1. *Answers should be written in continuous prose. Credit will be given for biological accuracy, the organisation and presentation of the information and the way in which the answer is expressed.*

Cancer may be treated by chemotherapy. This involves using drugs which kill cancer cells but have no effect on normal healthy cells. Unfortunately. cancer cells develop from normal cells so the two types of cell are similar to each other. Trials have begun which involve adding a new gene to the normal cells in the body. This gene makes a protein which protects these healthy cells against the drug being used. The cancer cells do not produce this protein. so they are killed.

(a) Describe the features of a gene which enable it to code for a particular protein.

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(4)

(b) Explain how enzymes and vectors may be used to isolate genes and insert them into another organism.

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(6)

(c) Describe how the new protein is made once the gene has been inserted into the cell.

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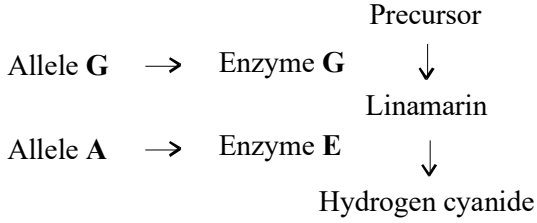
(7)
(QWC 3)
(Total 20 marks)

2. (a) The inheritance of the ability to produce hydrogen cyanide is controlled by two genes which are located on different chromosomes. The dominant allele of one gene, **G**, controls the production of enzyme **G** which converts a precursor to linamarin. The dominant allele of the other gene, **E**, controls the production of enzyme **E** which converts linamarin to hydrogen cyanide. This is summarised in the diagram.



Explain why plants homozygous for the allele **g** will not produce hydrogen cyanide when their tissues are damaged.

(2)

Recently a strain of genetically engineered clover has been developed which has a high concentration of proteins rich in sulphur-containing amino-acids. A piece of DNA was prepared which contained the three different genes. This was inserted into a clover plant.

Gene **1** obtained from sunflower seeds. This gene codes for a protein rich in sulphur-containing amino acids.

Gene **2** ensures that the protein rich in sulphur-containing amino acids is produced in leaf cells.

Gene **3** prevents this protein being digested in the rumen of sheep.

(b) The copy of Gene **1** used in this experiment was obtained from the mRNA of the sunflower seeds.

(i) Explain how enzymes could be used to obtain the gene from the mRNA.

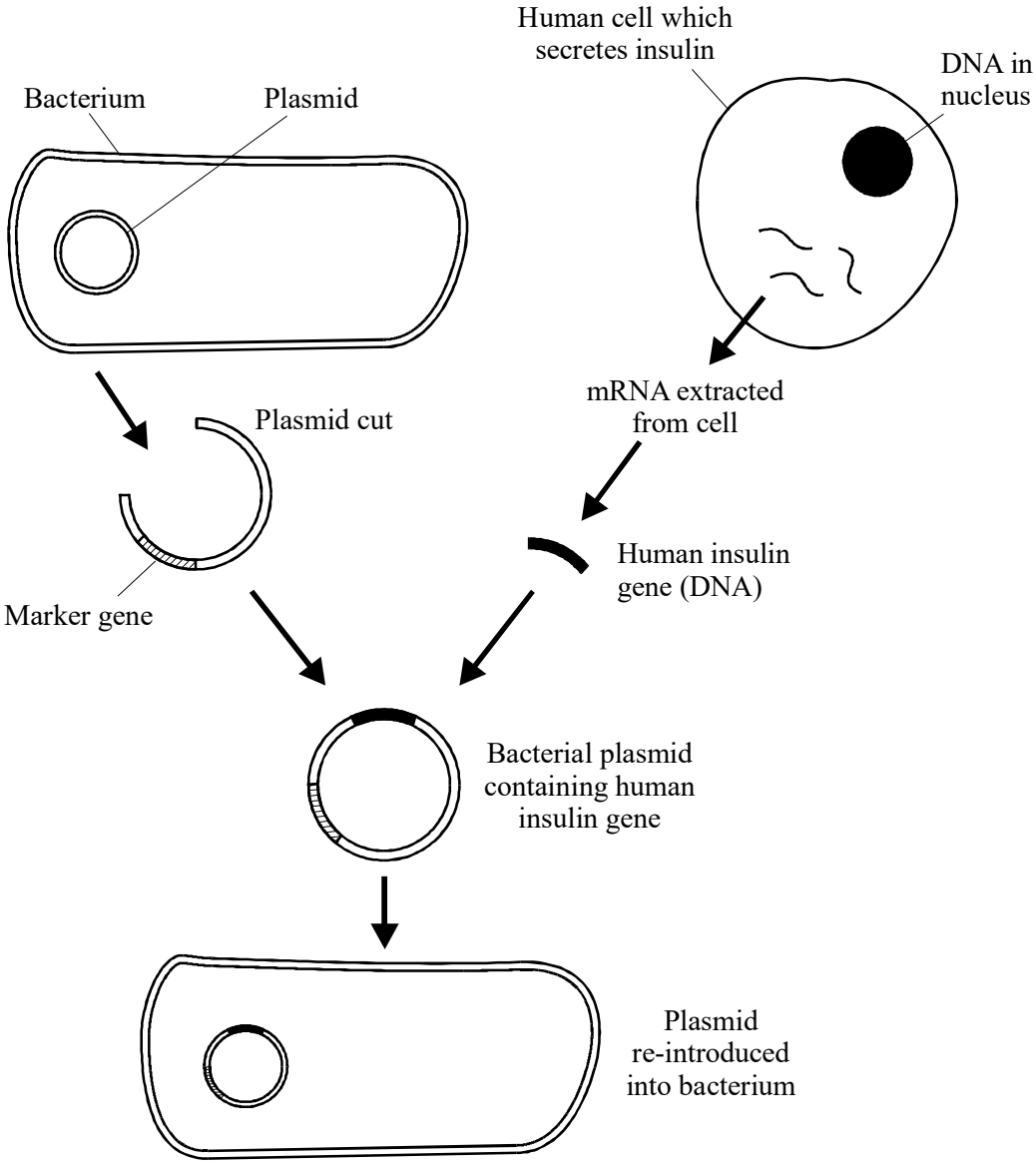
(3)

(ii) Explain why it would be an advantage to obtain the gene from the mRNA rather than from the DNA of the sunflower seeds.

(2)

(Total 7 marks)

3. The diagram shows how insulin can be made using genetically modified bacteria.



(a) (i) The human insulin gene is obtained from mRNA, rather than DNA. Suggest why.

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(1)

(ii) Name the enzyme used to make a single-stranded DNA copy of the mRNA coding for insulin.

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(1)

(iii) The table shows a sequence of bases from the mRNA coding for insulin. Complete the table to show the sequence of bases you would expect in the single-stranded DNA copy.

mRNA base sequence	U	C	A	A	C	C
DNA base sequence						

(1)

(b) What is the role of DNA ligase in producing genetically-modified bacteria?

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(1)

(c) The plasmid contains a marker gene coding for antibiotic resistance. Explain the importance of this marker gene.

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(2)

(Total 6 marks)

4. (a) Describe how a particular gene can be removed from the DNA of an animal cell.

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(2)

(b) Describe how this gene can then be inserted into the genetic material of a bacterium.

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(4)
(Total 6 marks)

5. Read the following passage.

Large numbers of possums in New Zealand are eating crops and spreading disease between cattle. The use of shotguns and poisons by farmers has not greatly reduced possum numbers. A better solution to the possum problem may have been found. A crop of carrots has been genetically modified to produce a „sterility protein". This sterility protein prevents possums producing offspring.

First, scientists identified the gene that codes for this sterility protein. Several copies of the sterility gene were cut out from long sections of DNA using a special enzyme. The same enzyme was also used to cut open plasmids which had been removed from bacterial cells. A different enzyme joined together the „sticky ends" of a plasmid and of a sterility gene to produce a recombinant plasmid.

The scientists then tried to put these plasmids back into the bacteria. Each plasmid also contained a gene, giving resistance to an antibiotic which normally kills bacteria. Because of this resistance gene, the scientists could identify bacteria containing the sterility gene and isolate them from bacteria which had not taken up this gene. Finally, carrot seedlings were sprayed with bacterial cells. The plasmids entered the carrot seedlings and carried copies of the sterility gene into the DNA of carrot cells.

The genetically modified crop will be harvested and the carrots scattered across land populated by possums.

Use information from the passage and your own knowledge to answer the following questions.

(a) Name the type of enzyme used to

(i) cut out the sterility gene (line 7)

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(1)

(ii) join together the plasmid and the sterility gene (lines 9 - 10)

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(1)

(b) Explain the meaning of the term „sticky ends" (line 9)

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(2)

(c) In this procedure the bacterial plasmids acted as vectors. Explain the function of a vector.

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(2)

(d) Explain how the presence of the antibiotic resistance gene allowed scientists to identify and isolate the bacteria which contain the sterility gene (lines 12 - 14)

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(3)

(e) Explain the arguments for and against using the genetically modified carrots to reduce the population of possums

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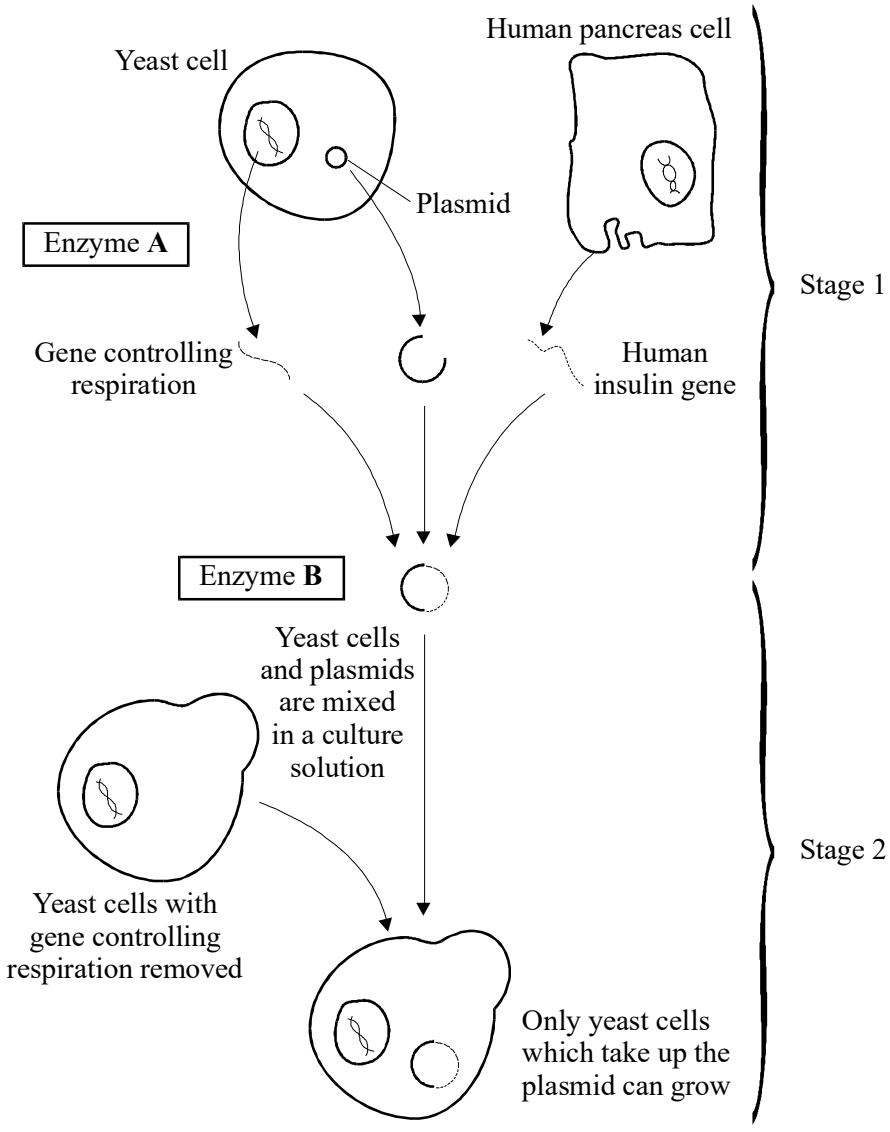
(6)

(Total 15 marks)

6. It is possible to genetically engineer yeast cells to manufacture human insulin. The flow diagram shows the main stages in this process.



(a) (i) Name

Enzyme **A** .....

Enzyme **B** .....

(2)

(ii) In **Stage 1,** the same enzyme is used to open the plasmid and to cut out the gene controlling respiration from the yeast DNA. Explain why

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(2)

(b) Explain why, in **Stage 2,** only yeast cells which are able to make insulin will survive.

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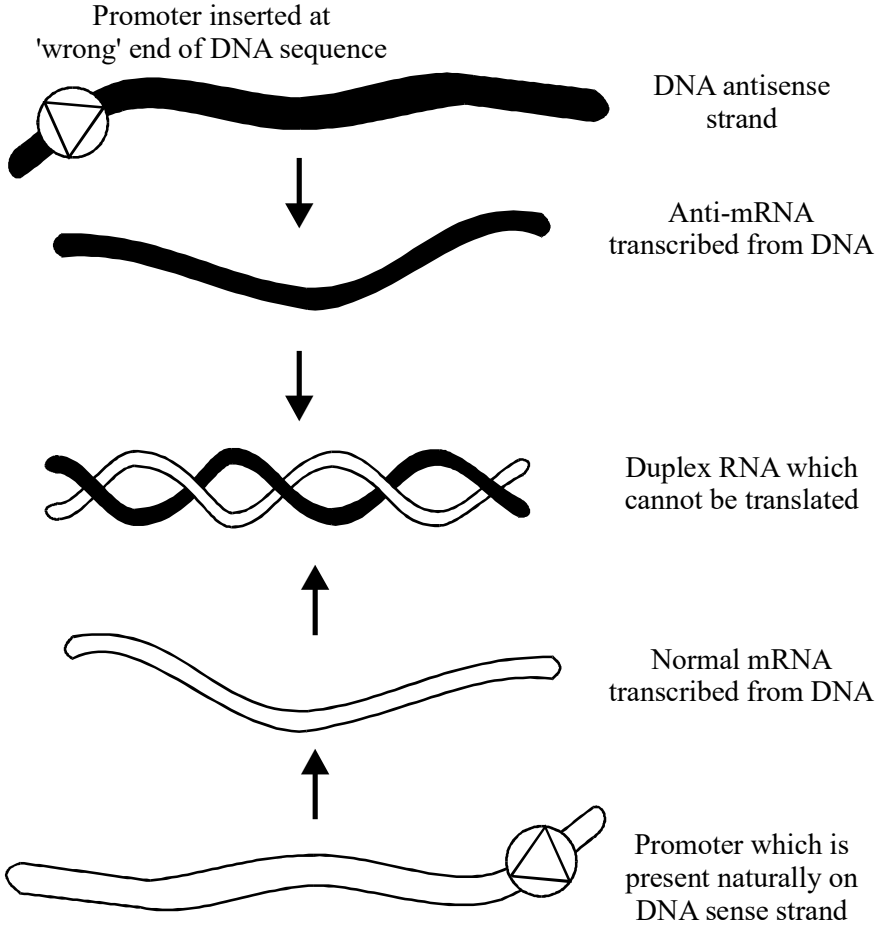
(4)

(Total 8 marks)

7. Read the following passage.

When a tomato ripens, it changes colour and develops its flavour. It also becomes softer. It gets softer because it produces an enzyme called polygalacturonase (PG). This enzyme causes the tomato cells to separate from each other. Genetic engineers have developed a way of producing tomatoes which are red and full of flavour but don't go soft and squashy. They have done this by producing tomato plants which produce less PG.
The diagram summarises the process. A molecule of DNA consists of two strands. The sense strand is the strand which is normally transcribed in the production of a protein. The other strand is the antisense strand. The method relies on producing an unreadable mRNA "duplex" by transcribing both the sense strand and the antisense strand of the DNA.



(a) Describe how transcription and translation result in the production of a protein from DNA.

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(6)

(b) The table shows the base sequence on part of the DNA sense strand. Complete the table by writing in the base sequence on the mRNA transcribed from:

(i) this part of the DNA sense strand;

(ii) the corresponding part of the DNA antisense strand.

Base sequence on DNA sense strand	A	T	G	G	C	A	T
(i) Base sequence on mRNA transcribed from DNA sense strand							
(ii) Base sequence on mRNA transcribed from DNA antisense strand							

(2)

(iii) Suggest why the promoter is needed.

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(1)

(c) (i) Explain why the two mRNA molecules transcribed from the sense and antisense strands of the DNA bind together to form a duplex.

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(2)

(ii) Give **two** ways in which the structure of the RNA duplex differs from the structure of DNA.

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(2)

(iii) The RNA duplex is double stranded. Explain why double stranded RNA cannot be translated to synthesise the enzyme PG.

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(2)

(Total 15 marks)

8. Read the following passage.

Scientists have recently determined the complete base-pair sequence of human DNA. They have found that only parts of the DNA molecule, called exons, are involved with the synthesis of proteins.

In order to find out what a particular part of the DNA molecule does, the DNA molecule must first be broken up into fragments. This is done by the use of restriction enzymes. These fragments can then be separated by electrophoresis. To help study the function of a particular DNA fragment, several copies of the fragment are required.

A plasmid from a bacterial cell can be used as a vector. The plasmid is removed from the bacterial cell to allow the insertion of the DNA fragment. The vector is replaced inside the bacterial host cell. Both the host cell and vector are then allowed to multiply under appropriate conditions. After multiplication, the vectors are removed from the host cell so that the copies of the DNA fragment can be extracted for further study.

Use information from the passage and your knowledge to answer the following questions.

(a) (i) What is an intron?

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(1)

(ii) Give **one** example of a base-pair found in DNA.

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(1)

(b) (i) Describe how a DNA fragment is inserted into a plasmid.

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(2)

(ii) A DNA fragment can be inserted into a plasmid because the plasmid also contains DNA. Suggest why the functioning of the plasmid DNA may be altered by the insertion of a DNA fragment.

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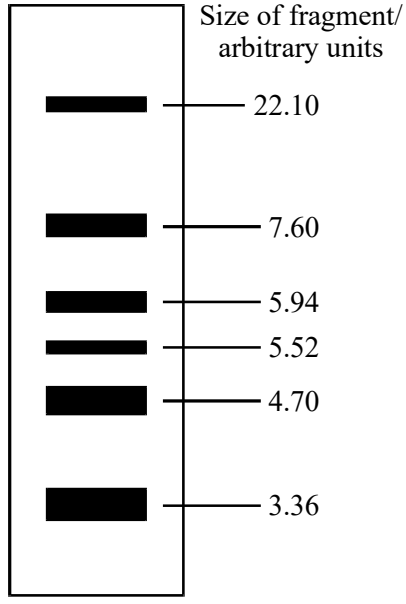
(1)

(c) The DNA molecule was split into fragments using the restriction enzyme *Eco*R1. Electrophoresis was used to separate these fragments of DNA. The diagram shows the result.



(i) Add an arrow to the diagram to show the direction in which the fragments moved during electrophoresis. Explain your answer.

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(1)

(ii) How many times does the sequence of bases recognised by the enzyme *Eco*R1occur in this section of DNA?

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(1)

(iii) The fragments of the DNA molecule contain genes. How can a DNA probe be used to show which fragment contains a particular gene?

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(2)

(d) A gene codes for the production of a specific protein. DNA extracted from a vector can be introduced into a different host cell where it can be transcribed and translated into a protein. Describe the processes of transcription and translation.

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(6)

(Total 15 marks)

9. Some snakes produce poisonous venom. Different poisonous snakes produce different types of venom. A person who has been bitten by a particular species of poisonous snake may be treated with the appropriate antivenom. Antivenoms consist of antibodies against venom. They are made by injecting an animal such as a horse with increasing doses of snake venom. The antibodies the horse produces are then extracted and purified.

The Brazilian pit-viper is an extremely poisonous snake. The main component of its venom is jararhagin. Jararhagin is a protein which breaks down tissues, rapidly causing the death of any animal which has been bitten.

DNA technology may soon provide a better way of making antivenom. Instead of injecting animals with venom, they are injected with DNA. In one trial antivenom was produced by injecting DNA, coding for jararhagin, into cells in mice. The mice responded by producing antibodies to the jararhagin.

(a) When a person is bitten by a poisonous snake, doctors try to identify the snake so that the correct type of antivenom can be used. Explain why the bite of a particular species of poisonous snake must be treated with the correct type of antivenom.

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(2)

(b) (i) The amino acid sequence of jararhagin is known. Explain how this information would enable a biologist to make an artificial gene which coded for jararhagin.

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(2)

(ii) The base sequence in this artificial gene may be different from the base sequence in the naturally occurring gene, even though they both code for the same protein. Use your knowledge of the genetic code to explain why.

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(2)

(iii) Describe how a cell from a mouse uses injected DNA to synthesise jararhagin protein (lines 10–12).

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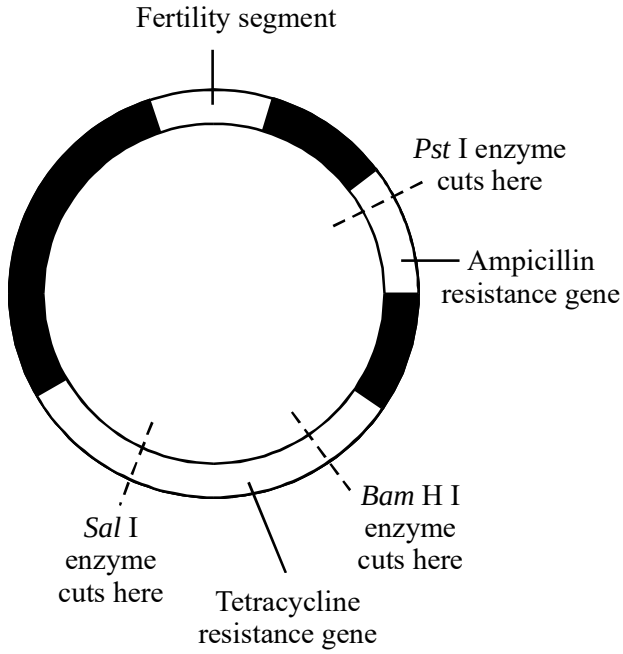
(6)
(Total 12 marks)

10. Read the following passage.

Recombinant DNA technology uses restriction enzymes to cut plasmid DNA at specific places. A gene from a different organism is located and then inserted in the plasmid. The plasmid is replaced in a bacterium which is then allowed to multiply. Plasmids may also contain marker genes. These make it possible to identify and isolate bacterial cells that
5. contain the plasmid with the relevant gene.

A plasmid from the bacterium *Escherichia coli* is often used as a vector in recombinant DNA technology. The diagram shows the structure of this plasmid. It also shows the sites where the plasmid can be cut by different restriction enzymes.



Use the information and your own knowledge to answer the following questions.

(a) Explain why the plasmid is described as a *vector* (line 6).

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(1)

(b) A DNA fragment, containing the gene for insulin, was inserted into this plasmid. To isolate bacteria that contained this recombinant plasmid, the bacteria were added to a culture medium containing the antibiotic ampicillin. Bacteria which contained the recombinant plasmid had lost resistance to ampicillin.

(i) Use the diagram to identify the restriction enzyme which had been used to insert the DNA fragment into the plasmid.

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(1)

(ii) Explain why resistance to ampicillin had been lost.

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(2)

(iii) Describe how a DNA probe could be used to confirm that the insulin gene was present in the DNA fragment.

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(3)

(c) Before a plasmid is used in recombinant DNA technology, the fertility segment is removed. This piece of DNA controls the process of conjugation in which different bacteria can link and pass DNA from one cell to the other. Explain why it is necessary to remove the fertility segment.

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(2)

(Total 9 marks)

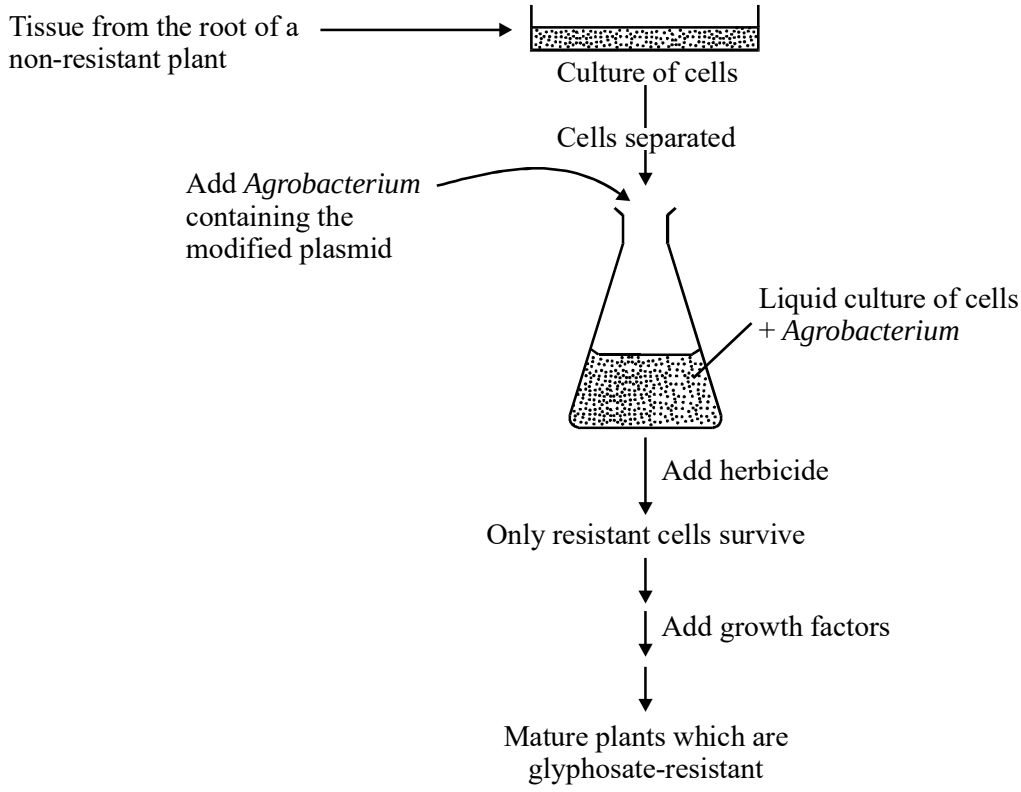
11. Read the following passage.

Genetic engineering is a technique for improving crop plants. It is a process by which a single new gene can be added to the genes already present in the plants.

One way of doing this is to use a natural genetic engineer, a soil microorganism called Agrobacterium. This bacterium possesses a plasmid which can be modified in the laboratory so that it becomes the carrier of new genetic information. For example, a gene coding for the ability to break down the herbicide glyphosate can be introduced with the assistance of the enzymes restriction endonuclease and ligase.

Once inside the crop plant, the gene for glyphosate breakdown makes the plant resistant to the effects of this herbicide. All surrounding weeds are destroyed when spraved with glyphosate.

The flow-chart shows how the plasmid carrying this gene can be used to produce glyphosate - resistant plants.



Use information from the passage and your own knowledge to answer the following questions.

(a) Describe how restriction endonuclease and ligase enzymes can be used in the formation of the modified plasmid (lines 4 – 7).

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(6)

(b) One mature plant grows from each glyphosate-resistant plant cell. Explain why all the cells of the mature plant contain the gene for glyphosate resistance.

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(2)

(c) Often in genetic engineering, plasmids containing genes for antibiotic resistance are used. These genes act as „markers".

(i) Explain why these markers are used.

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(2)

(ii) Use information from the flow-chart to explain why the use of such markers is **not** needed in the example described in the passage.

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(1)

(d) Suggest and explain **one** benefit and **one** possible problem associated with the use of herbicides together with genetically modified, herbicide-resistant crop plants.

Benefit .....

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Problem .....

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(4)

(Total 15 marks)

12. Every year, farmers in North America lose billions of dollars when their cattle get shipping fever. This is a disease caused by the bacterium *Mannheimia haemolytica*. It is often triggered by the stress of being transported and this is why it is called shipping fever. Unfortunately, controlling shipping fever by conventional vaccination is expensive, and also causes stress to the cattle.

The protein leukotoxin from *Mannheimia haemolytica* causes the symptoms of the disease. Scientists are working to develop an edible vaccine. They used a vector to insert part of the gene for leukotoxin into the DNA of white clover. White clover is a favourite food for cattle. Using the inserted DNA, the modified clover makes a polypeptide, which is part of the protein. Cattle injected with this polypeptide produce antibodies and these antibodies neutralise the leukotoxin.

(a) (i) Describe how an enzyme is used to remove part of the leukotoxin gene from *Mannheimia haemolytica*.

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(2)

(ii) Describe how this DNA could be inserted into white clover cells.

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(1)

(b) Only part of the leukotoxin gene was inserted into clover. Suggest **one** reason why the scientists did not attempt to insert the whole gene.

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(2)

(c) Describe how the modified clover plants made a polypeptide from the inserted DNA.

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(6)
(Total 11 marks)

13. **Figure 1** shows how the gene for human growth hormone (hGH) can be transferred into a bacterium.

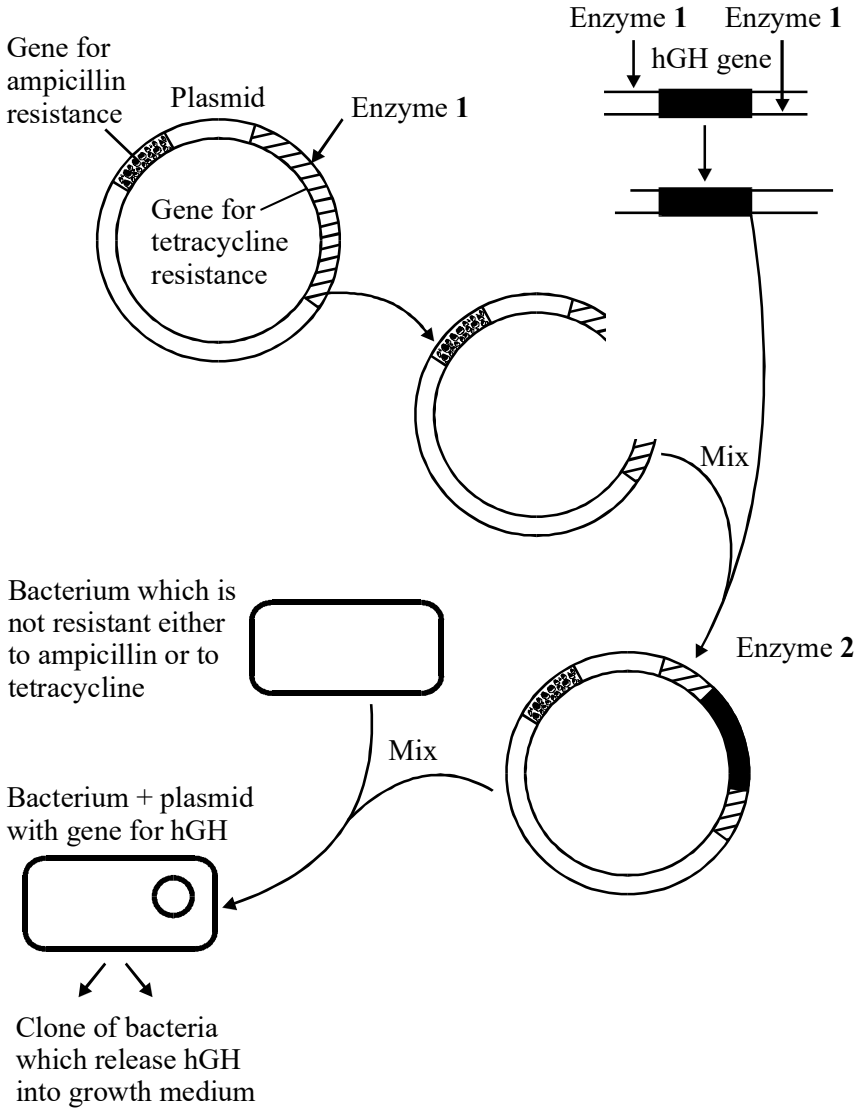

Figure 1

(a) Name

Enzyme **1**; .....

Enzyme **2**. .....

(2)

(b) After mixing with the plasmid, the bacteria are first grown in Petri dishes of agar containing ampicillin. What is the reason for this?

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(2)

(c) **Figure 2** shows how colonies of bacteria can be transferred from a Petri dish of agar containing ampicillin to identical positions on a Petri dish of agar containing tetracycline.

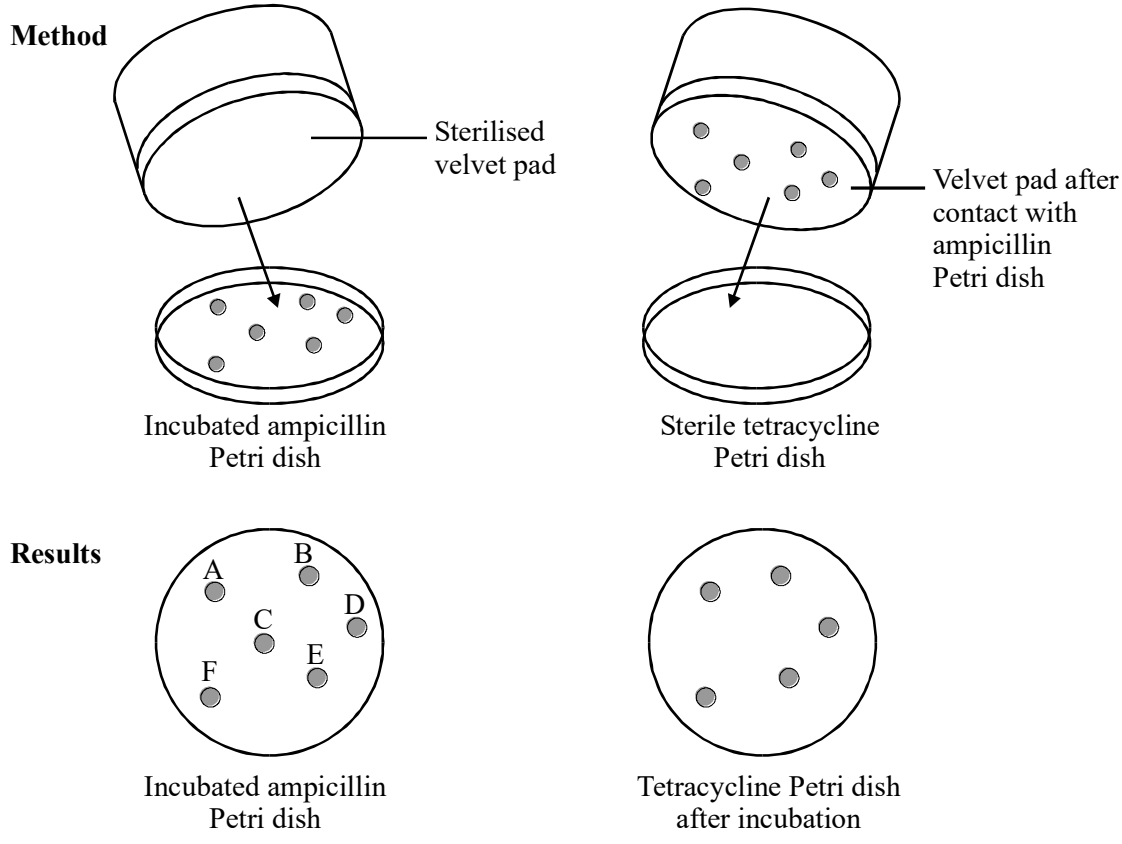

Figure 2

(i) Give the **letter** of **one** bacterial colony which contained a modified plasmid with the gene for hGH.

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(1)

(ii) Explain your answer to part (i)

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(2)

(d) Describe **one** possible danger of using plasmids which contain genes for antibiotic resistance.

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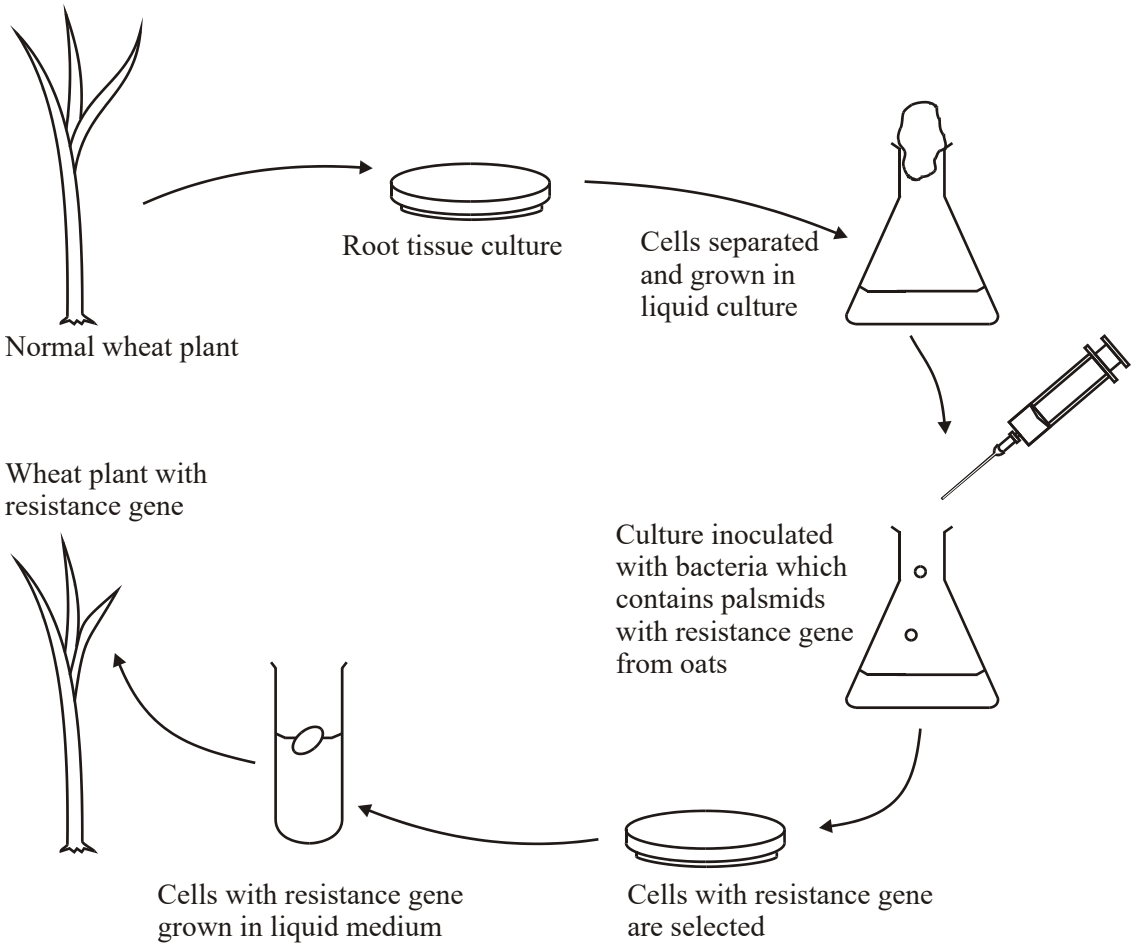
(2)

(Total 9 marks)

14. „Take-all" is a disease of wheat caused by a fungus. It can cause serious damage to the crop. There is no gene for resistance to this fungus in wheat. There is, however, a gene for resistance to this fungus present in oats.

The diagram shows how this gene might be transferred to wheat.



(a) (i) The wheat plant with the resistance gene contains recombinant DNA. What is *recombinant* DNA?

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(1)

(ii) The plasmids act as vectors for the resistance gene. What is a *vector*?

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(1)

(iii) Suggest how cells with the resistance gene might be selected.

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(2)

(b) A laboratory has oat plants containing the resistance gene and a supply of plasmids. Describe how bacteria may be produced which have the resistance gene in their plasmids.

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(6)
(Total 10 marks)

15. Read the following passage.

Malaria is a disease so deadly that it has devastated armies and destroyed great civilisations. It has been estimated that in the course of history malaria has been responsible for the death of one out of every two people who have ever lived. Even today, with all the advantages of modern technology, it is still responsible for some three million deaths a year.

The first half of the twentieth century was a time of hope for malarial control. The drugs chloroquine and proguanil had just been discovered and there seemed a real possibility of a malaria-free world. Unfortunately, this honeymoon ended almost as soon as it had started, with the emergence of drug-resistant parasite populations. Scientists now accept that whatever new drug they come up with, it is likely to have a very limited effective life. As a result, they are increasingly looking at combinations of drugs.

The approach to malaria control which holds the best hope is the production of a vaccine. One of these is being developed by a researcher in South America. His vaccine is based on a small synthetic polypeptide called SPf66 which is dissolved in a saline solution and given as an injection. A series of early trials on human volunteers produced confusing results. In one trial the effectiveness of the vaccine was claimed to be 80% while, in others, the results were statistically insignificant. Not only were the results inconclusive but the methods used were challenged by other scientists. In particular, the controls were considered inappropriate.

Another, possibly more promising, approach has been the development of a DNA-based vaccine. In theory, all that is required is to identify the DNA from the parasite which encodes key antigens. Unfortunately, scientists have hit snags. Although they have succeeded in sequencing the human genome, the genome of the malarial parasite has created major difficulties. This is partly because of the very high proportion of the bases adenine and thymine. In some places these two bases average 80%, and on chromosomes 2 and 3 nearly 100% of the bases present are adenine and thymine. Because of this, it has proved impossible to cut the relevant DNA with the commonly available restriction enzymes into pieces of a suitable size for analysis.

Use information from the passage and your own knowledge to answer the following questions.

(a) Explain how a resistant parasite population is likely to arise and limit the life of any new anti-malarial drug (lines 8 - 9).

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(3)

(b) A person has a 1 in 500 probability of being infected by a chloroquine-resistant strain of malarial parasite and a 1 in 500 probability of being infected by a proguanil-resistant strain. Use a calculation from these figures to explain why scientists are "increasingly looking at combinations of drugs" (lines 9 - 10).

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(2)

(c) (i) Explain why trials of the SPf66 vaccine needed a control.

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(1)

(ii) The controls for the SPf66 vaccine trials were considered inappropriate (line 17). Suggest how the control groups in these trials should have been treated.

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(2)

(d) In some of the DNA of a malarial parasite, the proportion of adenine and thymine bases averages 80% (lines 22 - 23). In this DNA what percentage of the nucleotides would you expect to contain

(i) phosphate; .....

(ii) guanine? .....

(2)

(e) (i) Use your knowledge of enzymes to explain why restriction enzymes only cut DNA at specific restriction sites.

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(3)

(ii) Restriction enzymes that can cut the DNA of chromosomes 2 and 3 produce pieces that are too small for analysis. Explain why these restriction enzymes produce small DNA fragments.

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(2)

(Total 15 marks)

16. (a) (i) Some human DNA was cut into separate pieces using a restriction enzyme which produced a staggered cut. A scientist wanted to insert these pieces of DNA into plasmids and used the same restriction enzyme to cut the plasmids.
Explain why the pieces of human DNA would be able to join to the cut DNA of the plasmids.

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(2)

(ii) Which other enzyme must the scientist have added to the mixture to form recombinant plasmids?

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(1)

(b) A plasmid may be used as a vector. Explain what is meant by a *vector*.

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(2)

(c) Molecular biologists often use plasmids which contain antibiotic resistance genes. Explain the reason for this.

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(2)
(Total 7 marks)

17. (a) (i) Some human DNA was cut into separate pieces using a restriction enzyme which produced a staggered cut. A scientist wanted to insert these pieces of DNA into plasmids and used the same restriction enzyme to cut the plasmids. Explain why the pieces of human DNA would be able to join to the cut DNA of the plasmids.

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(2)

(ii) Which other enzyme must the scientist have added to the mixture to form recombinant plasmids?

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(1)

(b) A plasmid may be used as a vector. Explain what is meant by a *vector* in this context.

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(2)

(c) Molecular biologists often use plasmids which contain antibiotic resistance genes. Explain the reason for this.

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(2)
(Total 7 marks)

18. Scientists are working to produce a genetically modified bacterium to treat patients suffering from a disease of the digestive system. They plan to collect mRNA from human cells. This will be used to produce the DNA of the gene for the protein interleukin. They will then transfer this human gene into the bacterium *Lactococcus*. The scientists intend patients to swallow the genetically modified bacteria. These bacteria will release interleukin inside the digestive system to treat the disease.

(a) (i) Name the type of enzyme which will be used to produce the DNA from the mRNA.

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(1)

(ii) It is easier to obtain the interleukin gene from mRNA rather than directly from the DNA removed from human cells. Explain why.

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(1)

(b) The scientists propose to put the gene directly into the DNA of *Lactococcus*. Describe the role of the enzyme ligase in this process.

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(1)
(Total 3 marks)

19. A gene was broken into fragments using enzyme **Z**. The mixture of fragments produced was then separated by electrophoresis.

(a) What type of enzyme is enzyme **Z**?

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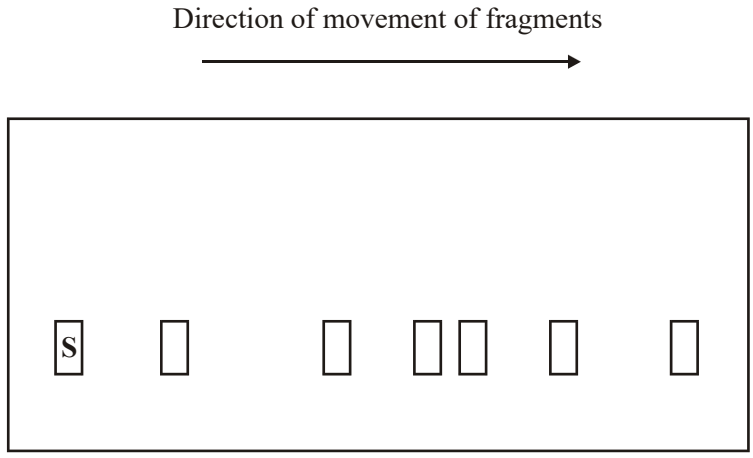
(1)

The table shows the number of base pairs present in the fragments.

Fragment	**Number of base pairs ($\times 10^3$)**
1	4.65
2	5.72
3	10.71
4	2.39
5	5.35
6	7.53

The diagram shows the electrophoresis gel used. The mixture of fragments was placed at the start point marked **S** and the process started. The boxes indicate the positions reached by the different fragments.



(b) Explain why base pairs are a suitable way of measuring the length of a piece of DNA.

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(2)

(c) (i) Write **6** above the appropriate box on the diagram to show the position you would expect fragment **6** to have reached.

(1)

(ii) Explain how you arrived at your answer.

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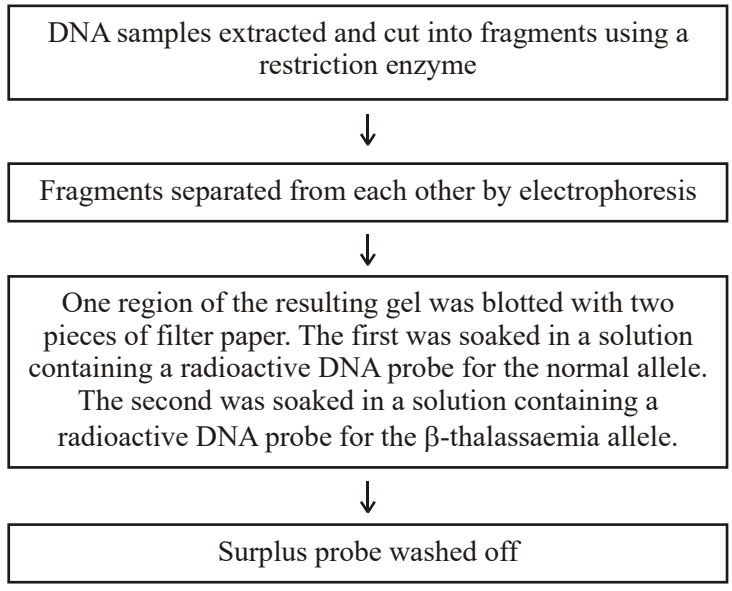
(1)

(d) Enzyme **Z** recognises a particular sequence of bases in the gene. How many times does this sequence appear in the DNA of this gene?

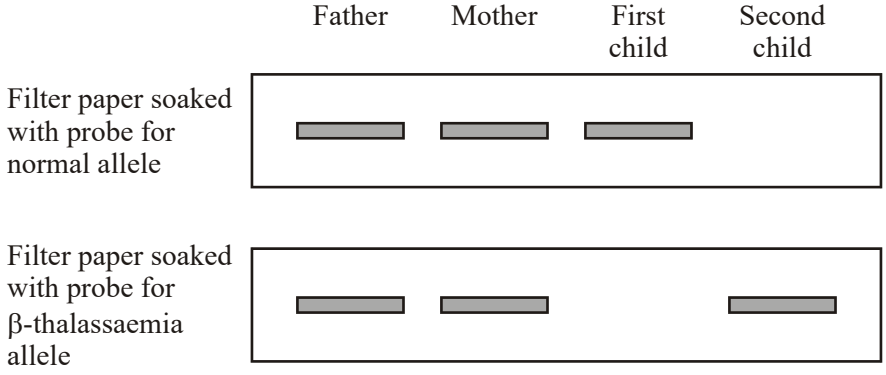
.....……..... **(1)**

(Total 6 marks)

20. β-thalassaemia is a genetic condition in which abnormal haemoglobin is produced. In one form, the recessive allele for β-thalassaemia, **t**, differs from the normal allele, **T**, by a single base-pair. A radioactive DNA probe was used to investigate the genotypes of four members of one family. The flowchart summarises the technique involved.



The diagram below shows the appearance of the two pieces of filter paper which resulted from the investigation.



(a) What is the probability that the next child that this couple have is a girl who has β-thalassaemia? Explain your answer.

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(3)

(b) (i) The fragment of DNA containing the normal allele and the fragment with the β-thalassaemia allele moved the same distance on the gel. Explain why.

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(2)

(ii) The allele for β-thalassaemia differs from the normal allele by only one base-pair. Explain why the probe used to identify these alleles consists of a piece of DNA twenty bases in length and not just one base.

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(2)

(Total 7 marks)

21. Read the following passage.

DNA tests were used to confirm the identity of deposed Iraqi leader Saddam Hussein, after his capture in December 2003. DNA tests were carried out to prove the suspect was not one of the many alleged "look alikes" of the former leader.

Firstly, the DNA was extracted from the mouth of the captured man using a swab. Great care was taken to check that the swab did not become contaminated with any other DNA. DNA extracted from the swab was then subjected to a standard technique called the polymerase chain reaction (PCR), which takes a couple of hours. Lastly, the sample was "typed" to give the genetic fingerprint. This was produced within 24 hours of Saddam Hussein's capture. Tests for use in criminal cases often take much longer because samples are very small or contaminated.

It appears that Hussein's genetic fingerprint was already stored away for comparison. This was obtained from personal items such as his toothbrush. DNA from the toothbrush would have been subjected to PCR before a DNA fingerprint could have been obtained.

Source: adapted from SHAONI BHATTACHARYA, *New Scientist* 15 December, 2003

Use information from the passage and your own knowledge to answer the questions.

(a) Describe how the technique of genetic fingerprinting is carried out and explain how it can be used to identify a person, such as Saddam Hussein.

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(6)

(b) Explain how DNA could be present on a toothbrush (line 12).

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(2)

(c) (i) Explain why the polymerase chain reaction was used on the sample of DNA from the toothbrush (lines 12-13).

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(2)

(ii) Explain **one** way in which the polymerase chain reaction differs from DNA replication in a cell.

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(2)

(d) Tests for use in criminal cases often take much longer because samples are very small or contaminated (lines 8-10). Explain why it takes longer to obtain a genetic fingerprint if the sample is

(i) very small;

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(1)

(ii) contaminated.

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(2)

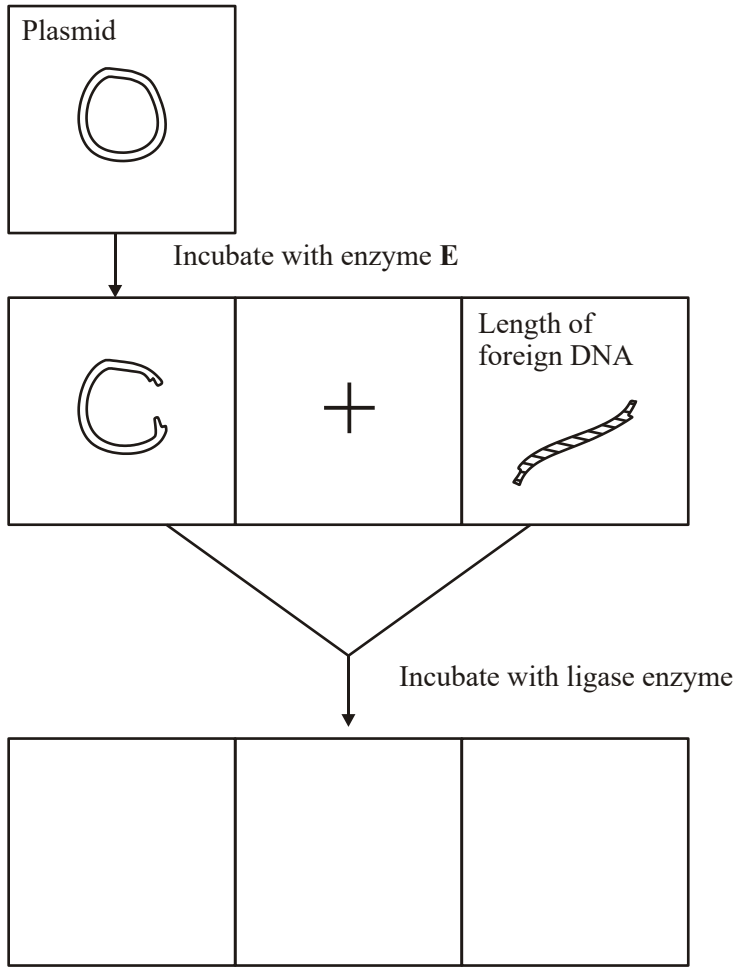
(Total 15 marks)

22. *Write an essay on the following topic. You should select and use information from different parts of the specification. Credit will be given for the biological content. It will also be given for the selection and use of relevant information, and for the organisation and presentation of the essay.*

Bacteria affect the lives of humans and other organisms in many ways.
Apart from causing disease, describe how bacteria may affect the lives of humans and other organisms.

(Total 25 marks)

23. Plasmids can be used as vectors to insert lengths of foreign DNA into bacteria. The diagram shows how this is achieved.



(a) Name enzyme **E**.

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(1)

(b) Cut plasmids and lengths of foreign DNA can join. What features of their ends allows them to join?

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(2)

(c) Draw **three** different structures that could be formed by incubating cut plasmids and lengths of foreign DNA with ligase. Use the spaces provided on the diagram.

(3)
(Total 6 marks)

24. Tobacco plants do not grow well in salty soil. Scientists have used genetic engineering techniques to insert a gene for salt resistance from the bacterium *Escherichia coli* into the DNA of tobacco plants.

(a) Describe how scientists could

(i) remove the gene for salt resistance from the DNA of the bacterium *Escherichia coli*;

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(2)

(ii) insert this gene into the DNA of a tobacco plant.

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(2)

(b) Briefly describe how you could test whether these genetically engineered tobacco plants do grow better than normal tobacco plants in salty soil.

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(3)
(Total 7 marks)

25. Epidemiology has shown that there is a link between high fat diets and cancer of the colon, but this type of research tells us nothing about how such a diet actually causes the disease. More recently, investigators have tried to identify the events which take place before a malignant tumour develops. They have been looking for biological markers which show that there is an increased risk of cancer. Some of these markers are related to stages along the pathway from being exposed to a risk factor to developing cancer. These markers include particular chemicals attached to molecules of DNA, mutation of specific genes and abnormal cell growth. Other markers are associated with genetic factors such as inherited inefficiencies in destroying carcinogens, repairing DNA or in the way in which the immune system recognises tumour cells. This work has helped us to understand that malignant tumours usually arise from accumulated damage to the genes present in a single cell.

(a) The base sequence of a specific gene is known. Explain how a mutation of this gene could be detected in a sample of cells from human blood.

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(3)

(b) Suggest how the information acquired through research on biological markers could be used to reduce deaths from cancer.

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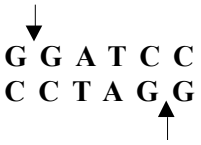
(3)

(Total 6 marks)

26. A restriction endonuclease cuts DNA at a particular base sequence. The restriction endonuclease, *Bam* H1, recognises the sequence of six bases as shown in the diagram and cuts the DNA to form sticky ends. The arrows show where *Bam* H1 cuts the DNA.



(a) Draw the sticky ends which are produced when *Bam* H1 has cut the DNA.

(1)

(b) Describe how the two polynucleotide chains of DNA are normally held together.

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(2)

(c) The enzyme DNA ligase is used to join together pieces of DNA from different sources. Explain why the DNA to be joined together must be cut with the **same** restriction endonuclease before DNA ligase is used.

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(2)

(Total 5 marks)

27. In 1987 an attempt was made to produce human insulin from genetically engineered yeast. Genes for human insulin were inserted into small loops of DNA called plasmids. These plasmids were then used to try to carry the insulin genes into yeast cells.

(a) Describe how an insulin gene could be removed from human DNA and inserted into the plasmid DNA.

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(4)

(b) The yeast used to produce insulin was a mutant strain that did not have the gene for an enzyme needed by the yeast in respiration. The missing enzyme is called triose phosphate isomerase. The DNA of the plasmids, however, did contain the gene for triose phosphate isomerase.

Explain the importance for insulin production of using plasmids that have the gene for triose phosphate isomerase and a mutant yeast that does not have this gene.

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(3)

(Total 7 marks)

28. (a) A herbicide, glyphosate. works by blocking the activity of an enzyme needed for amino acid synthesis. A mutant petunia plant was found which contained a gene giving resistance to glyphosate. This gene was transferred into tomato plants.

(i) Describe how the gene may have been transferred from the mutant petunia into tomato plants.

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(3)

(ii) Suggest how the gene from the mutant petunia may give resistance to glyphosate.

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(1)

(b) (i) Explain how growing herbicide-resistant plants may lead to a higher yield of tomatoes.

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(2)

(ii) Describe **one** environmental risk associated with growing herbicide-resistant tomato plants.

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(1)

(Total 7 marks)

29. Researchers have produced cows which make milk with high levels of α-lactalbumin, a protein found in human breast milk.
The gene for α-lactalbumin production was removed from human DNA. A fertilised egg was obtained from a cow and was injected with the gene. The fertilised egg was then placed in the uterus of the cow. The calf that developed contained the gene in all the cells of its body. This calf matured into a cow which produced milk containing α-lactalbumin. Half of her offspring were found to contain the α-lactalbumin gene.

(a) Describe how the α–lactalbumin gene could be removed from human DNA.

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(2)

(b) Explain why all the cells of the body of the calf contained the α–lactalbumin gene.

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(2)

(c) When the calf developed into a cow, half of her offspring contained the α–lactalbumin gene. Explain why.

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(2)
(Total 6 marks)

30. Genetic engineering has made it possible to transfer genes from one species to another. For example, a gene that gives resistance to herbicide and another gene which gives resistance to insect attack have been transferred into maize. Some people think that there will be great advantages in growing maize with these genes. Others are equally convinced that there are long-term dangers in growing crops of this maize.

Evaluate both of these viewpoints.

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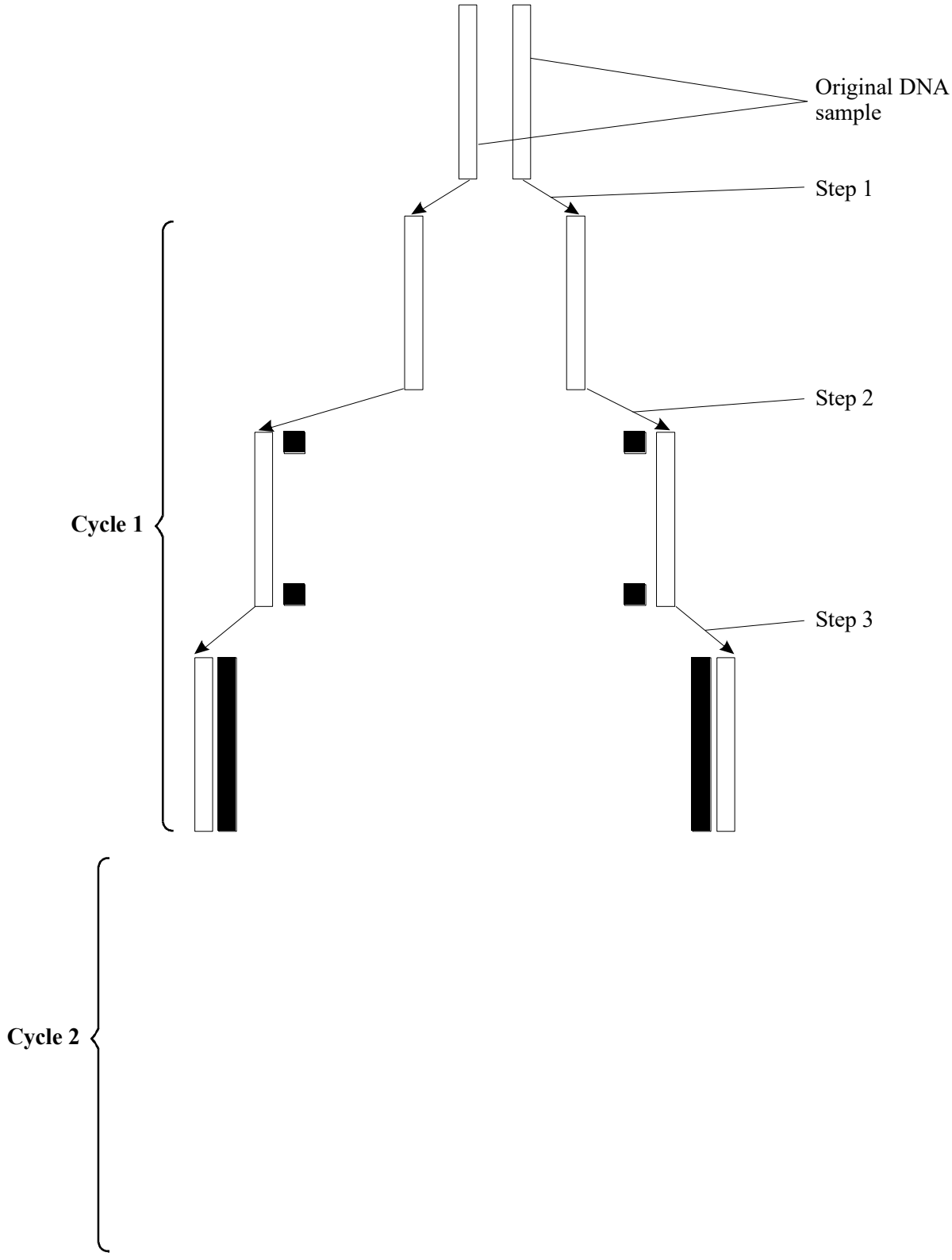
(Total 6 marks)

31. The diagram illustrates the polymerase chain reaction.



(a) (i) What method is used to split the original DNA sample into two strands during Step **1**?

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(1)

(ii) Describe what happens during Steps **2** and **3** in order to produce a new DNA strand.

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(3)

(b) On the diagram, draw the DNA molecules that would be present at the end of **Cycle 2.** Use the same method of shading to distinguish between original DNA strands and the new DNA strands.

(2)

(c) Describe **one** example of the use of the polymerase chain reaction.

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(2)

(Total 8 marks)

32. A new variety of tomato has been produced by genetic engineering. This variety contains a synthetic gene that blocks the action of a natural gene that would make the fruit soften rapidly once ripe. It also contains a marker gene.

The marker gene added by the scientists makes this variety of tomato resistant to the antibiotic, kanamycin. It is possible that this gene could be taken up by disease-producing bacteria in the human gut. In humans, kanamycin is used to treat certain types of gut infections.

Using information from the passage, explain the advantages and disadvantages of putting this new variety of tomato on the market.

Advantages

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Disadvantages

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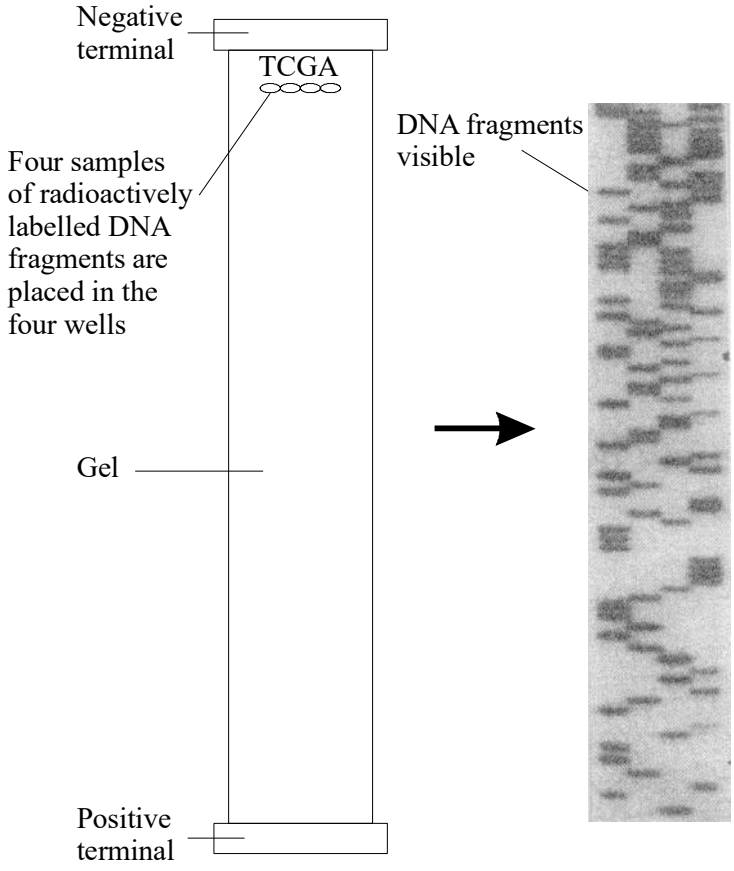
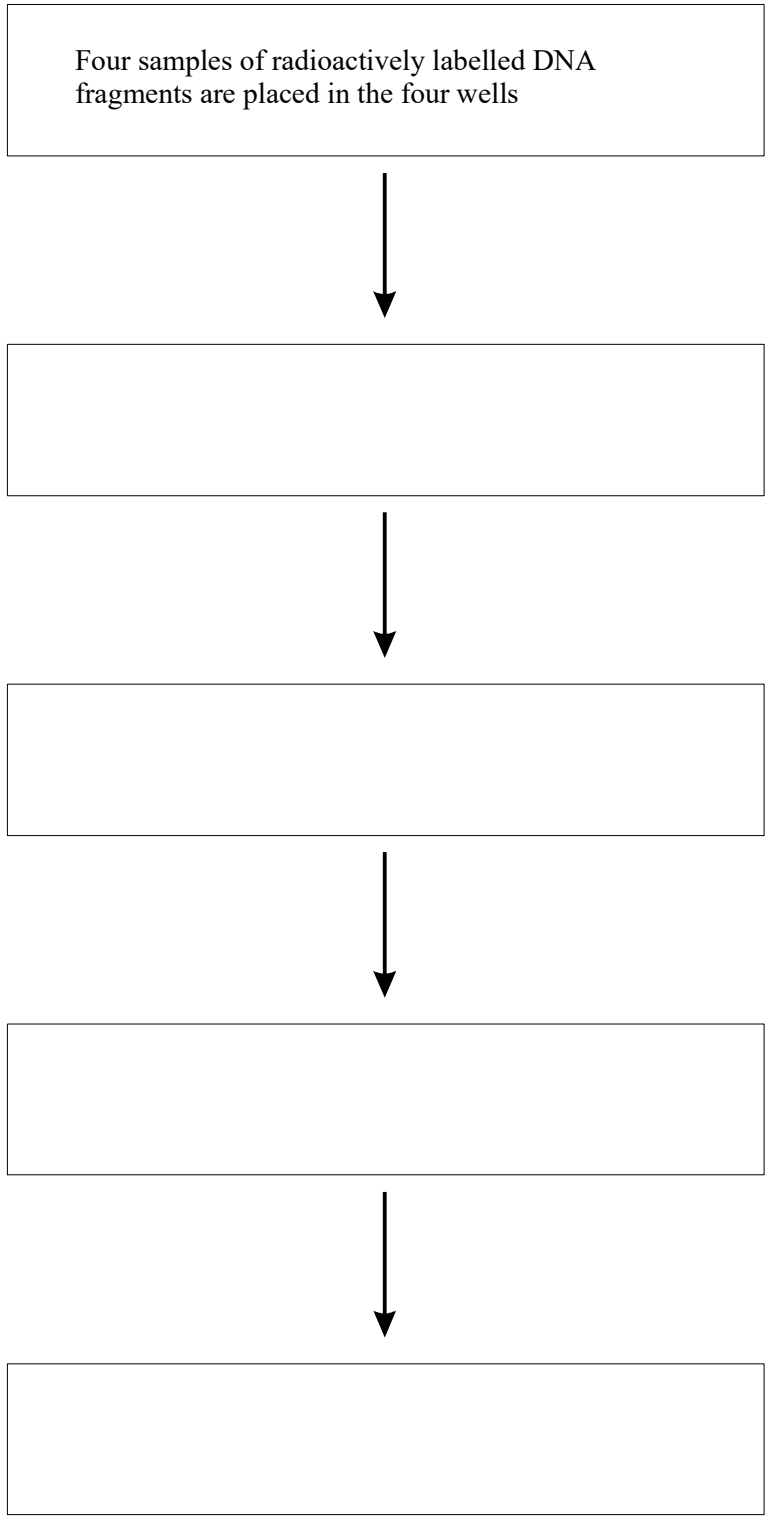
(Total 5 marks)

33. DNA sequencing is a technique used to find the sequence of nucleotides in a sample of DNA.
Enzymes are used to cut the DNA sample into fragments of different lengths.
The ends of these fragments are then labelled using radioactive probes.
Four different probes attach to the end of DNA fragments in which the terminal nucleotide is adenine, cytosine, thymine and guanine respectively. The labelled fragments are then separated.

The diagram shows apparatus used to separate the DNA fragments and the end result of the process.



(a) (i) Use the information from the diagram and your knowledge of DNA sequencing techniques to draw a flow chart for the process. The first box has been completed for you.



(4)

(ii) Why do the DNA fragments move different distances in the gel?

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(1)

(iii) This technique is being used to find the DNA sequences of human chromosomes. Give **one** advantage to humans of determining this DNA sequence.

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(1)

(b) Describe how genetic engineering is used to produce alpha-1-antitrypsin which is used to treat cystic fibrosis.

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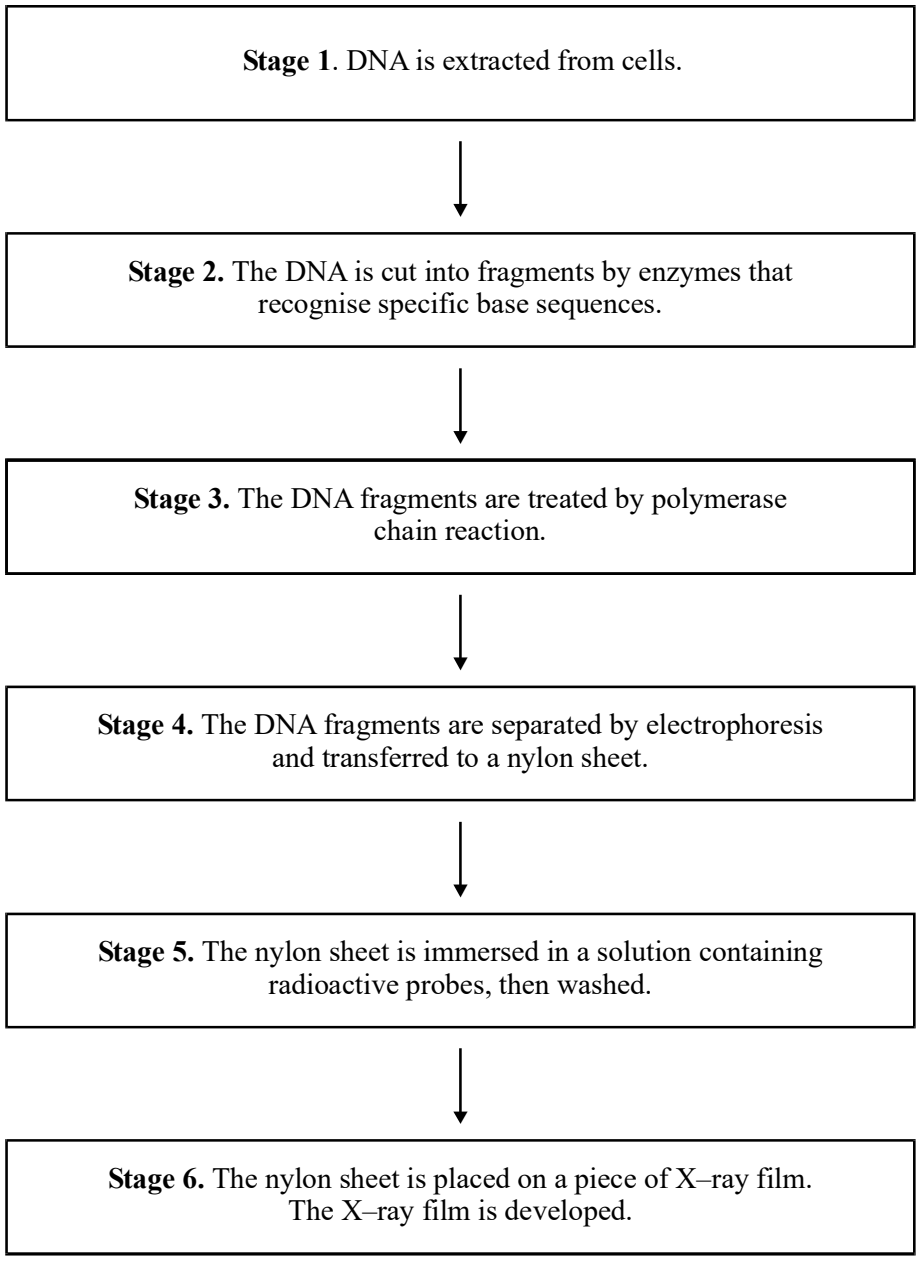
(6)
(Total 12 marks)

34. The flow chart shows one method of finding the sequence of nucleotides in DNA.



(a) Name the type of enzyme which is used in **Stage 2.**

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(1)

(b) Why is the polymerase chain reaction used in this procedure **(Stage 3)**?

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(1)

(c) What is meant by electrophoresis **(Stage 4)**?

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(1)

(d) (i) In Stage 5, the nylon sheet is immersed in a solution containing radioactive probes. Explain the function of these radioactive probes **(Stage 5)**.

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(ii) Explain why only some nucleotide sequences show up when the X-ray film is developed.

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(4)

(Total 7 marks)

35. (a) (i) Describe how a gene may be taken from a mammalian cell and inserted into bacterial cells.

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(5)

(ii) Describe how bacteria containing the transferred gene can be cultured on a large scale.

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(2)

(b) Mutation of the gene responsible for production of the enzyme galactosidase causes a liver disorder. The liver is unable to metabolise certain carbohydrates, and toxic products accumulate.

(i) Describe **one** way in which the structure of the DNA of a gene may be changed as a result of a mutation.

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(2)

(ii) The disorder can be treated by introducing the gene for galactosidase into a harmless virus, then injecting the transformed virus into the patient. A liver cell containing DNA from the transformed virus produces galactosidase. Describe how a cell synthesises a protein such as galactosidase.

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(5)

(iii) The transformed virus enters liver cells but does not usually enter other cells in the body.

Suggest **one** explanation for this.

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(1)

(Total 15 marks)

36. Haemophilia is a genetic disease in which blood fails to clot. Factor IX is a protein used to treat haemophilia.

(a) Factor IX can be made by cloned mammalian cells. Explain what is meant by a clone.

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.....

(1)

(b) The DNA sequence that codes for factor IX protein is extracted from human DNA. This human DNA sequence is then introduced into a fertilised egg from a sheep.
Describe **one** way, other than using enzymes, in which a DNA sequence could be introduced into fertilised eggs.

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(1)

(c) (i) Another technique is to treat a culture of human body cells chemically so that the cells will take up a DNA sequence. Explain **one** advantage of using a culture of human body cells rather than fertilised eggs from sheep.

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(1)

(ii) **An** antibiotic resistance gene is added to the cultured human body cells along with the gene for factor IX protein. A dose of antibiotic concentrated enough to kill human cells is then added to the cell structure.

Suggest **one** explanation for adding the resistance gene and using the antibiotic.

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(2)

(iii) Some scientists wish to use this technique to clone cells from human embryos. The cloned cells could then be implanted into humans to cure diseases such as muscular dystrophy or Parkinson's disease.
Use your knowledge and understanding of genetic engineering to explain the arguments for and against using cells from human embryos in this way.

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(6)
(Total 11 marks)

37. Insulin is a protein. **Table 1** shows the amino acids from a section of an insulin molecule and the mRNA nucleotide bases which code for them. The identity of one amino acid has not been given.

Amino acid	cysteine	serine	X	tyrosine
mRNA nucleotides	UGU	AGC	UAC	UAU

Table 1

(a) (i) What name is given to the sequence of three mRNA nucleotides which codes for one amino acid?

.....

(1)

Some of the DNA nucleotide sequences coding for amino acids are shown in **Table 2**.

Amino acid	DNA nucleotide sequence
Phenylalanine	AAA AAG
Cysteine	ACA ACG
Serine	TCA TCG
Tyrosine	ATA ATG

Table 2

(ii) Name amino acid X in **Table 1**.

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(1)

(b) Some mutations occur when one nucleotide is substituted by a different nucleotide in a strand of DNA.

(i) Explain how the substitution of a nucleotide may cause a gene to code for a different protein.

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(3)

(ii) Some nucleotide substitutions have no effect on the protein coded by the gene. Use information given in **Table 2** to explain why.

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(2)

(c) A recombinant plasmid is produced by inserting a fragment of foreign DNA into a plasmid. Explain how enzymes are used to produce a recombinant plasmid.

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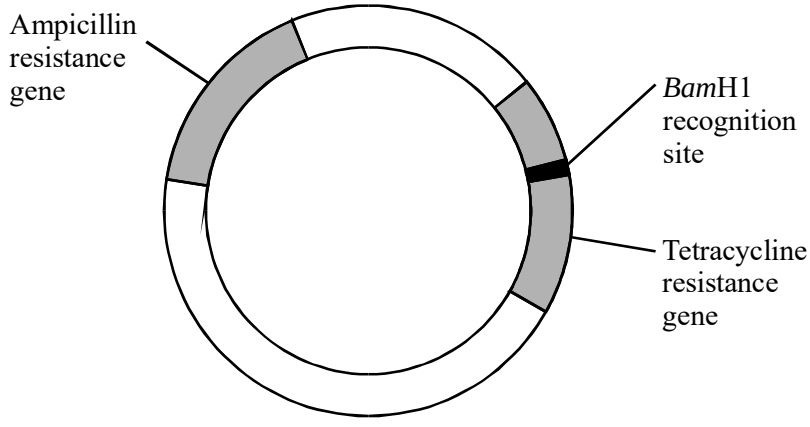
(3)

(d) The plasmid pBR322 is used in genetic engineering. The plasmid contains resistance genes for the antibiotics ampicillin and tetracycline and a recognition site for the restriction endonuclease *Bam*H1.



Recombinant plasmids were produced by inserting a fragment of foreign DNA into pBR322 using *Bam*H1. The recombinant plasmids were mixed with a culture of bacteria. Some bacteria took up the plasmid.

Explain how bacteria that contain the pBR322 recombinant plasmid could be separated and identified using the technique of replica plating.

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(5)
(Total 15 marks)

38. The gene for alpha-1-antitrypsin was isolated from human white blood cells and multiplied by the polymerase chain reaction.

(a) Explain the reason for each of the following stages in the polymerase chain reaction.

(i) heating the sample of DNA above 90 °C

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(1)

(ii) adding primers

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(2)

(iii) using polymerase enzymes obtained from bacteria that live at high temperatures

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(2)

(b) One cycle of the polymerase chain reaction takes 5 minutes to complete. Starting with a single gene, calculate the number of copies of the gene produced in 40 minutes.

Answer: .....

(1)

(c) Genetic engineering has been used to introduce the human gene for alpha-1-antitrypsin into sheep. The sheep produce alpha-1-antitrypsin in their milk.

(i) Give **one** advantage of producing alpha-1-antitrypsin in this way.

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(1)

(ii) Suggest **three** reasons why people may be concerned about using genetic engineering in this way.

1 .....

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2 .....

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3 .....

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(3)

(Total 10 marks)

39. Liposomes are tiny droplets made of lipid molecules. They are sometimes used in gene therapy to treat people suffering from cystic fibrosis.

(a) Explain the meaning of *gene therapy*.

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(1)

(b) A defective protein causes the symptoms associated with cystic fibrosis.

How does the sequence of amino acids in the defective protein differ from the sequence in the protein of a healthy person?

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(1)

(c) (i) Explain how it may be possible for a person with a genetic abnormality to be cured using gene therapy.

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(1)

(ii) Explain why a person cured by gene therapy may still have children who suffer from the abnormality.

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(2)
(Total 5 marks)

W €**40.** Locations where the restriction endonuclease enzymes *Eco*RI and *Hpa*II cut a plasmid are shown in the diagram.

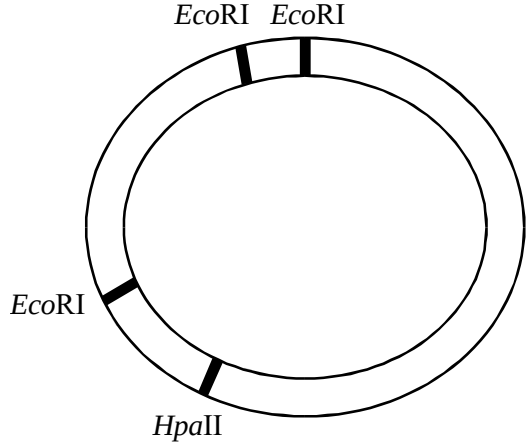

Figure 1

(a) Explain why *Eco*RI cuts the plasmid at different locations from *Hpa*II.

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(1)

(b) *Eco*RI was used to cut the plasmid into DNA fragments.

(i) Explain why the cut ends of the plasmids are able to join to each other to form new plasmids.

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(2)

(ii) Suggest why these new plasmids differ in size from one another.

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(1)

(iii) Name the type of enzyme used to join the DNA fragments.

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(1)

(c) In a different experiment, *Eco*RI was used to cut the plasmid shown in **Figure 1** but the fragments obtained were not rejoined. These fragments were separated by electrophoresis. The pattern of bands shown in **Figure 2** was obtained.

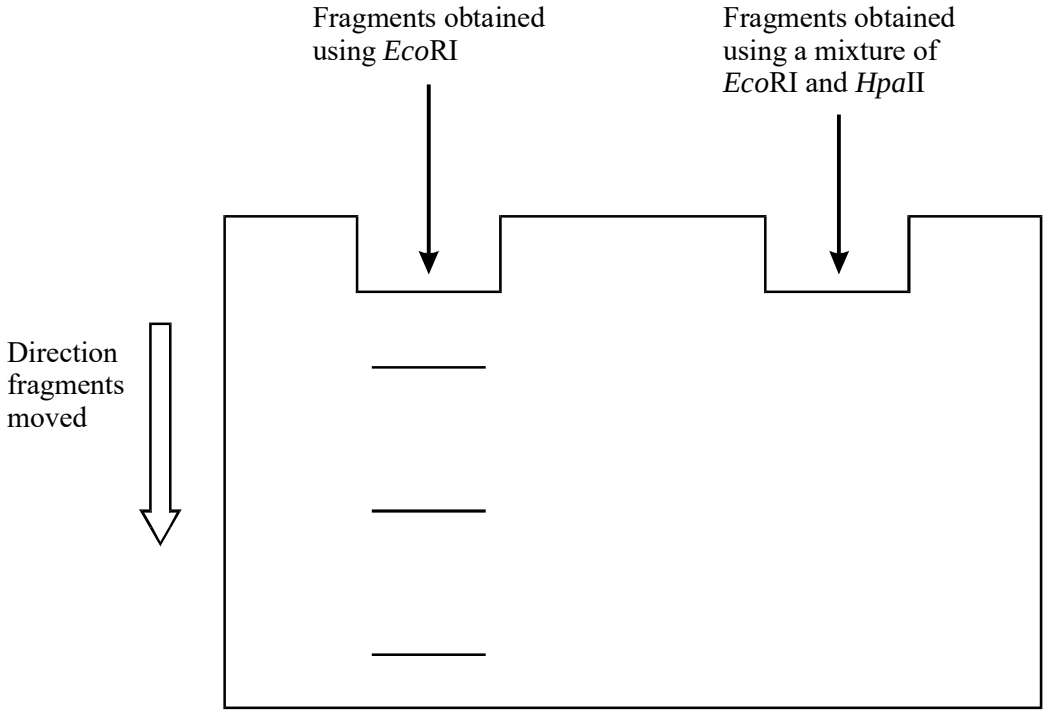

Figure 2

(i) Explain why some DNA fragments move further than other DNA fragments in electrophoresis.

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(1)

(ii) Draw bands on **Figure 2** to show the positions of the DNA fragments that would be obtained if a mixture of *Eco*RI and *Hpa*II was used to cut the plasmid.

(2)

(Total 8 marks)

41. In gene therapy, normal genes are put into cells which contain defective genes. In attempts to treat cystic fibrosis, a virus has been used to put the normal gene into cells.

(a) (i) Give **one** reason for using a virus to introduce genes into cells.

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.....

(1)

(ii) Give **one** disadvantage of using a virus to introduce genes into cells.

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(1)

(b) Genetic engineering could be used to replace the defective gene in either body cells or gametes. At present, gene therapy is limited to replacing genes in body cells.

(i) Suggest **one** advantage of replacing a defective gene in a gamete rather than in a body cell.

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(1)

(ii) Suggest why replacing genes in gametes is not allowed in the United Kingdom.

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(1)

(c) Some people are concerned about using gene therapy to treat genetic disorders in humans.

Give **one** possible argument against the treatment of disorders by gene therapy.

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(1)

(Total 5 marks)

42. Date palms may also be grown from seeds, but until recently it has not been possible to determine the sex of a date palm until it is about 10 years old. However, it is now possible to determine the sex of a date palm seedling using the polymerase chain reaction (PCR). This process uses primers which attach to the DNA. The DNA between the primers is then replicated by an enzyme, and a large number of these DNA fragments is produced. The fragments from male and female seedlings are different sizes.

What is a primer?

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(Total 2 marks)

43. Bacteria can be genetically modified to produce insulin for human use. To achieve this, human insulin genes are transferred into bacteria. Plasmids containing two antibiotic resistance genes, one coding for resistance to tetracycline and one for resistance to ampicillin, are used to carry out this transfer.

A restriction enzyme was used to cut up the human DNA and plasmids. **Figure 1** shows the different fragments of human DNA and the type of cut plasmid that was produced.

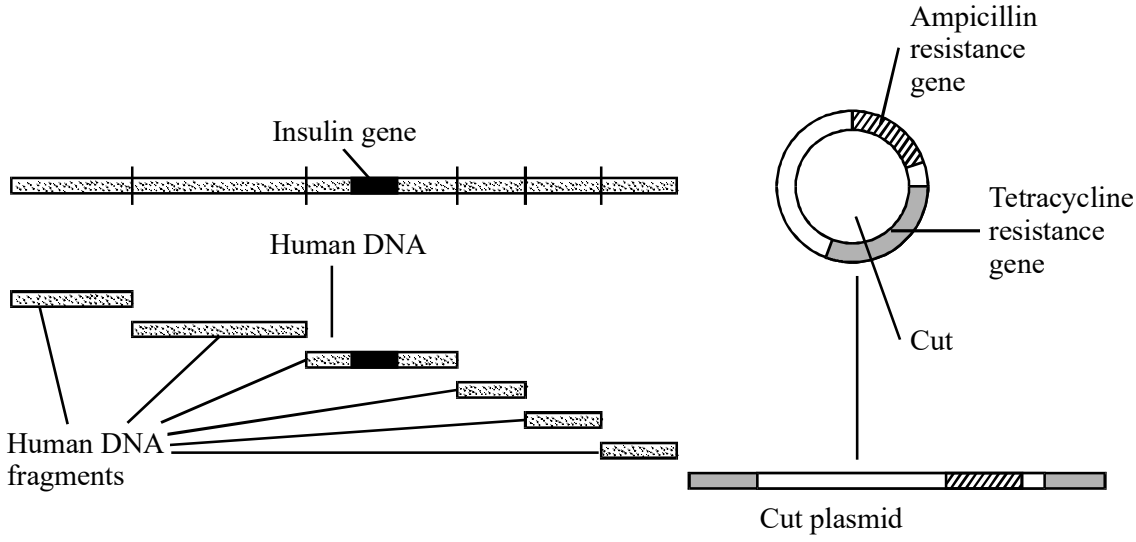

Figure 1

(a) Describe a plasmid.

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(2)

(b) Suggest why the restriction enzyme has cut the human DNA in many places but has cut the plasmid DNA only once.

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(2)

The fragments of human DNA and the cut plasmids were mixed together with DNA ligase. Several types of plasmid were formed. Some contained human DNA in the centre of the gene coding for resistance to tetracycline. The different types of plasmid are shown in **Figure 2**.

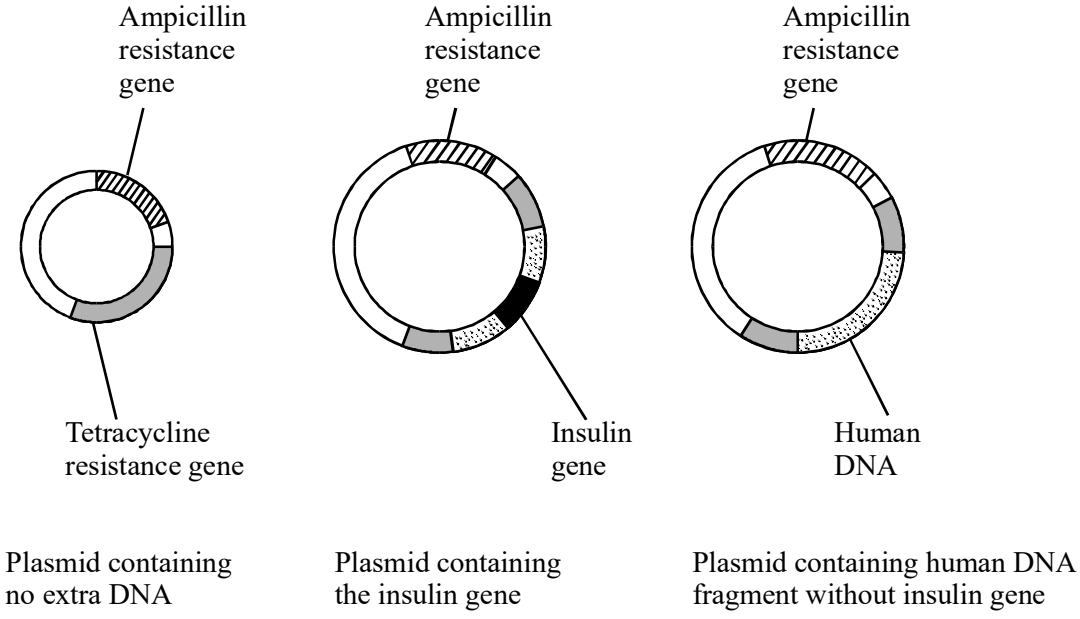

Figure 2

(c) Explain what causes several types of plasmid to be formed.

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(2)

(d) The plasmids are mixed with the bacteria. Some bacteria take up the plasmids.

(i) Explain how it is possible to distinguish between bacteria which have taken up a plasmid with human DNA and those which have taken up a plasmid without any extra DNA.

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(4)

(ii) How is it possible to determine which bacteria have taken up the human insulin gene?

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(1)

(Total 11 marks)

44. In the production of genetically engineered bacteria, a human gene was first combined with a bacterial plasmid containing two antibiotic resistance genes. One gene coded for resistance to tetracycline and one for resistance to ampicillin. The human gene was inserted in the centre of the gene coding for resistance to tetracycline as shown in **Figure 1**.

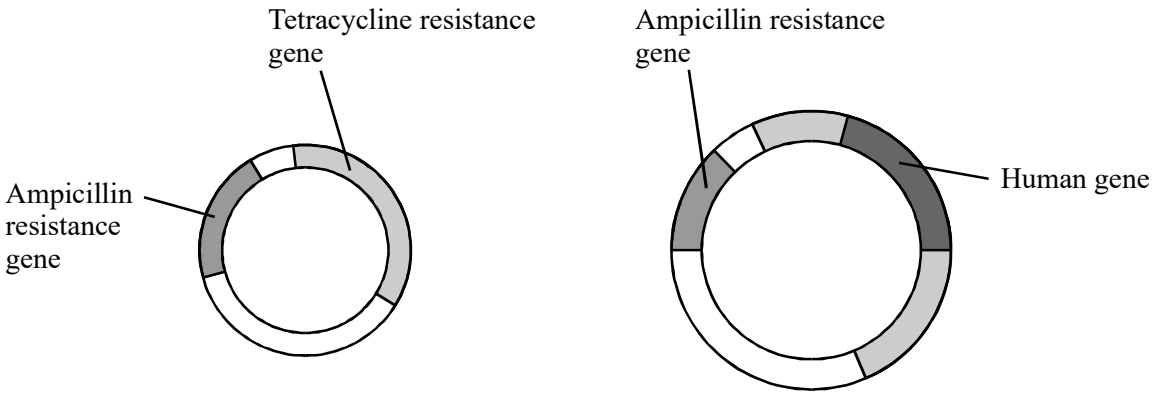

Figure 1

(a) The human gene has split the gene coding for resistance to tetracycline. What effect will this have?

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(1)

The plasmids were then added to a bacterial culture. Replica plating was used to find out which bacteria had taken up the plasmid containing the gene. This is shown in **Figure 2**.

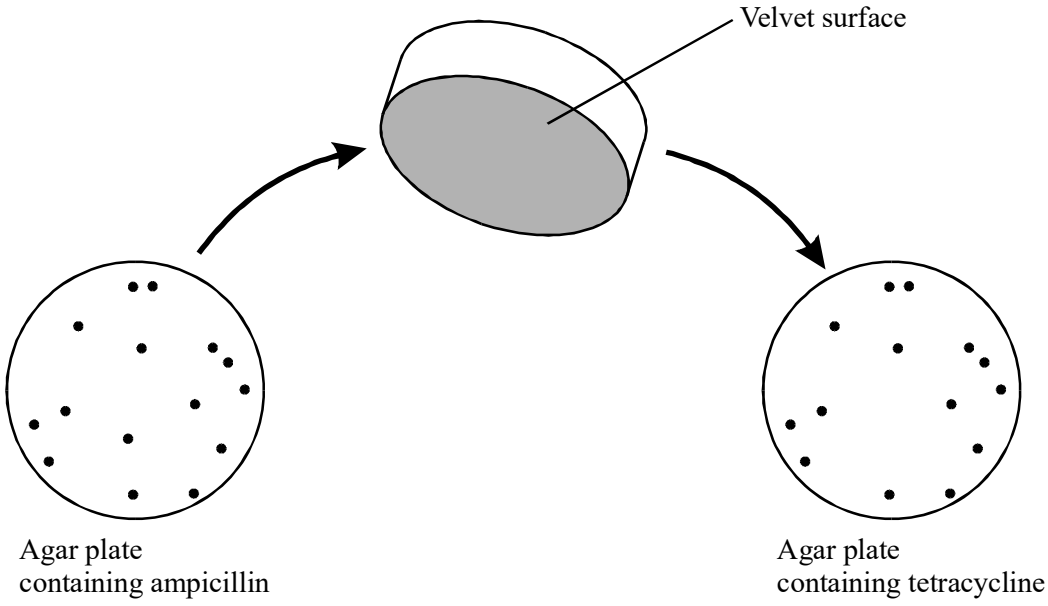

Figure 2

(b) Explain why each of the following was used.

(i) The velvet surface.

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(1)

(ii) The agar plate containing ampicillin.

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(2)

(iii) The agar plate containing tetracycline.

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(2)

(c) Draw a circle around the colony containing the human gene.

(1)
(Total 7 marks)

45. Alpha-l-antitrypsin (αAT) is a protein needed to prevent the breakdown of the elastic tissue in the lungs. Some people have a different form of the gene for αAT. These people cannot produce the αAT protein.

(a) What term is used to refer to different forms of a gene?

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(1)

(b) People who are unable to produce αAT can be treated with αAT extracted from the milk of genetically engineered sheep. These sheep are produced by inserting the human αAT gene into the fertilised eggs of sheep. These eggs are allowed to develop into embryos which are then implanted into surrogate sheep.

Suggest **one** reason why

(i) only a few live births result from the large number of embryos implanted;

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(1)

(ii) only 1 in 20 of the female sheep born produce αAT in their milk.

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(1)

(c) When sheep capable of producing αAT in their milk are allowed to breed, some of their female offspring are also able to produce milk containing αAT.

(i) Explain how the ability to produce milk containing αAT is passed from mother to offspring.

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(2)

(ii) Suggest why only some of the female offspring produce milk containing αAT.

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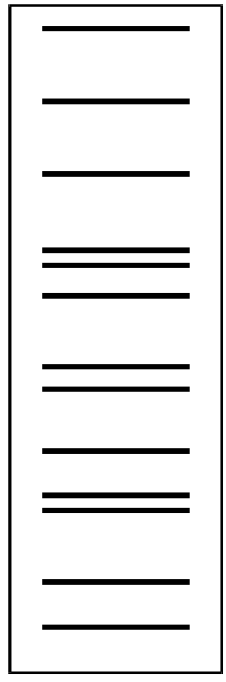
(1)

(Total 6 marks)

46. This question should be answered in continuous prose.
Quality of Written Communication will be assessed in these answers.

DNA fingerprinting can be used to identify an individual from a small spot of blood. The diagram shows a DNA fingerprint.



(a) After the DNA has been extracted from the blood, it is copied by the polymerase chain reaction (PCR). The DNA is then cut into fragments, which are separated by electrophoresis. Describe the main stages in the copying, cutting and separation of the DNA.

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(6)

(b) The position of the fragments is determined by the use of radioactive probes. These probes consist of short lengths of DNA with specific base sequences, which bind onto the fragments. Suggest why several different probes have to be used to produce a DNA fingerprint with a large number of bands.

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(2)

(Total 8 marks)

47. (a) Describe how a gene can be isolated from human DNA.

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(2)

(b) Describe how an isolated gene can be replicated by the polymerase chain reaction (PCR).

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(4)

(c) (i) Describe how a harmless virus, genetically engineered to contain a CFTR gene, can be used to insert the gene into a cystic fibrosis sufferer.

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(2)

(ii) A virus used in gene therapy has RNA as its genetic material and has an enzyme called reverse transcriptase. Inside a human cell, reverse transcriptase uses viral RNA to make viral DNA.

Explain why the enzyme is called *reverse transcriptase*.

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(1)

(Total 9 marks)

48. This question should be answered in continuous prose.
Quality of Written Communication will be assessed in the answer.

(i) Starting with mRNA, describe how the process of translation leads to the production of a polypeptide.

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(4)

(ii) Normal tomato plants have an enzyme that softens tomatoes as they ripen. Genetically engineered tomatoes ripen and soften more slowly. A gene was inserted which reduces the amount of softening enzyme produced.

The diagram shows matching parts of the base sequences for the mRNA produced by the gene for the softening enzyme and that produced by the inserted gene.

Softening gene mRNA …AAUCGGAAU…

Inserted gene mRNA …UUAGCCUUA…

Suggest how the inserted gene reduces the production of the softening enzyme.

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(2)
(Total 6 marks)

49. (a) Explain the reason for each of the following in the polymerase chain reaction (PCR).

(i) DNA is heated to 95 °C.

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(1)

(ii) DNA polymerase used is heat-stable.

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(1)

(iii) The reaction mixture is cooled to 40 °C.

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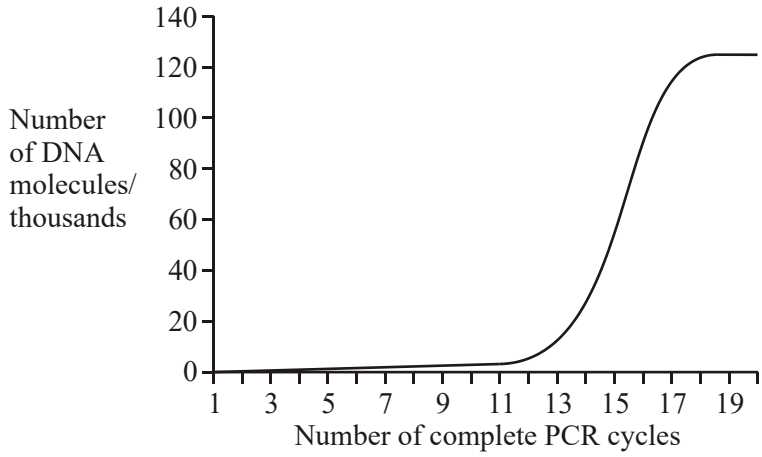
(1)

(b) The graph shows the number of DNA molecules made using PCR, starting with one molecule.



(i) Explain the shape of the curve from cycles 1 to 16.

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(2)

(ii) Suggest **one** explanation for the levelling out of the curve from cycles 17 to 20.

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(2)

(Total 7 marks)

50. (a) CFTR is a transmembrane regulator protein. Its molecules have 1480 amino acids. People with cystic fibrosis produce defective CFTR protein which is missing one amino acid from its structure.

(i) What is the minimum number of bases on DNA which would code for the normal CFTR protein? Explain your answer.

Number of bases .....

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(2)

(ii) Which type of gene mutation produced the cystic fibrosis allele?
Explain your answer.

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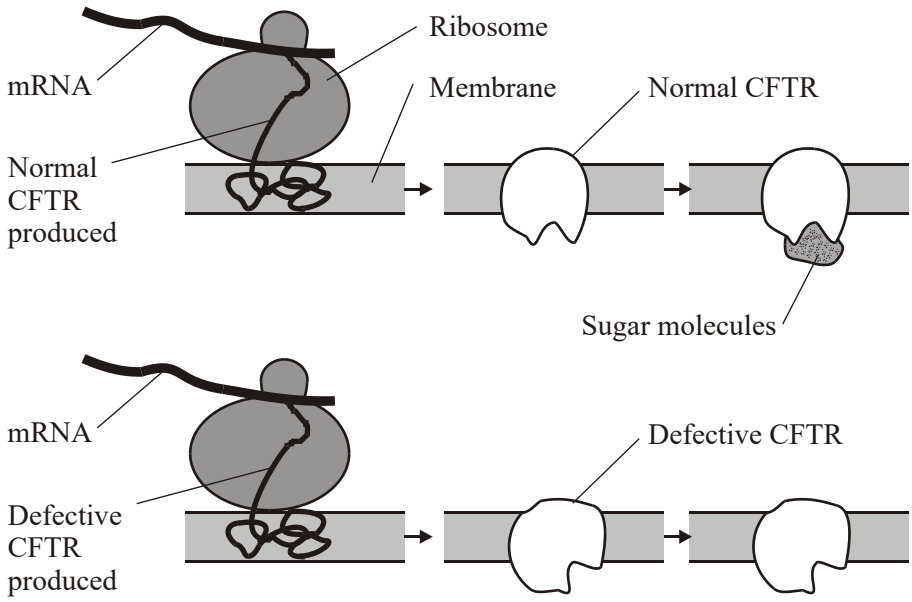
(2)

(b) The diagram shows part of the process of making normal and defective CFTR in a cell. A normal CFTR protein molecule has sugar molecules attached to it which make it functional.



(i) Describe how the information on mRNA is translated into CFTR at the ribosome.

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(4)

(ii) Using information in the diagram and your own knowledge, suggest why defective CFTR, missing one amino acid, is not functional.

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(2)
(Total 10 marks)

51. (a) Plasmids can be modified by genetic engineering and inserted into bacteria.
These bacteria can then make useful substances normally made by another organism.
Explain how modified plasmids are made by genetic engineering and how the use of markers enable bacteria containing these plasmids to be detected.

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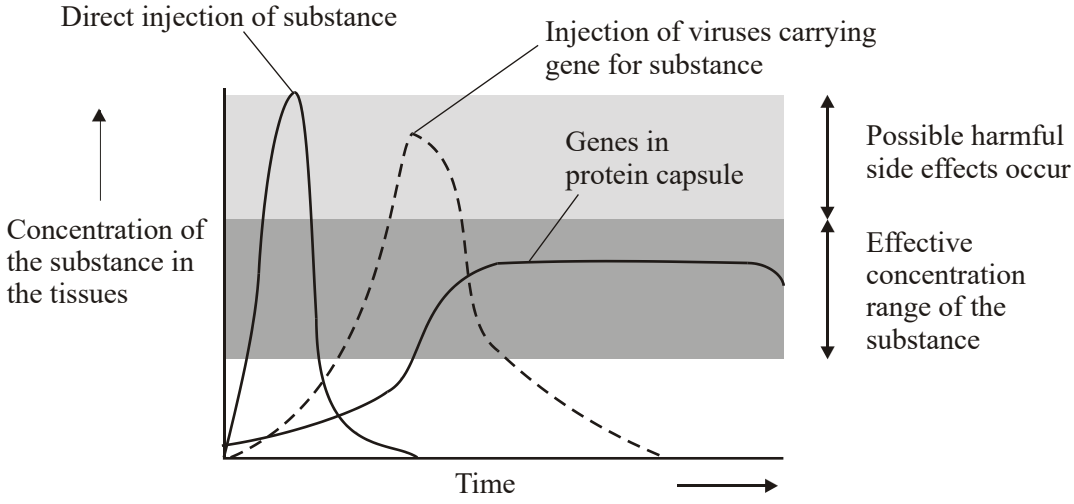
(6)

(b) In gene therapy, genes are introduced into a person who has defective genes which do not produce an important substance. Three experiments were done to compare techniques for introducing an important substance into a person with defective genes.

1. The substance was injected directly.
2. Harmless viruses carrying genes coding for the substance were injected.
3. The genes were put into a protein capsule which was inserted into the tissues.

The graph shows results of the experiments.



Takahiro Ochiya et al, Biomaterials for Gene Delivery: Studies on Metastasis, (National Cancer Centre, Research Institute, Tokyo, Japan) 1999

(i) Describe the results of the three experiments.

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(3)

(ii) Using the information in the graph, suggest **one** advantage and **one** disadvantage of the capsule method compared to the others.

Advantage .....

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Disadvantage .....

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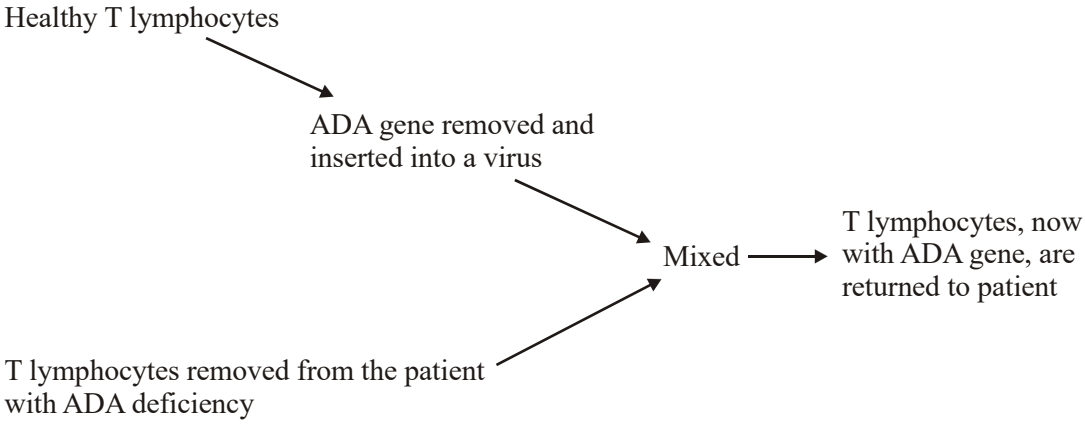
(2)
(Total 11 marks)

52. Gene therapy is used to treat the genetic disorder, ADA deficiency. Affected individuals are unable to produce the enzyme adenosine deaminase (ADA). Without this enzyme, T lymphocytes, a type of white blood cell, cannot provide immunity to infection.
The diagram shows the processes involved in the treatment of ADA deficiency by gene therapy.



(a) What is meant by *gene therapy*?

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(1)

(b) The ADA gene is inserted into a virus. Give **two** advantages of using a virus in gene therapy.

1 .....

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2 .....

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(2)

(c) Individuals who have been treated by this method of gene therapy do not pass on the ADA gene to their children. Explain why.

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(1)

(d) T lymphocytes are produced in bone marrow. A bone marrow transplant from a genetically matched donor can provide a permanent cure for ADA deficiency.

(i) Suggest why bone marrow for a transplant is obtained from a genetically matched donor.

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(1)

(ii) Explain why treatment of ADA deficiency by gene therapy must be repeated at regular intervals, whereas a single bone marrow transplant can provide a permanent cure.

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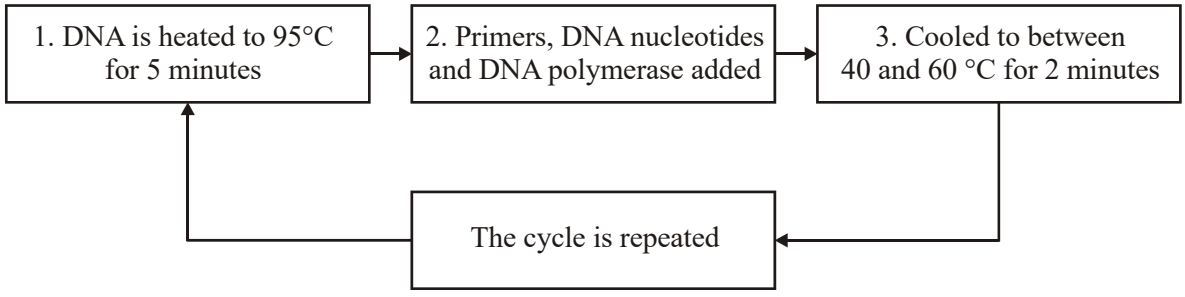
(2)

(Total 7 marks)

53. The polymerase chain reaction is a process which can be carried out in a laboratory to replicate DNA. The diagram shows the main stages involved in the polymerase chain reaction.



(a) Explain why DNA is heated to 95°C.

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(1)

(b) What is the role of

(i) a primer in this process;

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(1)

(ii) DNA polymerase?

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(1)

(c) (i) How many DNA molecules will have been produced from one molecule of DNA after 6 complete cycles?

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(1)

(ii) Suggest **one** use of the polymerase chain reaction.

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(1)

(d) Give **two** ways in which the polymerase chain reaction differs from the process of transcription.

1 .....

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2 .....

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(2)
(Total 7 marks)

54. Research scientists can increase the nutritional value of potatoes by genetically engineering potato plants. A gene which results in increased protein production has been removed from cells of an amaranth plant and inserted into cells of a potato plant.

(a) Describe how a gene could be removed from cells of an amaranth plant and inserted into cells of a potato plant.

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(6)

(b) Describe the advantages of using vegetative propagation rather than sexual reproduction to reproduce genetically engineered potato plants.

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(3)

(c) Whole potato plants can be produced from genetically identical potato cells grown in a tissue culture. Use your knowledge of genes to suggest how different cells, such as leaf and root cells, can develop from genetically identical cells.

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(2)
(Total 11 marks)

55. One technique used to determine the sequence of nucleotides in a sample of DNA is the Sanger procedure. This requires four sequencing reactions to be carried out at the same time.
The sequencing reactions occur in four separate tubes. Each tube contains

- a large quantity of the sample DNA
- a large quantity of the four nucleotides containing thymine, cytosine, guanine and adenine
- DNA polymerase
- radioactive primers

A modified nucleotide is also added to each tube, as shown in **Figure 1**.

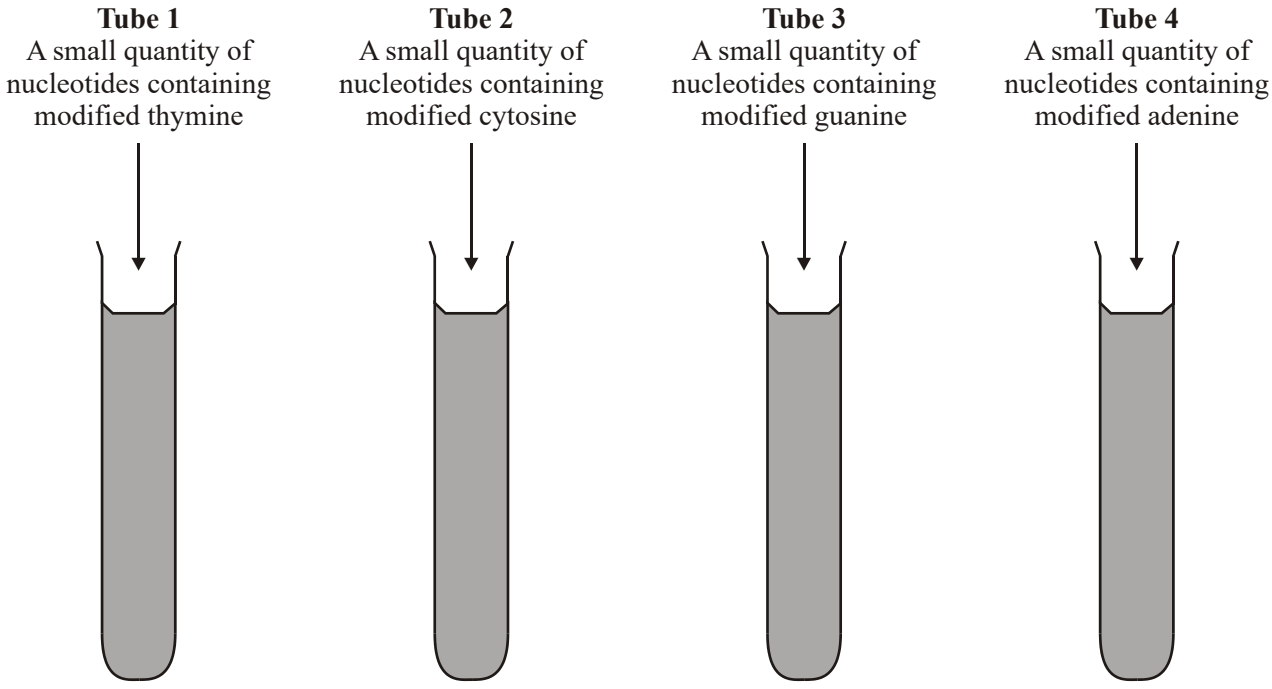

Figure 1

(a) A large quantity of the DNA sample is required for this procedure. Name the reaction used to amplify small amounts of DNA into quantities large enough for this procedure.

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(1)

(b) Explain the reason for adding each of the following to the tubes.

(i) DNA polymerase

.....

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(1)

(ii) Primers

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(1)

(c) (i) When a modified nucleotide is used to form a complementary DNA strand, the sequencing reaction is terminated. Suggest how this sequencing reaction is terminated.

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(1)

(ii) A sample of DNA analysed by this technique had the following nucleotide base sequence.

T G G T C A C G A

Give the base sequence of the shortest DNA fragment which would be produced in **Tube 2**.

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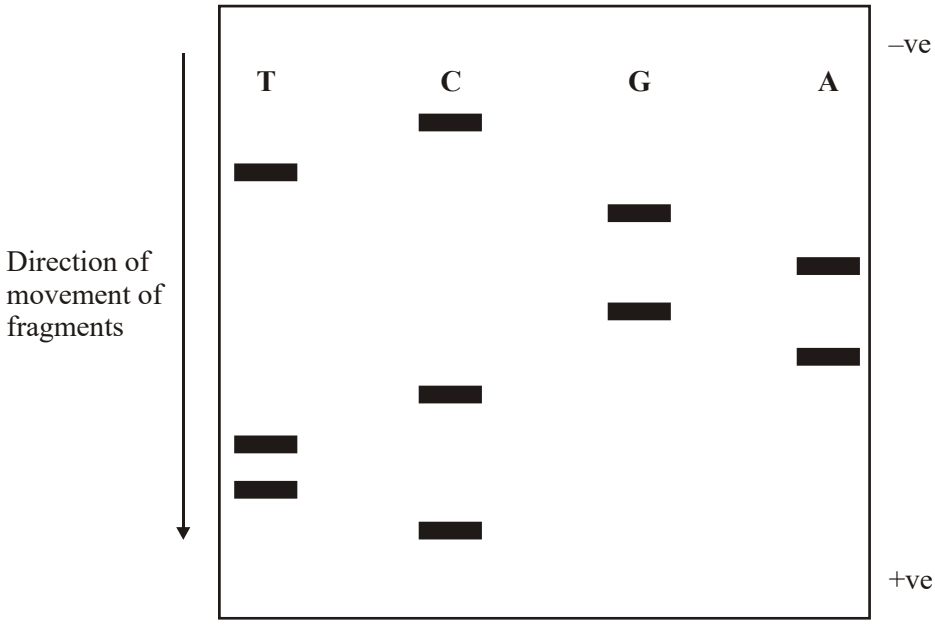
(1)

(d) A different sample of DNA was then analysed. The DNA fragments from the four tubes were separated in a gel by electrophoresis and analysed by autoradiography. **Figure 2** shows the banding pattern produced.



(i) Explain why the DNA fragments move different distances in the gel.

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(1)

(ii) What makes the DNA fragments visible on the autoradiograph?

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(1)

(iii) Use **Figure 2** to determine the sequence of nucleotides in this sample of DNA.

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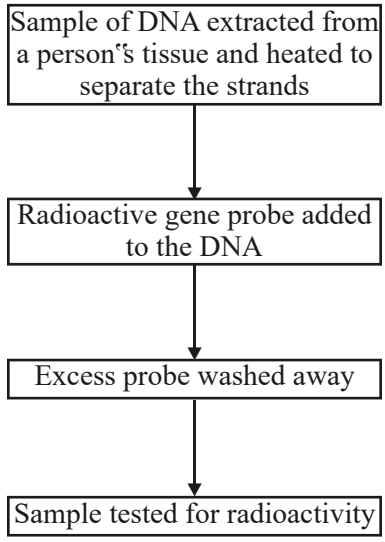
(1)

(Total 8 marks)

56. (a) Cystic fibrosis can be caused by any one of several mutant alleles of the cystic fibrosis gene. The most common of these mutant alleles accounts for about 70% of cases of cystic fibrosis. The use of gene probes can identify individuals carrying this allele. Gene probes are single strands of DNA which are radioactively labelled. They have a base sequence that is complementary to a mutant allele. The main stages in using a gene probe are shown in the diagram.



Using the information given, explain how the use of a gene probe could enable the presence of a mutant allele of the cystic fibrosis gene to be detected.

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(4)

(b) Sheep have been genetically engineered to produce alpha-1-antitrypsin which is used to treat cystic fibrosis. Use your knowledge of this process to explain **one** argument for and **one** against using sheep in this way.

For

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.....

Against

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(2)

(Total 6 marks)

57. A protein produced by a species of bacterium is toxic to caterpillars. The gene coding for this protein was removed and transferred into a crop plant.

(a) (i) Describe how the gene could have been removed from the bacterial DNA.

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(2)

(ii) Many copies of the isolated gene were required. Name the process used in a laboratory to produce many copies of DNA from a small amount.

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(1)

(b) The gene was injected into isolated cells from the crop plant. These cells were then cloned and new plants grown from the cloned cells. Explain the advantage of inserting the gene into isolated plant cells rather than directly into cells within a whole plant.

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(3)
(Total 6 marks)

58. (a) Plasmids are often used as vectors in genetic engineering.

(i) What is the role of a vector?

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(1)

(ii) Describe the role of restriction endonucleases in the formation of plasmids that contain donor DNA.

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(2)

(iii) Describe the role of DNA ligase in the production of plasmids containing donor DNA.

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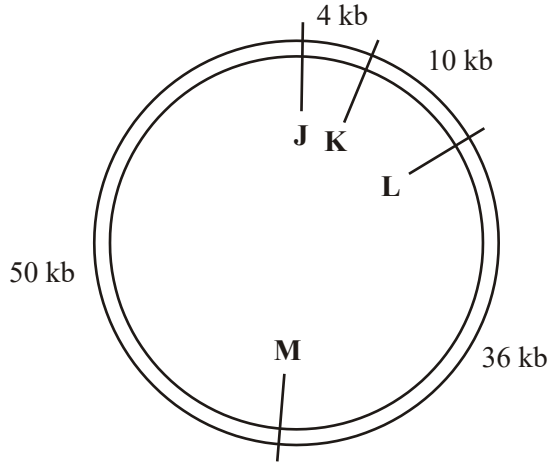
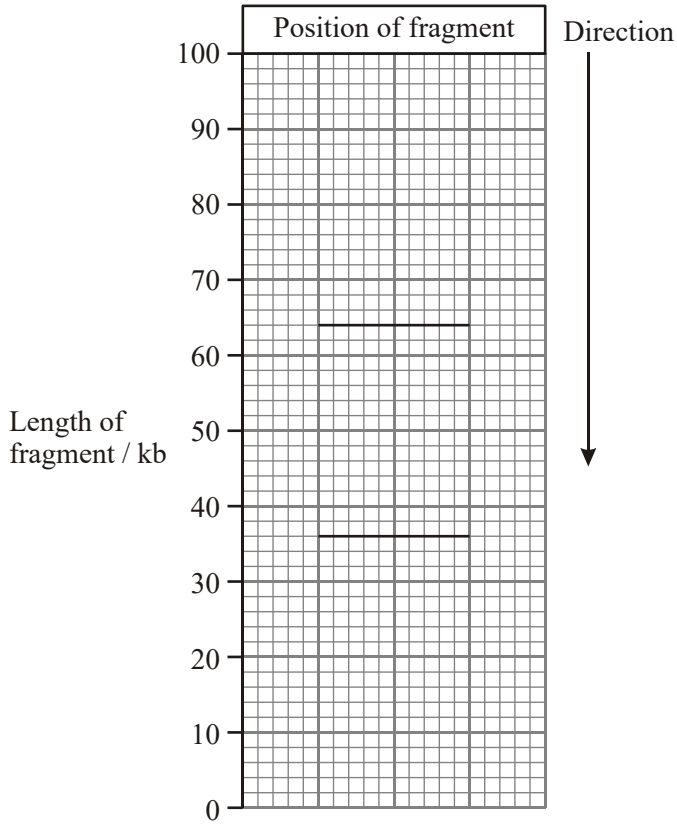
(1)

(b) There are many different restriction endonucleases. Each type cuts the DNA of a plasmid at a specific base sequence called a restriction site. The diagram shows the position of four restriction sites, **J**, **K**, **L** and **M**, for four different enzymes on a single plasmid. The distances between these sites is measured in kilobases of DNA.



1 kb = 1 kilobase

The plasmid was cut using only two restriction endonucleases. The resulting fragments were separated by gel electrophoresis. The positions of the fragments are shown in the chart below.



(i) Which of the restriction sites were cut?

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(1)

(ii) Explain your answer.

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(1)

(Total 6 marks)

59. (a) The polymerase chain reaction (PCR) can be used to produce large quantities of DNA. Describe how the PCR is carried out.

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(6)

(b) About twenty percent of the DNA produced by the PCR is copied inaccurately. Suggest and explain why it is not safe to use the PCR to clone the CFTR gene for use in treating cystic fibrosis.

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(4)

(Total 10 marks)

60. (a) What is a gene probe?

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(3)

(b) Give **two** ways in which the information obtained from the use of gene probes might be helpful to a doctor who is counselling someone with a family history of cancer.

1 .....

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2 .....

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(2)
(Total 5 marks)

61. Restriction enzymes are used in DNA technology. They digest DNA at specific recognition sequences on the DNA molecule.

(a) **Figure 1** shows the recognition sequence of a restriction enzyme called *Eco*RI.

Figure 1

--G-A-A-T-T-C--
--C-T-T-A-A-G—

(i) Name the type of reaction that occurs when *Eco*RI digests DNA.

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(1)

(ii) Explain why *Eco*RI digests DNA only at the specific recognition sequence shown in **Figure 1**.

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(1)

(iii) The recognition sequence shown is referred to as a 6 bp palindromic sequence. Use evidence from **Figure 1** to explain what this means.

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(1)

(b) The piece of DNA shown in **Figure 2** was labelled using a radioactive nucleotide. The piece of DNA is 10 kilobases long.

Figure 2

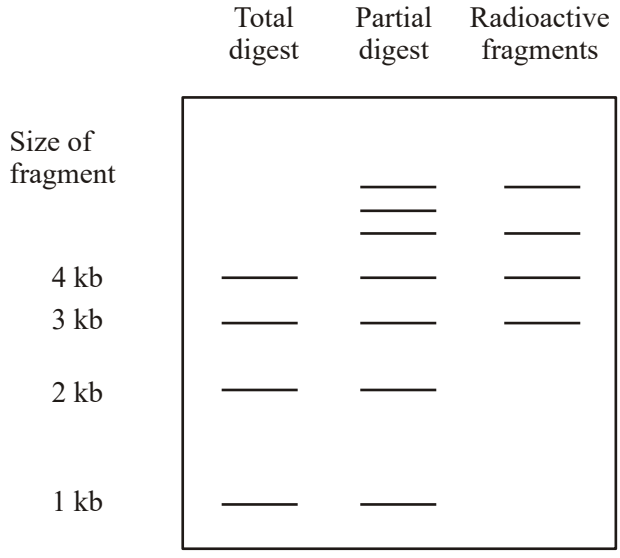
Position of radioactive nucleotide

This piece of DNA was then digested using *Eco*RI and the resulting fragments of DNA were separated using electrophoresis.

Figure 3 shows the results of elecrophoresis. The three lanes of the electrophoresis gel show:

- the fragments of DNA formed after total digestion of the piece of DNA
- the fragments of DNA formed after partial digestion of the piece of DNA
- those fragments after partial digestion that contained radioactivity.

Figure 3



(i) The total digestion lane shows four fragments. How many times did the recognition sequence for *Eco*RI appear in the piece of DNA?

.....

(1)

(ii) Explain the presence of the three additional fragments in the partial digestion lane.

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(2)

(iii) The evidence from **Figure 3** was used to construct a restriction map of the piece of DNA. The map is shown in **Figure 4**

Figure 4

Explain why it is possible to map the positions of

the 3kb fragment .....

.....

the 1 kb fragment .....

.....

(2)
(Total 8 marks)