1. One product manufactured using microorganisms is insulin. The process involves genetically engineering bacteria to synthesise human insulin.

(i) Describe how the isolated human insulin gene is inserted into a bacteria plasmid.

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[4]

(ii) Suggest **two** ways in which the bacteria which take up the modified plasmids can be identified.

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[2]

[Total 6 marks]

2. Suggest **one** reason why it is considered preferable to use genetically engineered sources of human insulin rather than insulin obtained from pigs.

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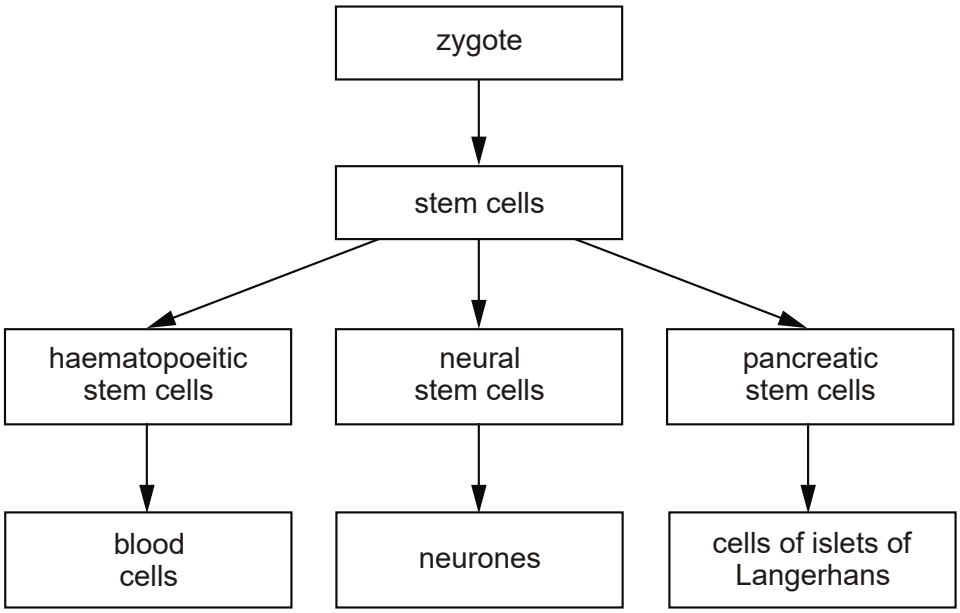
[Total 1 mark]

3. A human zygote divides to produce stem cells. Stem cells have the ability to develop into any cell type, in a similar way to meristematic cells in plants.

The figure below shows development of three cell types from human stem cells.



There are many potential medical uses of stem cells from human embryos. One potential use is to make cells of the islets of Langerhans for transplantation, as a treatment for diabetes mellitus.

(i) Suggest **one** ethical objection to the use of stem cells from human embryos.

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[1]

(ii) Suggest **two** other medical conditions which could be treated using the embryonic stem cells shown in the figure.

1 .....

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2 .....

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[2]

[Total 3 marks]

4. Recombinant DNA technology, using restriction enzymes, enables bacteria such as *Escherichia coli* to produce human proteins.

(i) Explain what is meant by a *restriction enzyme*.

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[3]

(ii) Outline the formation of recombinant DNA.

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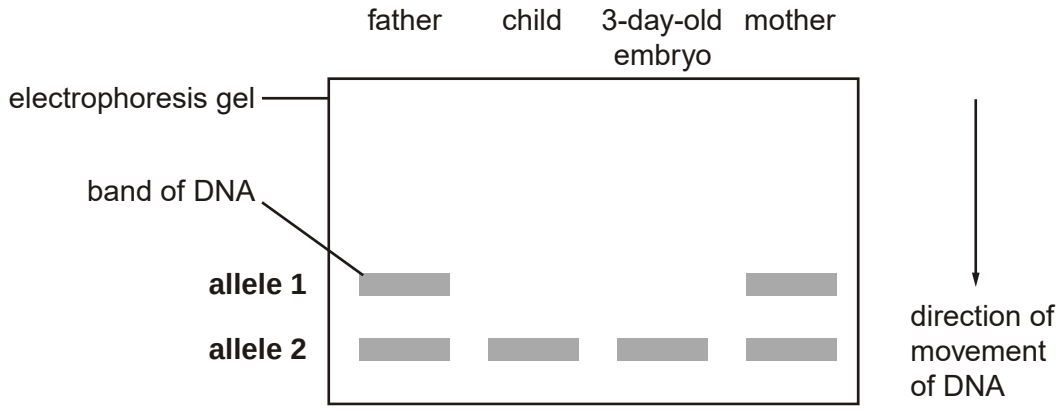
[4]

[Total 7 marks]

5. A couple who already had a child affected by Cystic fibrosis (CF) underwent pre-implantation genetic diagnosis.

In this process, a single cell, taken from one of several three-day-old embryos created by *in vitro* fertilisation (IVF), can be tested for CF.

The resulting DNA banding pattern produced by electrophoresis is shown in the figure below.



(a) Using the information above,

(i) explain why the three-day-old embryo will develop CF;

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[2]

(ii) explain why the position of **allele 2** on the electrophoresis gel indicates that it contains a deletion in comparison with **allele 1**.

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[3]

(b) State the probability of another of the couple's three-day-old embryos having CF.

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[1]

[Total 6 marks]

6. In this question, one mark is available for the quality of the use and organisation of scientific terms.

People who have one form of diabetes are unable to make insulin. In order to control blood sugar concentration, these people need to receive insulin. Originally, insulin was obtained from animals, such as pigs. Now, bacteria are transformed by genetic engineering to make proteins, such as insulin. This is the source of the majority of insulin now used by diabetics.

Describe how genetic engineering has been used to produce human insulin **and** the advantages of obtaining insulin in this way.

[8]

Quality of Written Communication [1]

[Total 9 marks]

7. The DNA target sites of four restriction enzymes are shown in the table below. The points at which the strands of DNA are cut are shown by arrows and lines.

restriction enzyme	target site
Sau3AI	↓G – A – T – C – – C – T – A – G ↑
BamHI	– G ↓ G – A – T – C – C – – C – C – T – A – G ↑ G –
HinfI	– G ↓ A – N – T – C – – C – T – N – A ↑ G – 'N/N' may be any complementary base pair

With reference to the information above,

(i) describe the characteristics of a restriction enzyme's target site;

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[2]

(ii) explain whether or not a piece of DNA cut by **Sau3AI** could join with one cut by **BamHI**;

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[3]

(iii) show on the figure below the result of exposing this piece of DNA to **HinfI.**

–G – A – T – T – C – A –G – A – A – T – T – T – C – G– A – A – T – C –

– C – T – A – A –G – T – C – T – T – A – A – A – G – C – T – T – A – G –

[1]

[Total 6 marks]

8. In this question, one mark is available for the quality of use and organisation of scientific terms.

Describe the roles of restriction enzymes and other enzymes in genetic engineering.

[8]

Quality of Written Communication [1]

[Total 9 marks]

9. In an experimental gene therapy for insulin-dependent diabetes, the insulin gene was combined with a glucose-sensitive promoter and inserted into liver cells of diabetic rats. The mean concentration of insulin was then measured at three different concentrations of blood glucose. The results are shown below.

concentration of blood glucose / mg dm^{-3}	mean concentration of insulin / ng cm^{-3}
100	0.3
300	5.0
500	7.0

With reference to the table above, explain the role of the glucose-sensitive promoter in this gene therapy.

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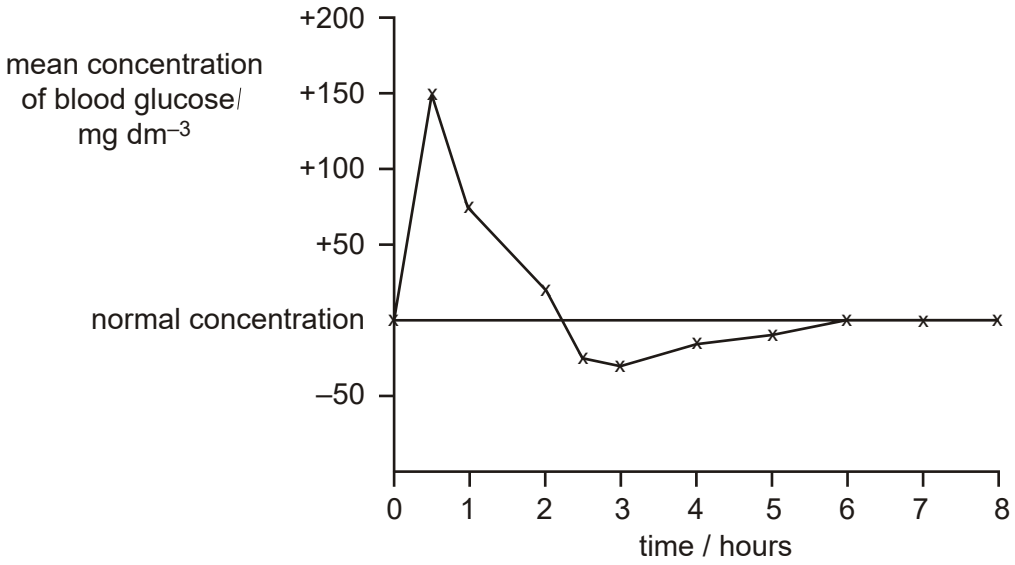
[Total: 3 marks]

10. Treated rats were given a glucose meal and the concentration of blood glucose measured immediately and at intervals for eight hours. The results of this investigation are shown in the figure below.



With reference to the figure, discuss the possible **benefits** and **problems** of using this gene therapy in the treatment of diabetes in humans, rather than taking insulin.

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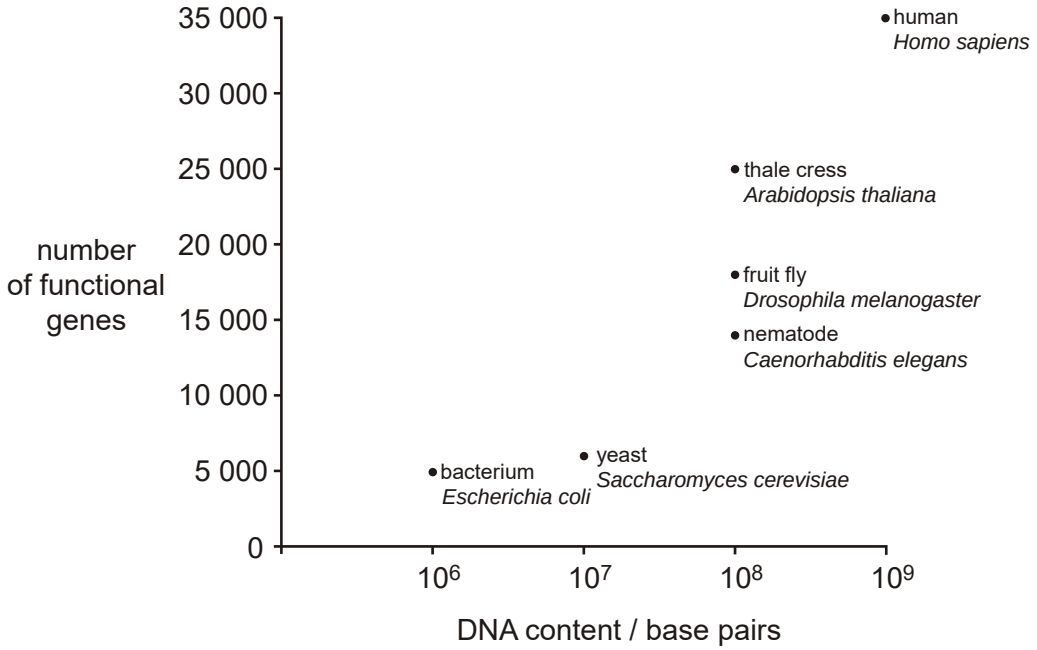
[Total 4 marks]

11. Recent data shows that organisms vary widely in the size of their genomes. The figure below shows the number of functional genes plotted against the total length of DNA in six organisms. The length of DNA is measured in numbers of base pairs.



Adapted from Teresa Attwood, Bioinformatics: What use is it?, Biological Sciences Review, April 2003. Reproduced by kind permission of Philip Allan Publishers Ltd.

The figure shows that the human genome contains only about seven times as many functional genes as the bacterium *Escherichia coli*, but consists of about a thousand times as much DNA.

Suggest why humans have so much extra DNA despite having only seven times as many functional genes as the bacterium *E. coli*.

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[Total 2 marks]

12. Read the following passage and then answer the questions that follow.

Human Factor VIII is a glycoprotein found in blood plasma. It is involved in blood clotting.

This glycoprotein contains 2332 amino acids linked into a single chain. This chain is folded and coiled into a secondary structure and then further folded. The chain forms six individual regions, each with its own function.

An artificial source of Factor VIII, created using genetic engineering, is now used to treat patients with haemophilia, a medical condition in which the blood clots more slowly than normal. The Factor VIII gene is first removed from the genome of human cells. It is then inserted into the genome of hamster cells.

Cancer cells or cells taken from an ovary are usually used to produce Factor VIII as these grow very well in industrial tanks. The Factor VIII that is produced is then removed from the tanks and purified before use in treating patients.

(i) State the type of enzyme used to remove the gene for Factor VIII from the rest of the human genome (lines 8 and 9).

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[1]

(ii) Name the enzyme used to insert the gene for Factor VIII into the genome of hamster cells (line 9).

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[1]

[Total 2 marks]

13. (a) In this question, one mark is available for the quality of spelling, punctuation and grammar.

Britain's only native species of carp, the crucian carp, is very hardy and can live in conditions that would be fatal for most freshwater fish. It can survive in water temperatures from 1 °C to 38 °C and can live in water with a very low oxygen concentration and a low pH.

The crucian carp can interbreed with two other species of carp:

- common carp, a non-native species which was introduced into Britain to increase the stock of fish for freshwater fishing;
- goldfish, which are often illegally released into the wild.

Explain the importance of maintaining a population of crucian carp that has not interbred with other species.

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[8]

Quality of Written Communication [1]

(b) Interbreeding between the three species of fish can be detected by genetic fingerprinting.

A repetitive sequence of DNA has been found in all three species. This sequence has been isolated by using a restriction enzyme. The length of the sequence differs in the three species:

- goldfish - 100 base pairs
- common carp - 75 base pairs
- crucian carp - 65 base pairs.

(i) Explain briefly what is meant by a *restriction enzyme*.

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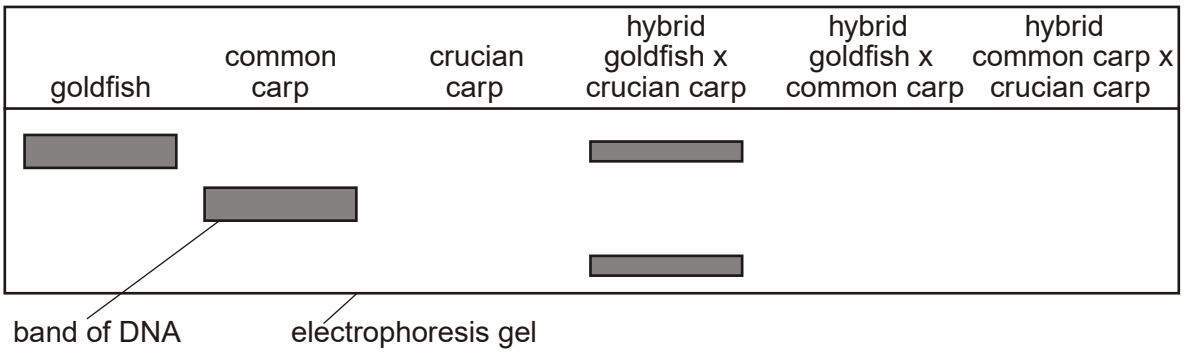
[3]

(ii) The figure below shows an electrophoresis gel on which bands of DNA produced by genetic fingerprinting have been revealed by staining. **Only** the bands produced from goldfish, common carp and hybrid goldfish × crucian carp are shown.



Draw onto the figure the bands expected from:

- crucian carp;
- hybrid goldfish × common carp;
- hybrid common carp × crucian carp.

[3]

[Total 15 marks]

14. The synthesis of caffeine in coffee plants involves enzymes which add methyl groups (CH_3) to convert xanthosine to caffeine:

In an attempt to produce caffeine-free coffee, cells of a coffee plant, *Coffea canephora*, were grown in tissue culture and genetically modified to suppress expression of the gene for theobromine synthase.

DNA was constructed to code either for short or for long lengths of RNA with the **complementary** base sequences to parts of the messenger RNA (mRNA) produced by the gene for theobromine synthase.

(a) Explain how lengths of RNA that are complementary to mRNA may suppress the expression of a gene.

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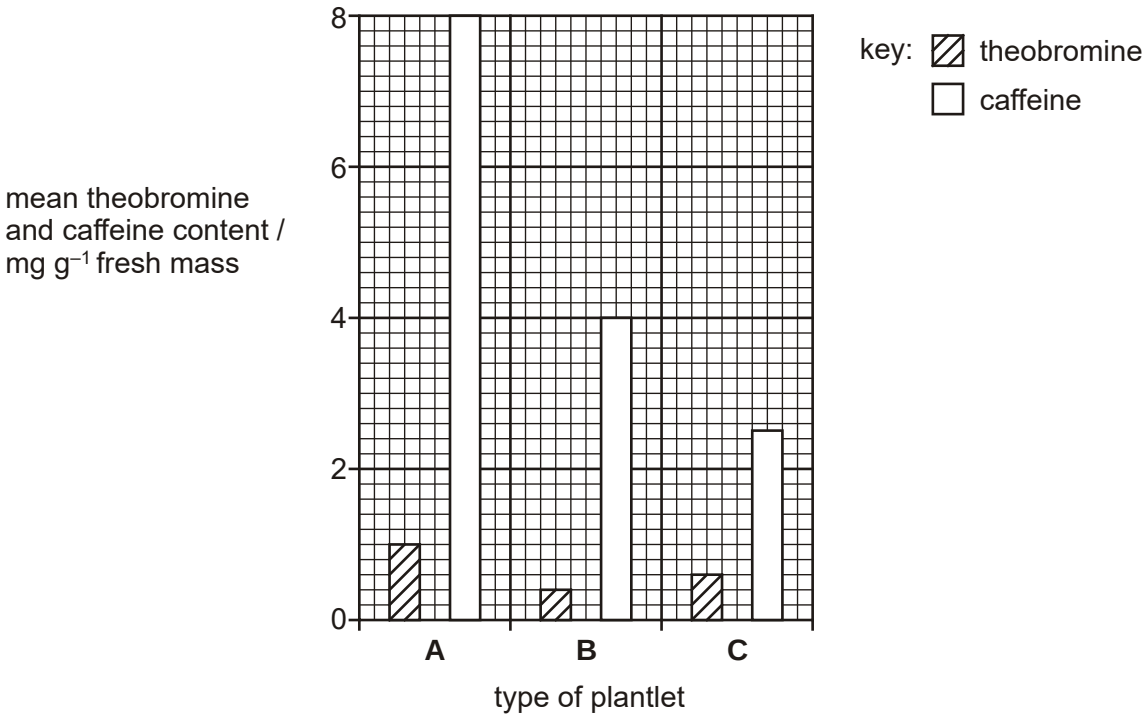
[4]

(b) Three types of cell were then cloned in tissue culture into plantlets:

A - unmodified (control) cells
B - genetically modified cells with the DNA code for short lengths of RNA complementary to mRNA for theobromine synthase
C - genetically modified cells with the DNA code for long lengths of RNA complementary to mRNA for theobromine synthase.

Samples of each of the three types of plantlet were analysed to measure their theobromine and caffeine content. The results of the analysis are shown below.



(i) Describe the results shown in the figure above.

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[3]

(ii) Suggest an explanation for the difference in the results of the two experimental treatments, **B** and **C**.

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[1]

(iii) Describe briefly how plants are cloned by tissue culture.

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[4]

(iv) Explain the advantages of using cloned plants in experiments such as this.

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[3]

[Total 15 marks]

15. (a) Duchenne muscular dystrophy (DMD) is a genetic disease caused by the absence of the protein dystrophin in muscle fibres. In the absence of dystrophin, muscle fibres gradually die.

A potential gene therapy for DMD involves injecting muscles with a viral vector carrying recombinant DNA (rDNA) for part of the normal allele for dystrophin.

Outline the formation of recombinant DNA.

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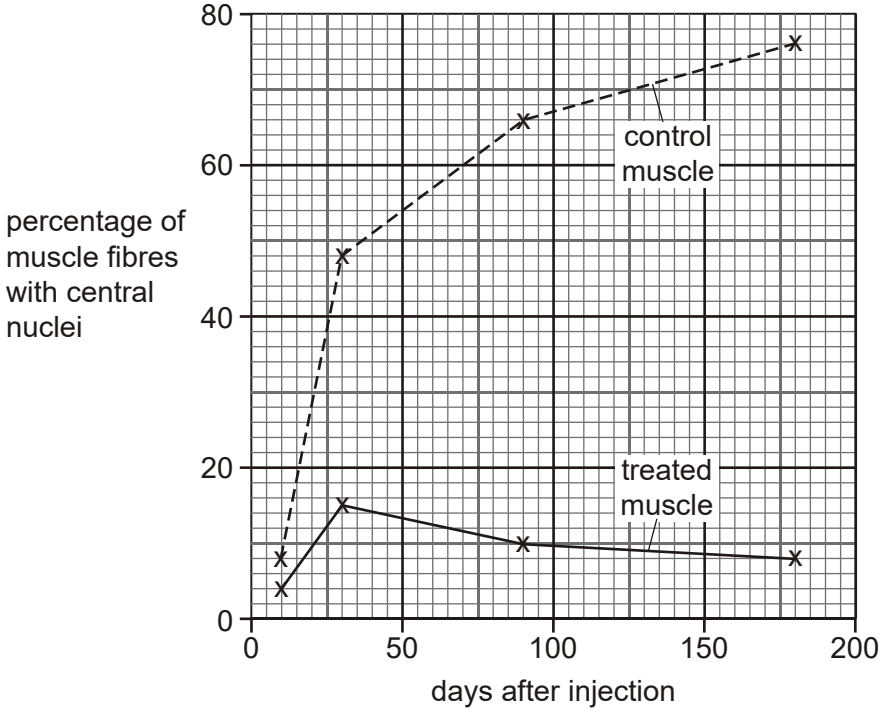
[3]

(b) Mice with the symptoms of DMD were given this gene therapy shortly after birth. Each mouse was injected with the viral vector in a muscle of one hind limb. The corresponding muscle of the other hind limb was injected with a buffer solution to provide a control.

The nuclei of muscle fibres that do not produce dystrophin move from the edge of the fibre to the centre. The fibres eventually die.

The percentage of muscle fibres with centrally placed nuclei was measured in fibres from treated and control muscles at different times after injection. The results are shown in the figure below.



Using the information above, describe the results of the experiment.

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[3]

(c) In this question, one mark is available for the quality of use and organisation of scientific terms.

Genetic screening is available for families with a history of DMD.

Discuss the advantages and disadvantages of genetic screening.

[8]

Quality of Written Communication [1]

[Total 15 marks]

16. In this question, one mark is available for the quality of use and organisation of scientific terms.

Describe the sequence of steps that can be used to produce a protein of medical importance, such as human growth hormone (HGH), on a large scale.

Include in your answer details of how

- a microorganism can be genetically modified to produce such a protein
- large amounts of the protein can then be produced.

[6]

Quality of Written Communication [1]

[Total 7 marks]

17. Read the passage below and answer the questions which follow.

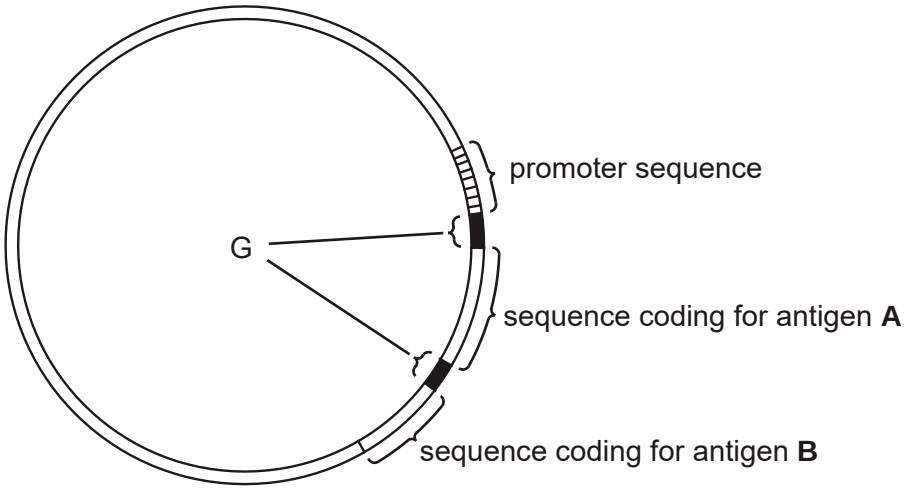
DNA vaccines

Mice and monkeys have been successfully immunised against several important infectious diseases using experimental DNA vaccines, in the form of plasmids. Plasmids are small circular DNA molecules.

During the 1990s, researchers found that mouse muscle and other mouse tissues were able to absorb plasmids which had been injected into the animals. Any genes that were part of this plasmid DNA were transcribed and translated. The resulting proteins were transferred to the plasma membranes (cell surface membranes) of the mouse muscle cells. The proteins were exposed on the muscle plasma membranes together with receptor molecules that allow the immune system to recognise cells as self or non-self. Proteins that are presented at the cell surface in this way stimulate the lymphocytes of the immune system very effectively.

This discovery allows plasmid DNA to be used as a vaccine, even though the DNA does not itself act as an antigen. Most vaccines contain proteins, or fragments of proteins, that are extracted from the surface of pathogens. It is a complex and costly procedure to purify these protein antigens.

The figure below shows a simplified diagram of a DNA vaccine. This plasmid codes for two antigens, **A** and **B**.



(a) State **three** ways in which the structure of plasmid DNA differs from the structure of a protein molecule.

1 .....

2 .....

3 .....

[3]

(b) (i) Define the term *antigen*, as used in the passage.

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[1]

(ii) Suggest why proteins presented at the cell surface are able to stimulate an immune response more effectively than proteins dissolved or suspended in the blood or tissue fluids.

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[1]

(c) (i) Explain why a promoter sequence is needed as part of the plasmid if the vaccine is to work.

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[2]

(ii) Suggest why it may be desirable to include nucleotide sequences coding for more than one antigen in a DNA vaccine.

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[2]

(iii) Sequences of nucleotides, labelled G on the figure, code for groups of amino acids at the beginning of each polypeptide. These amino acid sequences direct the newly synthesised polypeptides to the Golgi apparatus of the muscle cell.

Explain how this makes the vaccine effective.

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[2]

(d) Suggest **three** reasons why researchers may be more concerned about the potential risks of DNA vaccines as compared with protein-based vaccines.

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2 .....

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3 .....

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[3]

[Total 14 marks]

18. A number of different crop plants have been genetically engineered to express a gene for an insecticidal toxin (*Bt* toxin) from a bacterium, *Bacillus thuringiensis*, that kills many insect species.

In China, *Bt* cotton has been grown since 1997. A survey at the end of 2001 showed that it was being grown by over two million farmers on fields totalling more than 7000km^2.

Some further findings of the survey are shown in the table below.

survey finding	percentage of reported cases of insecticide poisoning among cotton farmers	cost of producing 1kg of cotton / US $
farmers growing non-*Bt* cotton	22	2.23
farmers growing *Bt* cotton	5	1.61

Comment on the findings of the survey.

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[Total 4 marks]

19. Babies born with severe combined immune deficiency (SCID) have no defence against common infections and quickly become ill when the protection from maternal antibodies is lost.

SCID is caused by a mutant allele of the gene coding for an enzyme, adenosine deaminase (ADA).

Gene therapy for SCID has been carried out using the procedure shown in the figure below.

bone marrow cells removed from baby with SCID
↓
stem cells of immune system isolated
↓
stem cells infected with harmless genetically engineered virus containing the normal, dominant, allele for ADA
↓
stem cells take up normal allele for ADA
↓
stem cells transfused back into baby
↓
immune system develops T and B lymphocytes

(i) Describe how the DNA of the harmless virus referred to above can be genetically engineered to carry the normal allele of the human gene for ADA.

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[4]

(ii) Explain why it is easier to perform gene therapy when the normal allele is the dominant allele of the gene concerned.

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[2]

[Total 6 marks]

20. Many human proteins, such as growth hormone, are now produced in large quantities by genetically engineered cells. Previously, growth hormone was extracted from animals.

State **two** advantages of producing growth hormone by genetically engineered cells.

1 .....

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2 .....

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[Total 2 marks]

21. Scientists have produced strains of genetically engineered yeast that are capable of producing proteins and adding the branching arrangement of sugars characteristic of human cells. Each strain of yeast produces a different specific protein.

The process involves:

- removing the yeast gene that is responsible for adding the yeast sugars to the protein;
- adding to the yeast a gene from roundworms that builds short chains of mannose sugar units;
- adding two further genes, one from humans and one from a fungus, that add other sugars, such as galactose, to the short chains and make branched chains.

(i) State the type of enzyme that is used to remove a gene from the rest of an organism's DNA.

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[1]

(ii) Describe how the foreign genes can be inserted into DNA.

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[3]

(iii) Suggest how the gene from roundworms is responsible for the building of short chains of mannose sugar units.

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[2]

[Total 6 marks]

22. Chromosome 22 was the first chromosome to be decoded as part of the human genome project. This chromosome is known to carry genes involved in the functioning of the immune system, congenital heart disease, several cancers and certain mental disorders, such as schizophrenia.

Explain how knowledge of particular genes, such as those found on chromosome 22, may be used in the field of modern medicine.

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[Total 4 marks]

23. It has been suggested that future testing of our DNA will show our susceptibility to certain diseases and could create a genetic underclass.

Explain the arguments **against** extensive genetic screening of the population.

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[Total 4 marks]

24. Much of the world's irrigated farmland has become too salty for growing many crops.

Two varieties of tomato plant have been found that are tolerant of salty soil.

- Variety 1 can tolerate high concentrations of NaC*l* in its tissues but has little ability to prevent the ions from entering the plant. The tomatoes produced are large, but not very tasty.
- Variety 2 cannot tolerate high concentrations of NaC*l* in its tissues, but is able to prevent excess ions from entering the plant. The tomatoes produced are small, but tasty.

(a) In this question, one mark is available for the quality of spelling, punctuation and grammar.

Describe a programme for selectively breeding these two varieties to give tomato plants with high salt tolerance and large, tasty tomatoes.

(*Allow one line page*)

[8]

Quality of Written Communication [1]

(b) Another variety of tomato plant has been genetically engineered to grow in a concentration of 0.2mol dm^{-3} NaC*l* by increasing the expression of a gene coding for a protein in the vacuole membrane that pumps excess Na+ into the vacuoles of the leaf cells.

(i) Explain how such proteins pump ions into a plant cell vacuole.

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[3]

(ii) Describe the advantages of producing salt-tolerant tomato plants by genetic engineering rather than by selective breeding.

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[3]

[Total 15 marks]

25. Malarial parasites infect mosquitoes and are then transmitted to humans. An artificial gene has been synthesised to reduce transmission of malarial parasites by mosquitoes.

Recombinant DNA containing this gene was constructed using enzymes and inserted into mosquitoes.

(i) Explain what is meant by *recombinant* DNA.

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[2]

(ii) Describe briefly the use of enzymes in constructing recombinant DNA.

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[3]

[Total 5 marks]

26. The anti-malarial parasite gene is switched on when the mosquito takes a blood meal.

The protein coded for by the gene inhibits the malarial parasite from passing through the epithelia of both the gut and the salivary gland of the mosquito.

The genetically engineered mosquitoes and unaltered (control) mosquitoes were fed on the same mouse which was infected with malarial parasites.

The mosquitoes' abilities to be infected by and to transmit the parasites were then compared.

The results of the investigation are shown in the table below.

type of mosquito	percentage of mosquitoes in which malarial parasites have passed across the midgut	percentage of mosquitoes with malarial parasites in the salivary glands	percentage of mosquitoes that transmitted malarial parasites to uninfected mice
control	88	76	62
genetically engineered	46	26	10

(i) Use the data in the table to compare the abilities of control and genetically engineered mosquitoes to act as vectors of the malarial parasite.

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[3]

(ii) Suggest **one** potential benefit and **one** potential hazard of controlling the spread of malaria by such genetically engineered mosquitoes.

benefit .....

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hazard .....

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[2]

[Total 5 marks]

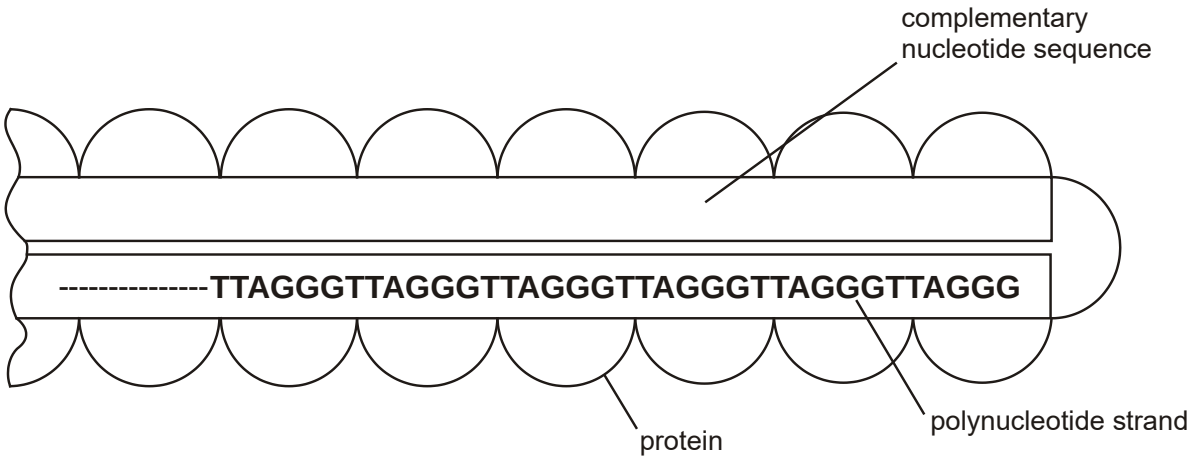
27. Telomeres are the lengths of DNA double helix at the **ends** of all eukaryotic chromosomes.

They have a nucleotide sequence in which the order of the bases in one of the single strands is:

Thymine Thymine Adenine Guanine Guanine Guanine.

This sequence is repeated as many as 2000 times. This repetition is shown in the figure below.

Attached to the DNA of the telomere are protein units that protect the DNA and enable homologous chromosomes to pair during meiosis.



What sequence of bases is **repeated** in the complementary polynucleotide shown in the figure above?

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[Total 1 mark]

28. Genetic engineers have tried to introduce genes for nitrogenase into wheat, which is **not** a legume.

Suggest the possible advantages of developing this wheat.

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[Total 2 marks]