way by selective breeding (artificial selection) and MON810 is an example of a genetically modified (GM) organism.

(a) MON810 produces a chemical that is toxic to insect pests. It is described as insect-resistant.

Outline how genetic engineering gave MON810 the trait of insect resistance.

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27 **(c)** In 2015 pigs were produced that had part of their genome altered by a new technique. The technique involved:

- an RNA sequence designed to bind to a specific targeted pig gene
- a gene-editing enzyme that is able to cut out sections of DNA.

The technique was used on pig zygotes that had been created by IVF. All the zygotes treated grew into piglets and these all showed large deletions in the targeted gene.

This gene coded for a specific cell surface membrane protein. The piglets did not express the protein and this gave them resistance to infection by a virus that causes a serious disease in pigs.

(i) Describe **two** advantages of the gene-editing technique compared to the traditional genetic modification technique used to make transgenic pigs in 1985.

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(ii) A scientist stated that this new technique is a form of selective breeding, so is not genetic engineering.

Discuss whether this statement is true **and** whether public groups who oppose transgenic animals will be more or less likely to accept the new technique.

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