

Q1.

- 1 Factor VIII is a glycoprotein synthesised in liver cells. Many haemophiliacs, who are deficient in Factor VIII, are now treated by regular injections of genetically engineered Factor VIII. Fig. 1.1 shows the molecular structure of Factor VIII.

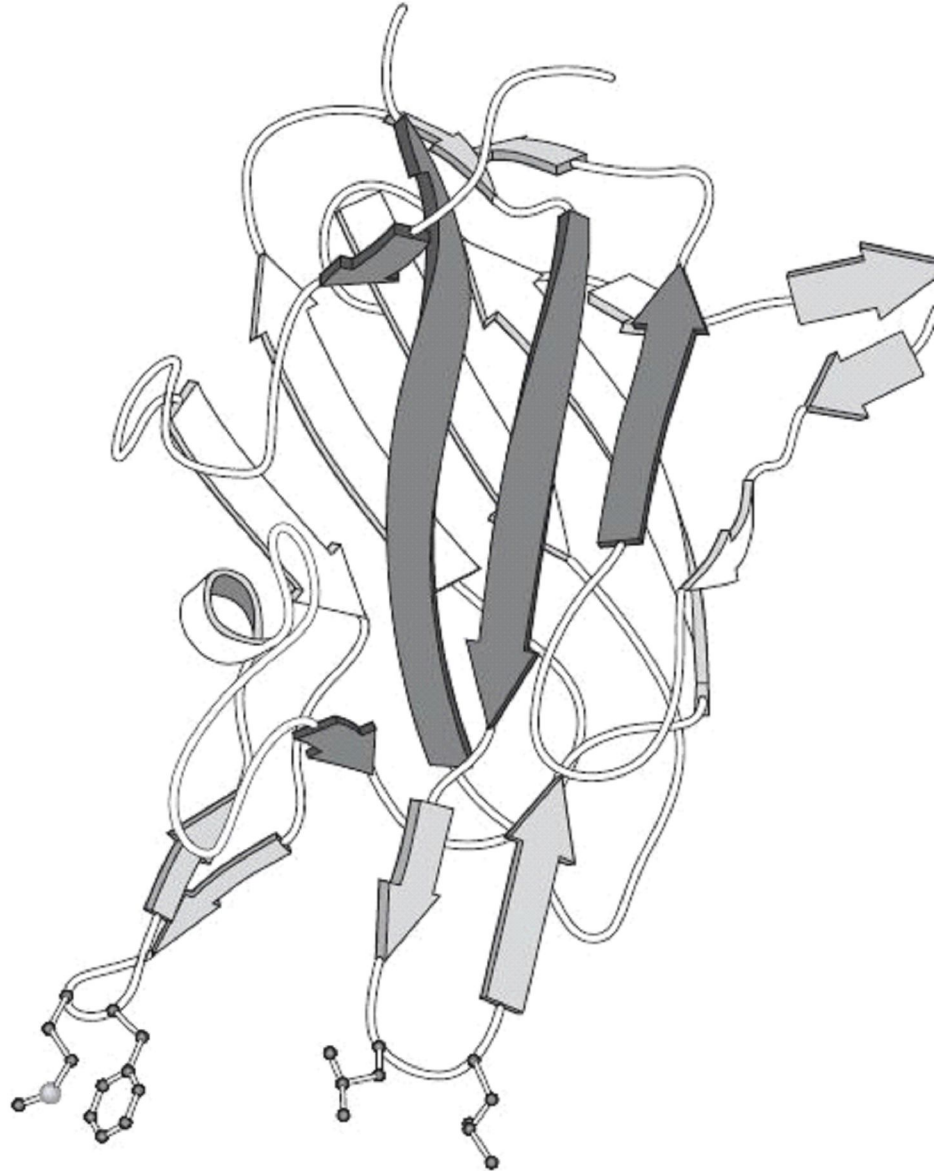


Fig. 1.1

Use

(a) Explain how the shape of the Factor VIII protein molecule shown in Fig. 1.1 is maintained.

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.....[3]

(b) Outline how the isolated gene for human Factor VIII is obtained and inserted into a host cell.

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.....[4]

(c) State **one** advantage of using recombinant Factor VIII instead of blood derived Factor VIII.

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.....[1]

(d) Suggest why the host cell used to produce genetically engineered Factor VIII must be a mammalian cell and not a bacterial cell.

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.....[1]

[Total: 9]

Q2.

(b) Fig. 4.1 shows some of the steps involved in the production of bacteria capable of synthesising human insulin.

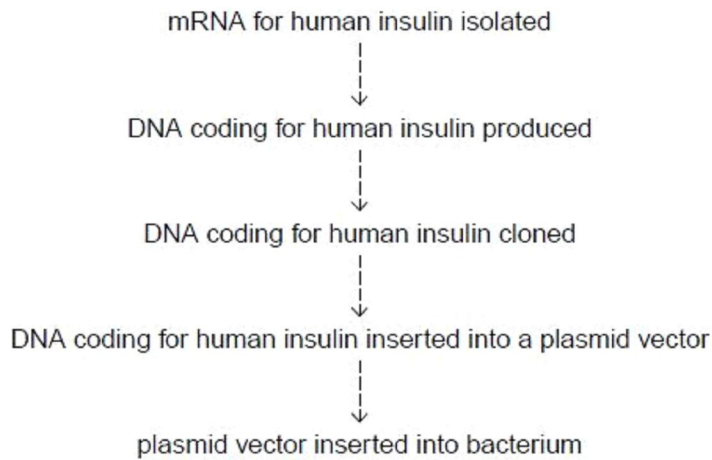


Fig. 4.1

State the role of each of the following enzymes in the production of bacteria capable of synthesising human insulin,

reverse transcriptase

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DNA polymerase

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restriction enzymes (restriction endonucleases)

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DNA ligase.

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[6]

Q3.

- (b) Cultivated maize is related to a wild Mexican grass, teosinte. Teosinte looks very different from maize. In particular, teosinte has a very hard layer surrounding the fruit, making it impossible to use as an edible grain.

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Archaeological excavations have found that maize in its edible form dates back at least 4000 years. It has been argued that the structural differences between teosinte and maize grains are so great that it is unlikely that maize could have been bred from teosinte so long ago.

Investigations have been carried out into the genetic differences between teosinte and maize. A gene on chromosome 4, *tga 1*, containing 1042 base pairs, was found to be responsible for all of the structural differences between teosinte and maize.

Fig. 3.2 shows the DNA base sequence of the only part of *tga 1* that always differs between teosinte and maize.

teosinte	GAT	TGG	GAT	CTC	AAG	GCG	GCG	GGC	GCG	TGG
maize	GAT	TGG	GAT	CTC	AAC	GCG	GCG	GGC	GCG	TGG

Fig. 3.2

- (i) Outline the principles of electrophoresis as used in sequencing this DNA.

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..... [4]

(ii) Suggest how the difference in the base sequence of the *tga 1* gene shown in Fig. 3.2 could cause large differences in phenotype between teosinte and maize. Ex

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..... [2]

(iii) With reference to Fig. 3.2, explain how these results support the suggestion that it would have been relatively easy for early farmers in Mexico to have bred maize from teosinte.

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..... [3]

Q4.

6 (a) Describe the role of insulin in the regulation of blood glucose concentration. Exa

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..... [3]

(b) State two advantages of treating diabetes with insulin produced by gene technology.

1

2

..... [2]

- (c) One of the steps in the production of bacteria capable of producing human insulin is the insertion of the gene coding for human insulin into a plasmid vector.

Fig. 6.1 shows one of the artificial plasmids constructed to act as a vector.

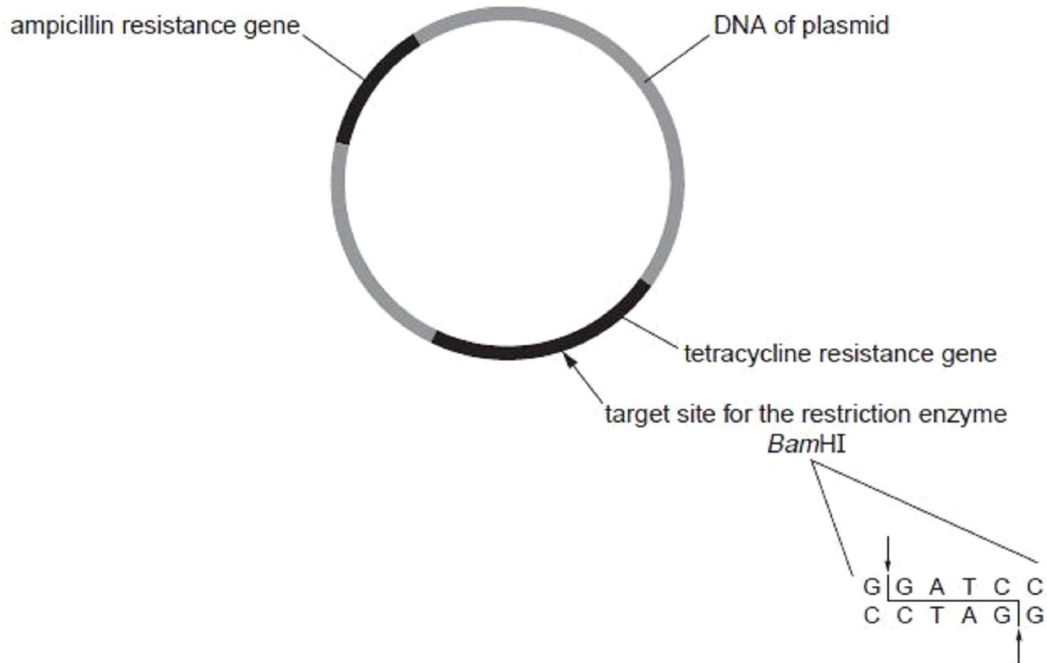


Fig. 6.1

- (i) With reference to Fig. 6.1, explain the importance of the plasmid having a single target site for a particular restriction enzyme, such as *Bam*HI.

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..... [2]

- (ii) The genes for ampicillin resistance and tetracycline resistance on the plasmid allow the genetic engineer to distinguish between bacteria that have taken up different circles of DNA.

Complete the table to show whether bacteria which have taken up each different circle of DNA are, or are not resistant to ampicillin, to tetracycline or to both. Show presence of resistance with a tick (✓) and absence of resistance with a cross (X).

circle of DNA taken up by bacteria	bacteria resistant to ampicillin	bacteria resistant to tetracycline
unaltered plasmids		
recombinant plasmids that have taken up the wanted gene		
circles of the wanted gene		

[3]

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(d) (i) Explain why genes for antibiotic resistance are now rarely used as markers in gene technology.

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..... [3]

(ii) Describe the use of **one** alternative marker gene that can be used instead of an antibiotic gene.

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..... [2]

[Total: 15]

Q5.

8 (a) Cystic fibrosis (CF) is an inherited disease.

(i) Explain briefly how two parents who do not have CF may have a child with CF.

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..... [2]

(ii) Describe **two** ways in which CF affects the lungs.

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..... [2]

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(b) One potential way of treating CF is by using gene therapy.

(i) Outline, with reference to CF, what is meant by *gene therapy*.

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.....
..... [2]

(ii) Describe one possible advantage and one possible disadvantage of using gene therapy to treat CF.

advantage

.....

disadvantage

..... [2]

[Total: 8]

Q6.

8 Gene technology has many uses including the production of substances such as insulin.

(a) (i) Outline what is meant by *gene technology*.

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..... [2]

(ii) Explain why genes for enzymes that produce fluorescent substances are used as makers in gene technology.

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..... [2]

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(b) There is much controversy throughout the world regarding the use of genetically modified (GM) crops.

(i) Suggest **two** advantages of growing GM rice with an enhanced vitamin A content.

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..... [2]

(ii) Suggest **two** disadvantages of growing GM crops.

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..... [2]

[Total: 8]

Q7.

3 Variable number tandem repeats (VNTRs) are repetitive, non-coding sections of DNA. A particular VNTR is located at the same locus in different individuals, but the number of repeats in that VNTR varies between individuals. E

(a) Explain how, in the process of genetic fingerprinting, gel electrophoresis is able to distinguish between the VNTRs that occur at the same loci of different individuals.

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..... [3]

(b) Gel electrophoresis is also used in genetic screening.

The mutation of the β -globin gene which gives rise to sickle cell anaemia removes a recognition site of a restriction enzyme, **R**, as shown in Fig. 3.1. **R** cuts DNA at the sites indicated by arrows (\downarrow). The lengths of the resulting fragments are shown in kilobases (kb).

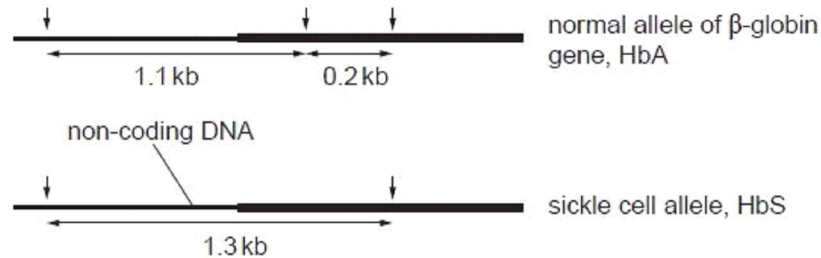


Fig. 3.1

Fig. 3.2 shows an electrophoresis gel with a stained band of DNA from an individual who was homozygous for the normal allele for β -globin, HbA HbA. This band is the 1.1 kb fragment shown in Fig. 3.1. The 0.2 kb fragment is **not** shown.

Complete Fig. 3.2 by drawing the stained DNA that would result from an individual who is heterozygous for the sickle cell allele, HbA HbS.

Put your answer on to Fig. 3.2.

[2]

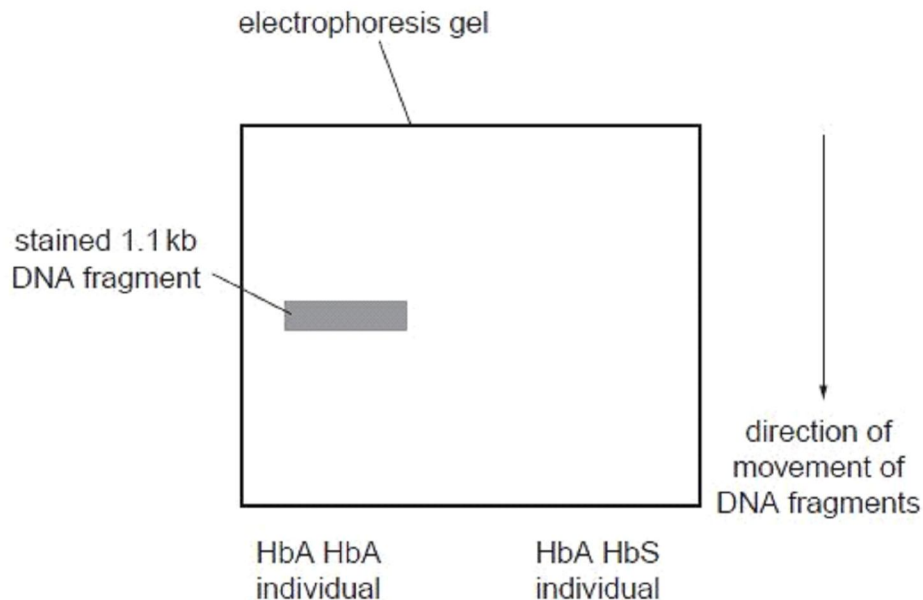


Fig. 3.2

(c) Describe the different circumstances in which this genetic screening for the sickle cell allele, HbS, might be used.

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[3]

[Total: 8]

Q8.

3 In order to sequence the DNA of a gene, it is first denatured to separate its two strands.

Then, in the presence of a large supply of each of the four nucleotides, the single-stranded DNA is replicated by DNA polymerase.

(a) Explain what determines the sequence of nucleotides in the newly replicated strand of DNA.

.....
.....
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[2]

Exa

(b) A low concentration of specially prepared nucleotides is also present. Once added to the chain, these nucleotides do **not** allow the chain to continue growing.

Each special nucleotide is labelled with a fluorescent dye, using a different colour for each of the four bases.

Fig. 3.1 shows a replicated DNA chain ending with one of the special nucleotides.

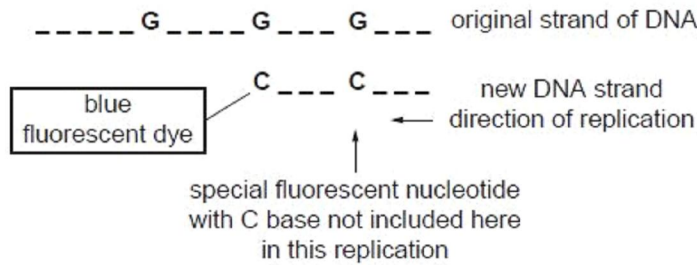


Fig. 3.1

With reference to Fig. 3.1 and to the information given, suggest why a special nucleotide with a C base was **not** included by DNA polymerase at the first site requiring a C nucleotide.

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.....[2]

- (c) This method of sequencing a gene produces as many DNA fragments as there are nucleotides in the gene, each fragment differing in length by one nucleotide.

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Fig. 3.2 shows part of a set of such fragments.

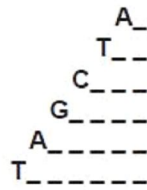


Fig. 3.2

These fragments are loaded onto a sequencing gel, shown in Fig. 3.3, and separated by electrophoresis.

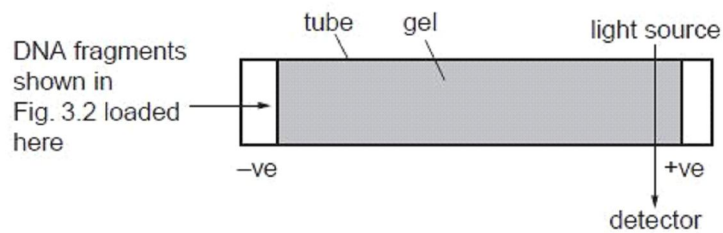


Fig. 3.3

(i) In what order will the fragments shown in Fig. 3.2 reach the light source and detector shown in Fig. 3.3?

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..... [1]

(ii) Explain how gel electrophoresis separates these fragments of DNA.

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..... [3]

[Total: 8]

Q9.

4 Golden Rice™ is a genetically modified form of rice that produces relatively large amounts of β carotene in the endosperm. β carotene is metabolised in the human body to produce vitamin A. Ex

(a) Explain why rice has been genetically modified to produce extra β carotene.

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..... [2]

- (b) The first types of Golden Rice™ produced only a very low mass of β carotene per gram of rice. Research continued to try to increase this.

Fig. 4.1 shows the metabolic pathway by which β carotene is synthesised in plants, and the enzymes that catalyse each step of the pathway.

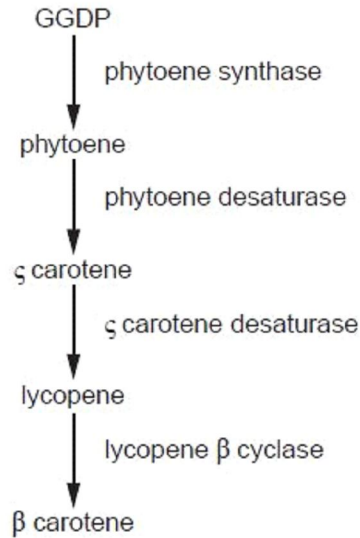


Fig. 4.1

The first types of Golden Rice™ contained a phytoene synthase gene, *psy*, from daffodils and a gene *crtI*, which produced the two desaturase enzymes, from the bacterium *Erwinia uredovora*.

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Measurements of the quantities of intermediates in this metabolic pathway in rice endosperm showed that there was always a large amount of GGDP present, and that no phytoene accumulated in the tissues.

Explain how this suggests it was **not** the enzymes produced by the *crtI* gene that were limiting the production of β carotene.

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..... [2]

- (c) Investigations were carried out to see if *psy* genes taken from species other than daffodils would enable rice endosperm to produce greater quantities of β carotene than the first types of Golden Rice™.
- *Psy* genes were isolated from the DNA of maize, tomatoes, peppers and daffodils. The genes were inserted into different plasmids.
 - The promoter *Ubi1*, and *crtI* genes from *E. uredoovora*, were also inserted into all of the plasmids.
 - The four types of genetically modified plasmids were then inserted into different cultures of rice cells.
 - The quantity of β carotene produced by these rice cells was measured.

The results are shown in Table 4.1.

Table 4.1

source of <i>psy</i> gene	total β carotene content of rice cells/arbitrary units
maize	14
pepper	4
tomato	6
daffodil	1

- (i) Name the type of enzyme that would have been used to cut the *psy* gene out of the DNA of the plant cells.

..... [1]

(ii) Explain why a promoter was inserted into the plasmids.

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..... [2]

(iii) Explain whether or not these results support the hypothesis that the *psy* gene, not the *crtl* gene, was limiting the production of β carotene in genetically modified rice.

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..... [2]

(d) The original choice of a *psy* gene from daffodils was made because daffodils produce large amounts of β carotene in their yellow petals, and because they are monocotyledonous plants, like rice.

Suggest explanations for the much lower production of β carotene in rice containing the *psy* gene from daffodils than in rice containing the *psy* gene from maize.

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..... [2]

(e) Describe the possible disadvantages of growing Golden Rice™.

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..... [3]

[Total: 14]

Q10.

- 2** A number of diseases, such as dengue fever, are spread by mosquitoes. The incidence of this disease has increased dramatically in recent years and this has been linked with the spread of the mosquito, *Aedes aegypti*. Ex

In an attempt to reduce the numbers of *A. aegypti*, genetically modified (GM) male mosquitoes were produced. One of the genes added to these mosquitoes, **when switched on**, results in the production of a protein which is toxic to mosquitoes.

In 2010, in the Cayman Islands and in Malaysia, GM male mosquitoes were released into the wild to mate with females. All the resulting offspring died in the larval stage.

- (a)** About 3 million GM male mosquitoes were released in the Cayman Islands.

Suggest why releasing such large numbers of male mosquitoes did not immediately increase the risk of transmission of dengue fever.

.....
..... [1]

- (b)** In Malaysia, both GM male and non-GM male mosquitoes were released in order to compare their dispersal and life span in the wild. The GM mosquitoes could be identified because they also carried a gene for green fluorescent protein (GFP).

Explain why, in many examples of gene technology, fluorescent markers are used in preference to antibiotic resistance genes.

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..... [2]

- (c) In addition to the gene for GFP, the DNA that has been added to the GM mosquitoes consists of
- a promoter
 - a gene coding for a toxic protein, tTA
 - a binding site for tTA.

When a GM mosquito larva hatches from an egg, the promoter induces the production of only a small amount of tTA, so that the larva does not die immediately. In a process of positive feedback, the tTA produced binds to the DNA as shown in Fig. 2.1. This increases the expression of the gene until the increased concentration of tTA kills the larva.

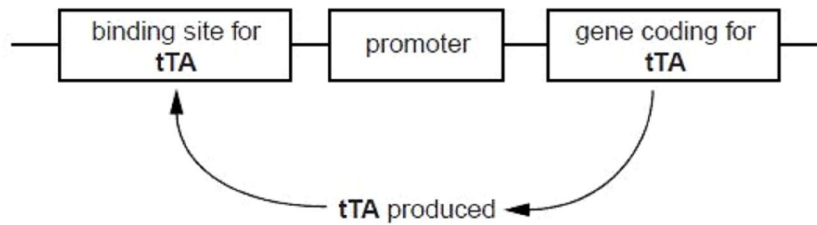


Fig. 2.1

- (i) Suggest why this process is called *positive feedback*.

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..... [1]

- (ii) Explain why, in gene technology, a promoter needs to be transferred along with the desired gene.

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..... [3]

(iii) Switching on the gene coding for tTA in the mosquito larvae, rather than in the eggs, increases the effectiveness of this method of controlling mosquito numbers. Ex

Suggest why this is so.

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..... [2]

(d) GM mosquitoes carrying the tTA gene can live and reproduce normally when fed on a diet containing an added chemical, **A**.

With reference to Fig. 2.1:

(i) suggest how **A** could prevent death of the GM mosquitoes

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..... [2]

(ii) suggest how large numbers of adult GM male mosquitoes can be produced for release into the wild, from an original stock of GM males

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..... [2]

(iii) suggest why there is little danger of the gene carried by these GM mosquitoes being passed to other organisms from GM mosquitoes which escape or are released into the wild.

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.....
..... [2]

[Total: 15]

Q11.

- 2 The pink bollworm moth, *Pectinophora gossypiella*, is a pest of cotton crops. The size of its population can be reduced by releasing large numbers of sterile male moths into cotton fields. The sterile male moths mate with wild females from the cotton fields, but no offspring are produced.

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Over a period of three years, 20 million genetically modified (GM) sterile male moths were released in the USA. Each insect contained a gene coding for a red fluorescent protein (DsRed) taken from a species of reef coral. The added DNA also included a promoter.

(a) Explain why, in gene technology:

- (i) genes for fluorescent proteins such as DsRed are now more commonly used as markers than are genes for antibiotic resistance

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..... [2]

- (ii) a promoter needs to be included when transferring a gene from a coral into an insect.

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..... [3]

(b) DsRed is visible at all stages of the life cycle of the moth, but the presence of the gene in a particular individual can be confirmed by genetic fingerprinting, using gel electrophoresis.

(i) Outline the principles of gel electrophoresis.

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..... [4]

(ii) Explain how the presence of the gene for DsRed in a moth can be confirmed once electrophoresis is complete.

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..... [2]

(c) DsRed allows sterile male moths to be distinguished from wild moths when caught in an insect trap in a field of cotton plants.

Suggest why it is important to be sure whether a moth caught in such a trap is a released sterile male or a wild insect.

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..... [2]

- (d) The United States Department of Agriculture has ruled that the release of sterile males to control insect pest numbers is environmentally preferable to all other alternatives.

Exs

Suggest what information would be needed to determine whether the release of the sterile male moths, carrying the gene for DsRed, has a damaging effect on the environment.

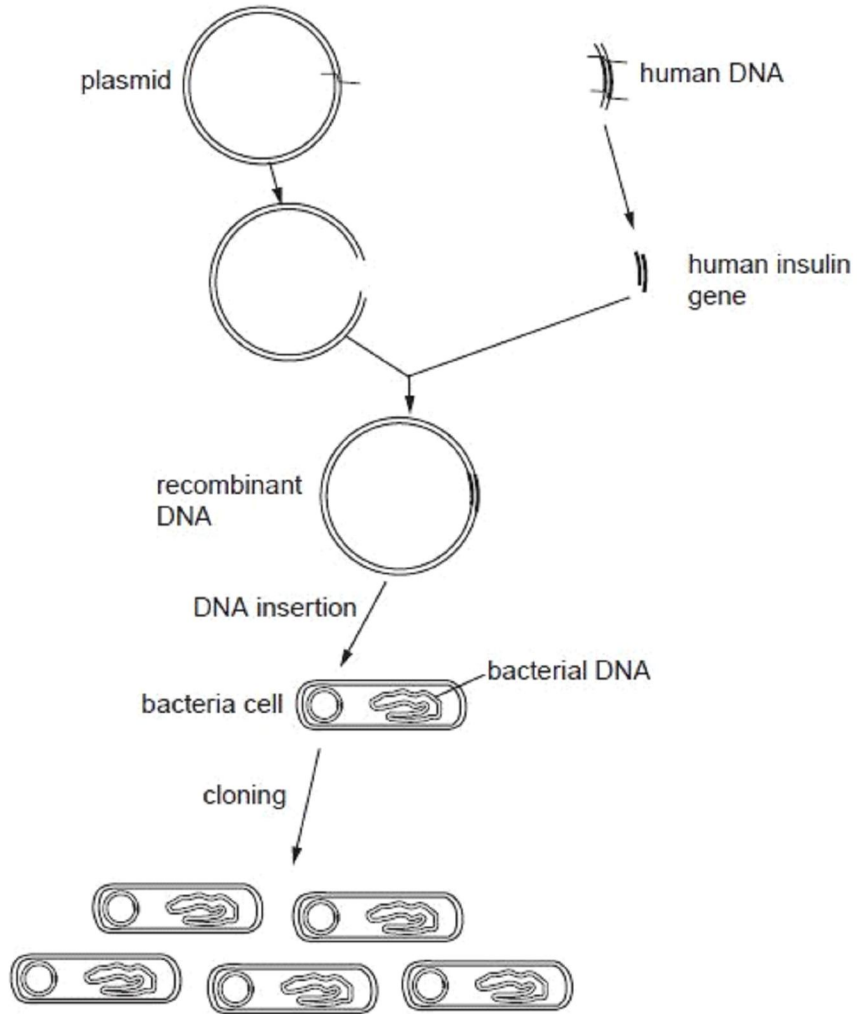
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[2]

[Total: 15]

Q12.

- 5 Fig. 5.1 outlines the way in which the gene for human insulin is incorporated into plasmid DNA and inserted into a bacterium.



(a) Describe how the plasmid DNA is cut.

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.....[3]

(b) Explain how the human insulin gene is joined to the plasmid DNA.

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.....[3]

(c) List **two** advantages of treating diabetics with human insulin produced by genetic engineering.

1.
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2.
.....[2]

[Total : 8]

Q13.

- 2 Fig. 2.1 shows the CFTR (cystic fibrosis transmembrane conductance regulator) protein in a plasma (cell surface) membrane.

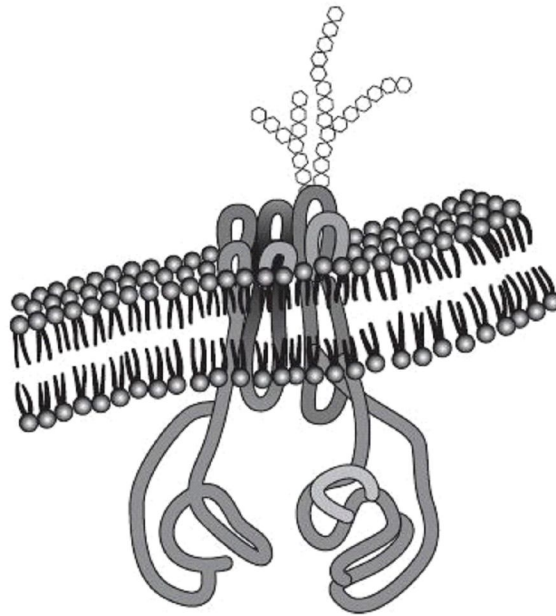


Fig. 2.1

- (a) (i) Describe the normal function of the CFTR protein.

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..... [2]

- (ii) On Fig. 2.1, use the letter **E** to indicate the external face of the membrane. State how you identified this face.

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..... [1]

- (b) Cystic fibrosis is caused by a recessive allele of the *CFTR* gene.

- (i) Explain the meaning of the term *recessive allele*.

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..... [2]

(ii) Explain how cystic fibrosis affects the function of the lungs.

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..... [3]

(c) As cystic fibrosis is caused by a recessive allele of a single gene, it is a good candidate for gene therapy. Trials were undertaken in the 1990s, attempting to deliver the normal allele of the *CFTR* gene into cells of the respiratory tract, using viruses or liposomes as vectors.

Explain how viruses deliver the allele into cells.

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..... [2]

(d) In some people with cystic fibrosis, the allele has a single-base mutation which produces a 'nonsense' (stop) codon within the gene.

Exa
t

(i) Explain how this mutation would prevent normal CFTR protein being produced.

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..... [2]

(ii) A new type of drug, PTC124, enables translation to continue through the nonsense codon. Trials in mice homozygous for a *CFTR* allele containing the nonsense codon have found that animals treated with PTC124 produce normal CFTR protein in their cells. The drug is taken orally, and is readily taken up into cells all over the body.

Using your knowledge of the progress towards successful gene therapy for cystic fibrosis, suggest why PTC124 could be a simpler and more reliable treatment for this disease.

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..... [3]

[Total: 15]

Q14.

- 6 (a) A husband and wife who already have a child with cystic fibrosis (CF) elected to have their second child tested for the condition while still a fetus in very early pregnancy. The results of the test, a DNA banding pattern, were discussed with a genetic counsellor.

The relevant DNA banding pattern produced by electrophoresis is shown in Fig. 6.1.

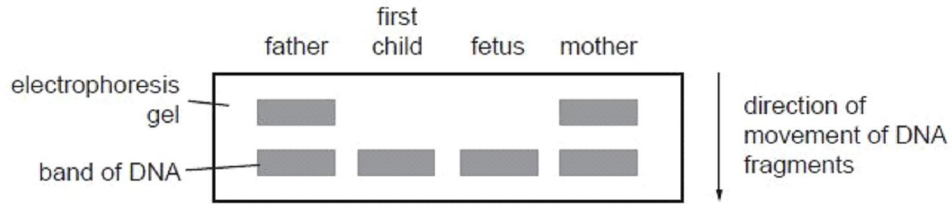


Fig. 6.1

With reference to Fig. 6.1, explain why,

- (i) the fetus will develop CF,

.....
..... [1]

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

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..... [2]

- (b) Explain briefly the need to discuss the result of the test with a genetic counsellor.

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..... [4]

[Total: 7]

Q15.

4 The secretion of insulin by the islets of Langerhans in the pancreas stimulates the liver to reduce the blood glucose concentration. Ex

(a) Describe how the **liver** reduces blood glucose concentration, when insulin is secreted.

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..... [3]

(b) Almost all insulin used to treat type I diabetes is produced by genetically engineered bacteria or yeast. A summary of this procedure is shown in Fig. 4.1.

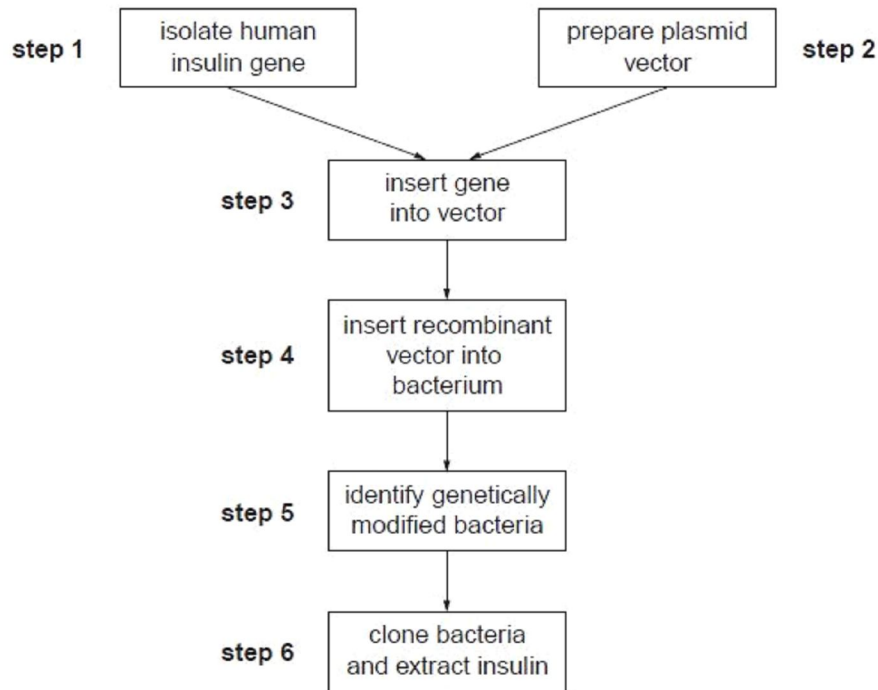


Fig. 4.1

- (i) One way of carrying out **step 1** is to collect mRNA from β cells from the pancreas. The relevant mRNA is then isolated and used to make DNA.

Suggest why isolating the mRNA coding for insulin in a β cell is easier than isolating the DNA for insulin in a β cell.

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..... [2]

- (ii) Outline the use of restriction enzymes in **step 2**.

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.....
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..... [2]

Exa
t

- (c) Most people with type I diabetes inject insulin. A recent product contains insulin that can be administered using a nasal spray. The spray is inhaled and the insulin is taken up through the lungs.

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Fig. 4.2 shows the concentration of insulin in the blood plasma in the 480 minutes after injecting or inhaling insulin. In both cases, the insulin was of the same type, obtained from genetically engineered *Escherichia coli*.

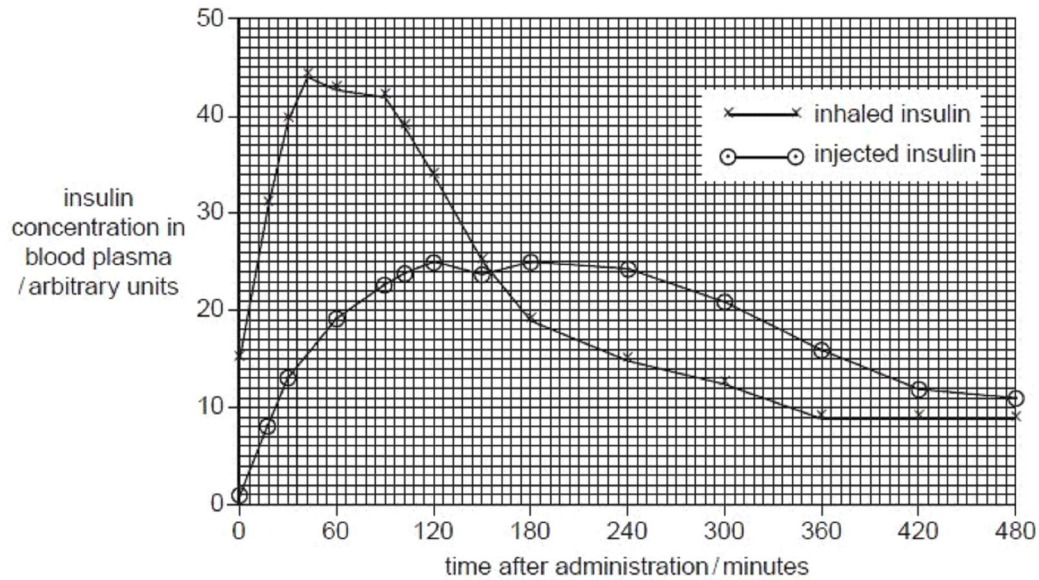


Fig. 4.2

Fig. 4.3 shows the concentration of glucose in the blood plasma in the 480 minutes after injecting or inhaling insulin.

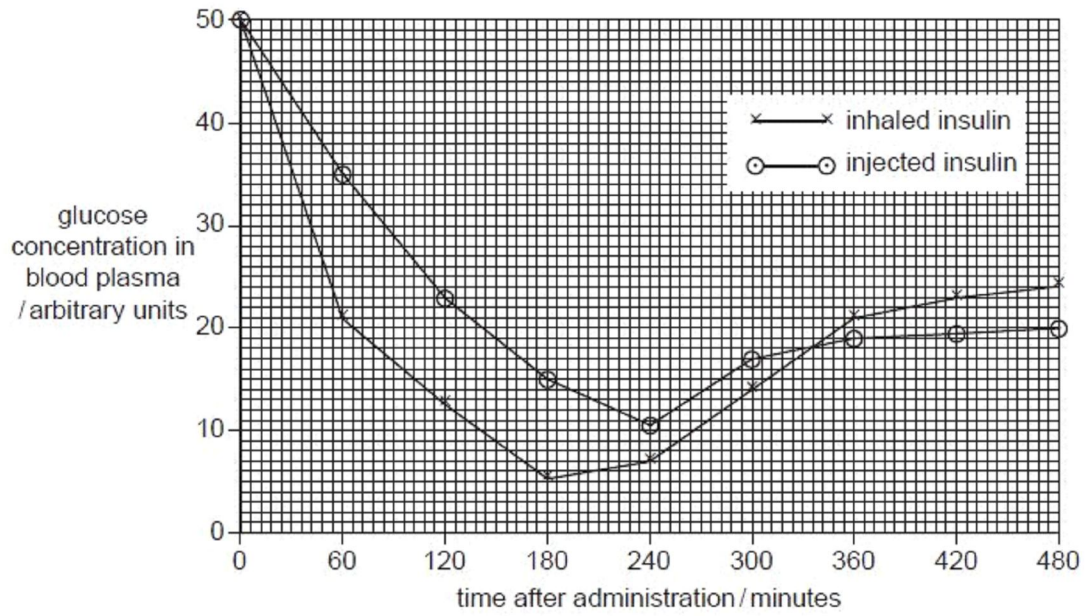


Fig. 4.3

(i) Compare the results for injected insulin and inhaled insulin shown in Fig. 4.2.

Ex

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..... [3]

(ii) With reference to Fig. 4.2, explain the differences in the blood glucose levels after injecting or inhaling insulin shown in Fig. 4.3.

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..... [3]

(iii) With reference to Figs. 4.2 and 4.3, suggest one advantage and one disadvantage of inhaling insulin rather than injecting it.

advantage

.....

disadvantage

..... [2]

[Total: 15]

Q16.

5 Many attempts have been made to find methods of using gene therapy to treat cystic fibrosis. One approach uses viruses to deliver normal alleles of the CFTR gene into epithelial cells of the airways. Viral delivery systems have two main problems:

- The virus may trigger an immune response which destroys the infected cells.
- Most non-pathogenic viruses are not very good at getting into cells, so very few cells receive the allele.

A team of researchers in the USA developed a new strain (AAV2.5T) of AAV, a non-pathogenic virus. AAV2.5T has an improved ability to bind with epithelial cells of the airways. Genes for the CFTR protein and for an enzyme, luciferase, were added to the DNA of the viruses. Luciferase produces a fluorescent green protein when luciferin is added.

The normal AAV strain and the AAV2.5T strain were added to cultures of epithelial cells from the airways. After adding luciferin, the numbers of cells that had taken up the viral genes was estimated using the intensity of the green fluorescence which developed.

The results are shown in Fig. 5.1.

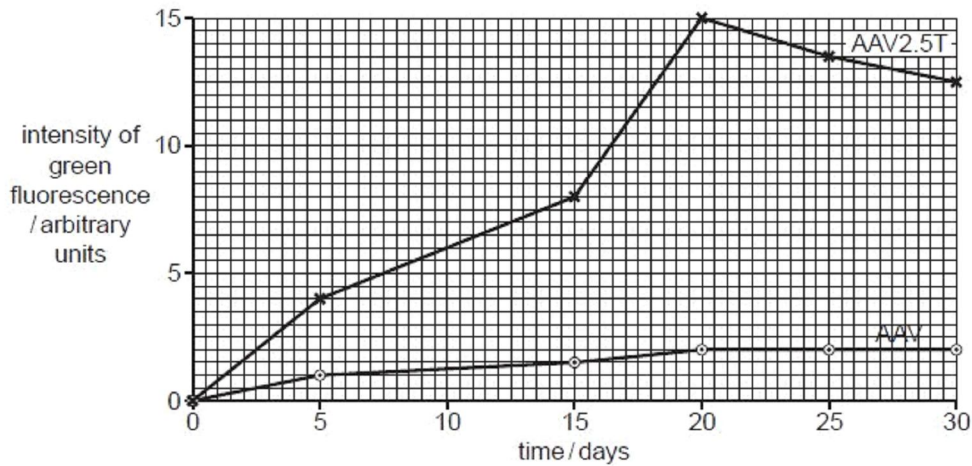


Fig. 5.1

(a) With reference to Fig. 5.1, compare the ability of the two viral strains, AAV and AAV2.5T, to infect epithelial cells from the airways.

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..... [2]

(b) Explain why the researchers added a gene for luciferase to the viral DNA.

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..... [2]

(c) Suggest how delivering normal alleles of the CFTR gene into epithelial cells in the airways could relieve the symptoms of cystic fibrosis.

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..... [4]

[Total: 8]

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Q17.

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

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

(a) Suggest and explain why LCA is suitable for treatment using gene therapy.

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.....[3]

(b) Six adults with this condition were used in the study. Genetically modified adenoviruses (a type of virus that can cause respiratory infections) were used as vectors. The vectors were injected beneath the retina of one eye of each of the participants.

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

1.
.....
2.
.....[2]

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

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

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.....[2]

[Total: 7]

Q18.

- 7 Insulin can be produced on a large scale using gene technology and prokaryotes such as *Escherichia coli*.

Ext

Table 7.1 summarises the sequence of steps in one method of production of insulin by *E. coli*.

Complete Table 7.1 by adding one statement in each of the empty boxes.

Table 7.1

step	reason for step
obtain copies of gene with sticky ends	the gene codes for the synthesis of insulin
	acts as a vector for the transfer of the gene into the host
use restriction endonuclease enzyme	
mix vector and gene	
	to seal the sugar-phosphate backbone
	to obtain transformed host <i>E. coli</i> cells
screen for, and obtain, successfully transformed cells	
	to obtain large amounts of insulin for extraction and purification

[7]

Q19.

- 5 (a) The steps involved in a method of production of human insulin by gene technology are listed in Table 5.1.
The steps are **not** listed in the correct order.

Exa

Table 5.1

step	description
A	DNA coding for human insulin inserted into cut plasmid vector
B	genetically modified bacteria identified
C	mRNA for human insulin isolated in β cells
D	plasmid vector inserted into bacterium
E	genetically modified bacteria cloned
F	DNA for human insulin cloned
G	human insulin harvested
H	cDNA coding for human insulin synthesised

- (i) Complete Table 5.2 to show the steps in the correct order.

Two of the steps have been done for you.

Table 5.2

correct order	letter of step
1	C
2	
3	
4	
5	D
6	
7	
8	

[4]

- (ii) Name the enzymes responsible for the following steps:

step A

step H

[2]

- (b) Explain **two** advantages of treating diabetes with human insulin produced by gene technology rather than using insulin from animals.

E

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..... [2]

[Total: 8]

Q20.

- 3 Green fluorescent protein (GFP) is a small protein that emits bright green fluorescence in blue light. It was first isolated from the jellyfish, *Aequorea victoria*.

E

The gene coding for GFP can be expressed in bacteria, such as *Escherichia coli*, and so it is often used as a marker to show successful uptake of a gene by the bacterium.

- (a) (i) Outline how a gene from another species can be inserted into *E. coli*.

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..... [3]

- (ii) Explain how a marker gene, such as the gene for GFP, is used to show successful uptake of a gene for a wanted protein.

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.....[3]

- (b) Genes for enzymes that produce fluorescent substances are often used as markers in gene technology.

GFP is **not** an enzyme.

Suggest **one** disadvantage of using the gene for GFP to produce easily detectable fluorescence, rather than using a gene for an enzyme that produces a fluorescent substance.

Explain your answer.

disadvantage
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explanation
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.....[2]

[Total: 8]

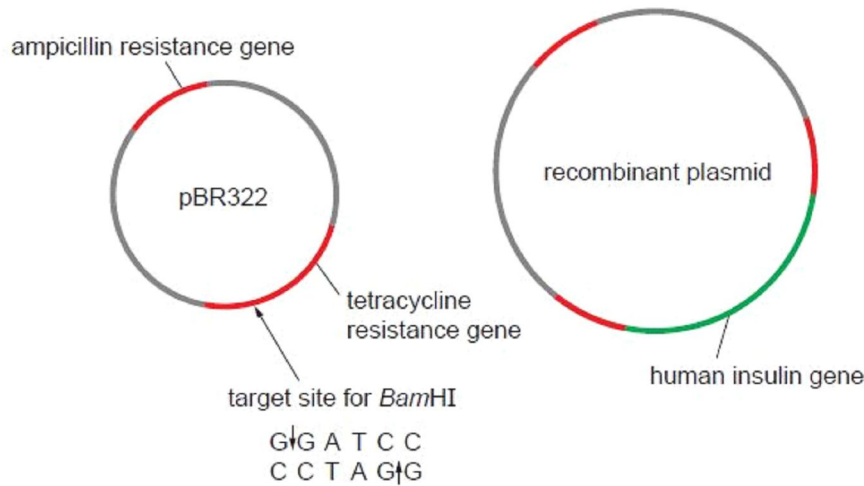
Q21.

- 2 The artificial plasmid, pBR322, was constructed to act as a vector. It has often been used to insert human genes, such as the human insulin gene, into the bacterium, *Escherichia coli*.

The plasmid was constructed to include two genes, each giving resistance to a different antibiotic: an ampicillin resistance gene and a tetracycline resistance gene. The plasmid also has a target site for the restriction enzyme, *Bam*HI, in the middle of the tetracycline resistance gene.

A pBR322 plasmid was cut using *Bam*HI and the cDNA gene for human insulin inserted into it.

Fig. 2.1 shows pBR322 and the recombinant plasmid.



- (a) With reference to Fig. 2.1, describe how a cDNA human insulin gene can be inserted into pBR322 that has been cut by *Bam*HI.

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[4]

Q22.

- 2 Russian scientists have discovered the fruits of a flowering plant, *Silene stenophylla*, in the food store in a burrow of a ground squirrel in frozen sediments in Siberia.

Dating techniques suggest that the fruits were stored by the ground squirrel about 32 000 years ago, shortly before the ground became permanently frozen.

Tissue samples were taken from the fruits and grown in a nutrient culture medium. After treatment with plant hormones to stimulate the growth of roots and shoots, 36 complete plants were produced.

These 'regenerated' plants, which looked identical to one another, flowered and after cross-pollination, produced seeds that were able to germinate.

- (a) Explain why cross-pollination produces more genetic variation among the offspring than self-pollination.

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.....[3]

- (b) The flowers of modern-day *S. stenophylla* look similar, but not identical, to the flowers of the 'regenerated' plants.

Outline how DNA sequencing could be used to compare the DNA of modern-day and 'regenerated' *S. stenophylla*.

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.....[4]

- (c) Suggest a simple experiment, using plants of modern-day and 'regenerated' *S. stenophylla* to find out whether, after 32 000 years, they are still the same species.

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.....[2]

[Total: 9]

Q23.

- 3 A group of membrane proteins which transport sugars out of cells have been identified and called SWEETs. They are found in the cell surface membranes of both animal and plant cells, including mammalian liver cells and rice mesophyll cells.

Each SWEET is a protein with seven coiled regions which together make a pore through a membrane bilayer as shown in Fig. 3.1.

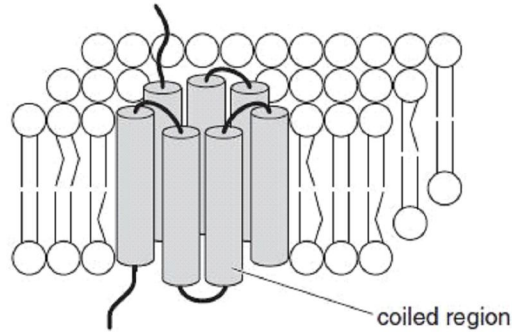


Fig. 3.1

- (a) (i) Explain why, to enter or leave a cell, sugars need molecules such as SWEETS.

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.....[2]

- (ii) Suggest how a SWEET is held within the membrane bilayer.

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.....[3]

- (b) The bacterium, *Xanthomonas oryzae* (Xoo), causes the disease known as bacterial leaf blight in rice plants. It infects the intercellular spaces of the leaves of the host plant. Then, by switching on *SWEET* genes in the mesophyll cells, it stimulates the secretion of glucose into the intercellular spaces.

Several different **recessive** alleles have been found, in rice plants from different countries, which give resistance to bacterial leaf blight. All these alleles have a mutation in the promoter of the *SWEET* gene.

The effect of Xoo on wild type and resistant rice plants is compared in Fig. 3.2.

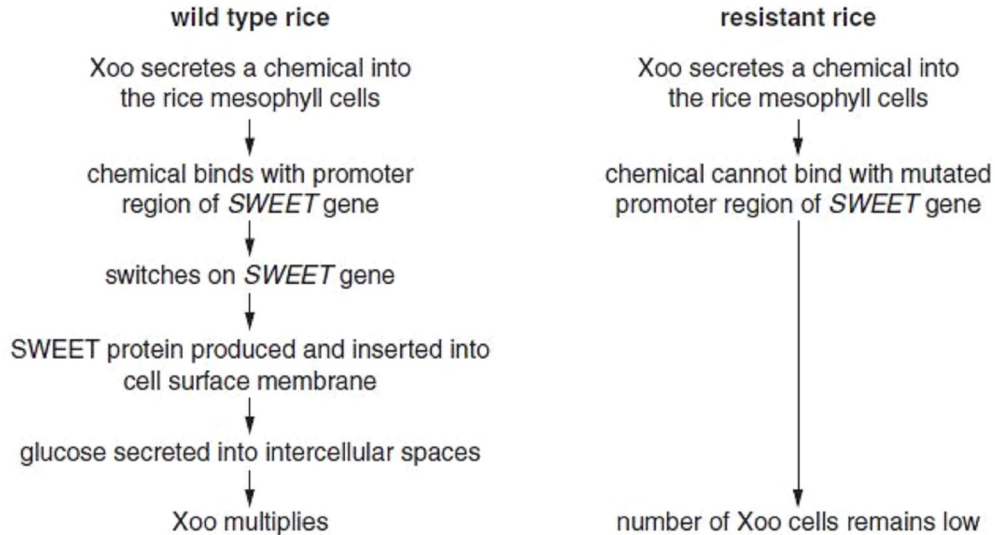


Fig. 3.2

- (i) Using the information in Fig. 3.2, explain this resistance of rice plants to Xoo.

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.....[3]

- (ii) Explain why it would be difficult to transfer this resistance into susceptible rice plants by genetic engineering.

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- (iii) Explain why the presence of large numbers of Xoo in the intercellular air spaces of rice plants affects the ability of the plants to grow with their roots submerged in water.

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.....[4]

- (ii) Explain why it would be difficult to transfer this resistance into susceptible rice plants by genetic engineering.

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.....[2]

- (iii) Explain why the presence of large numbers of Xoo in the intercellular air spaces of rice plants affects the ability of the plants to grow with their roots submerged in water.

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.....[4]

[Total: 14]

Q24.

- 3 Human insulin can be synthesised in a laboratory strain of *Escherichia coli* using recombinant DNA (rDNA) technology.

The starting point for the process is mRNA coding for insulin, isolated from human pancreas cells.

Four enzymes are needed:

- reverse transcriptase
- DNA polymerase
- restriction enzyme
- DNA ligase.

- (a) (i) State the role of each of these enzymes in producing rDNA carrying the gene for human insulin.

reverse transcriptase

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DNA polymerase

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restriction enzyme

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DNA ligase

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[4]

- (ii) Outline the role of insulin in a healthy human.

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[3]

(iii) Describe and explain **one** advantage of treating diabetics with human insulin produced by rDNA technology.

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.....[2]

(b) It is possible to use rDNA technology to produce insulin with a slightly different structure from that of human insulin. The effect of the changed structure can then be investigated.

The activities of equal quantities of two insulins, both produced by *E. coli*, were compared in healthy, non-diabetic subjects:

- human insulin
- insulin X, in which the positions of two amino acids, lysine and proline, were exchanged. Lysine has a hydrophilic R group and proline has a hydrophobic R group.

The results of the investigation are shown in Fig. 3.1.

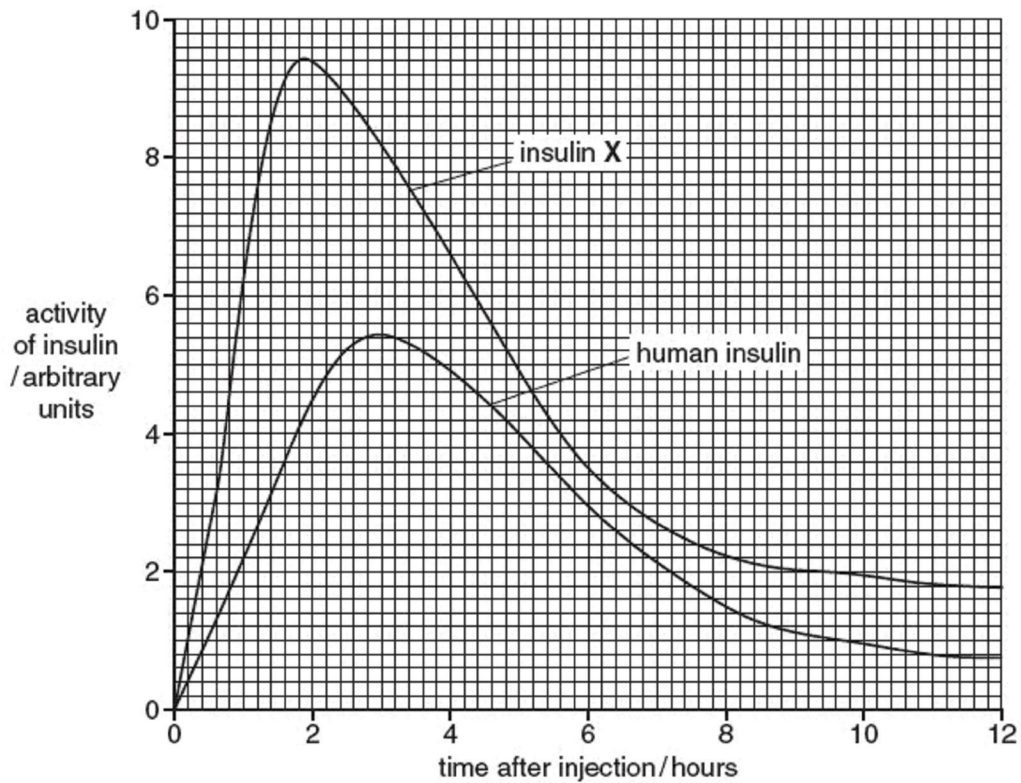


Fig. 3.1

- (i) With reference to Fig. 3.1 describe the differences in activity between human insulin and insulin X.

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.....[4]

- (ii) Suggest how exchanging the position of two amino acids in the insulin molecule can result in differences in activity.

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.....[2]

[Total: 15]

Q25.

- 3 (a) The diagram outlines how a gene coding for human insulin is produced by genetic engineering techniques.

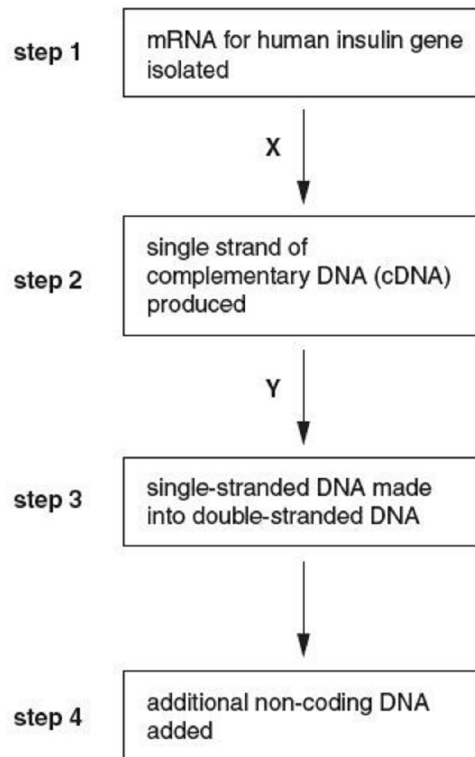


Fig. 3.1

- (i) Name the enzymes X and Y.

X

Y

[2]

- (ii) Explain why the starting point in this procedure is mRNA.

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.....[2]

(b) Suggest why some methods of manufacturing genetically engineered insulin use eukaryotic yeast cells rather than prokaryotic bacterial cells.

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.....[2]

(c) State **three** advantages of using human insulin produced by genetic engineering.

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.....[3]

[Total: 9]

Q26.

1 (a) Repeated self-pollination in some species of flowering plants leads to inbreeding.
Describe briefly the effect of inbreeding on genetic diversity.

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.....
.....[3]

Some species of tropical ginger plants have two distinct phenotypes, **A** and **B**, that coexist in all populations. Phenotypes **A** and **B** differ in the behaviour of the anthers and stigmas of their flowers. In both phenotypes, the flowers open for only one day and the behaviour of the flowers of each phenotype is synchronised in all plants in the population. The differences between the two phenotypes are shown in Table 1.1.

Table 1.1

time	phenotype	behaviour of flower
0600 – 1145	A	stigma above anther, avoiding contact with insects anther split to release pollen
	B	receptive stigma below anther anther not split to release pollen
1145 – 1330	A	stigma moves downwards below anther
	B	stigma elongates and moves above anther
1430 – 1500	A	receptive stigma below anther anther no longer releasing pollen
	B	stigma above anther, avoiding contact with insects anther split to release pollen

(b) With reference to Table 1.1,

(i) state the time of day at which phenotype **A** may be pollinated by phenotype **B**;

..... [1]

- (ii) explain how the behaviour of the two phenotypes of ginger plant helps to avoid inbreeding.
-
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-
- [4]
- (c) The number of phenotypes **A** and **B** in a natural population of one species of ginger plant was found to be 86 and 78 respectively. The ratio expected in the population was 1 : 1. The χ^2 (chi-squared) test was performed on these data, giving a calculated χ^2 of 0.39.
- (i) State the number of degrees of freedom applicable to these data.
- [1]
- (ii) Use the calculated value of χ^2 and the table of probabilities provided in Table 1.2 to find the probability of the observed ratio departing significantly from the expected ratio.
- probability [1]

Table 1.2

degrees of freedom	probability, p				
	0.10	0.05	0.02	0.01	0.001
1	2.71	3.84	5.41	6.64	10.83
2	4.61	5.99	7.82	9.21	13.82
3	6.25	7.82	9.84	11.35	16.27
4	7.78	9.49	11.67	13.28	18.47

- (iii) State what conclusion may be drawn from the probability found in (ii).
-
-
- [2]

- (iv) Suggest, using suitable symbols, genotypes for phenotypes **A** and **B** that would enable both phenotypes to be maintained in the ginger plant population for many generations. Explain the symbols you select.

explanation of symbols

.....

genotype A

genotype B[3]

[Total: 15]

Q27.

- 2 (a) Cystic fibrosis (CF) is caused by mutations of a gene coding for a transmembrane protein (CFTR) which acts as an ion pore. A large number of different mutations of the gene have been found.

- (i) Describe briefly the symptoms of CF in humans.

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..... [3]

- (ii) Explain how CF is inherited.

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..... [3]

- (iii) Explain why a gene test for CF may not reveal the presence of the disease.

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..... [2]

- (b) Ion transport by cells expressing the normal allele for CFTR was compared with that by cells expressing mutant alleles. The mutant cells chosen for study were all capable of producing a CFTR protein and correctly inserting it into their cell surface membrane. The abilities of the cells to transport HCO_3^- and Cl^- were measured and expressed as a $\text{HCO}_3^- : \text{Cl}^-$ transport ratio. The results are shown in Table 2.1.

Table 2.1

CF allele expressed by cells	$\text{HCO}_3^- : \text{Cl}^-$ transport ratio of cells
normal alleles	1.0 : 1.0
mutant alleles associated with CF with inadequate functioning of the pancreas	all < 0.1 : 1.0
mutant alleles associated with CF with adequate functioning of the pancreas	0.3 – 0.46 : 1.0

- (i) Explain why it was important that the CFTR protein was produced and correctly inserted into the cell surface membrane of all mutant cells chosen for study.

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..... [2]

- (ii) With reference to Table 2.1, explain the cause of inadequate functioning of the pancreas in CF.

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..... [3]

- (iii) Suggest two consequences of inadequate functioning of the pancreas in CF.

.....
.....
..... [2]

[Total: 15]

Section_B

1.

- 7 (a) Describe the use of recombinant DNA technology in the synthesis of human insulin by bacteria. [9]
- (b) Explain the advantages of treating diabetics with human insulin produced by genetic engineering. [6]

2.

- 10 (a) Explain what is meant by a **gene** mutation and outline the possible consequences of a gene mutation for an organism. [9]
- (b) Explain how faulty CFTR proteins in cell surface membranes can lead to the symptoms of cystic fibrosis. [6]

[Total: 15]

3.

- 10 (a) Cystic fibrosis (CF) is a genetic disease caused by an autosomal recessive allele. Gene therapy has been attempted to treat CF since 1993. Outline the basic principles of gene therapy for the treatment of CF. [8]
- (b) Describe the role of a genetic counsellor in dealing with genetic diseases in humans and discuss the circumstances in which a couple might be referred to a genetic counsellor. [7]

[Total: 15]

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