

Recombinant DNA technology

These practice questions can be used by students and teachers and is

Suitable for AQA A Level 7402 Biology Topic Question

Level: AQA A LEVEL 7402

Subject: Biology

Exam Board: AQA A Level 7402

Topic: Recombinant DNA technology

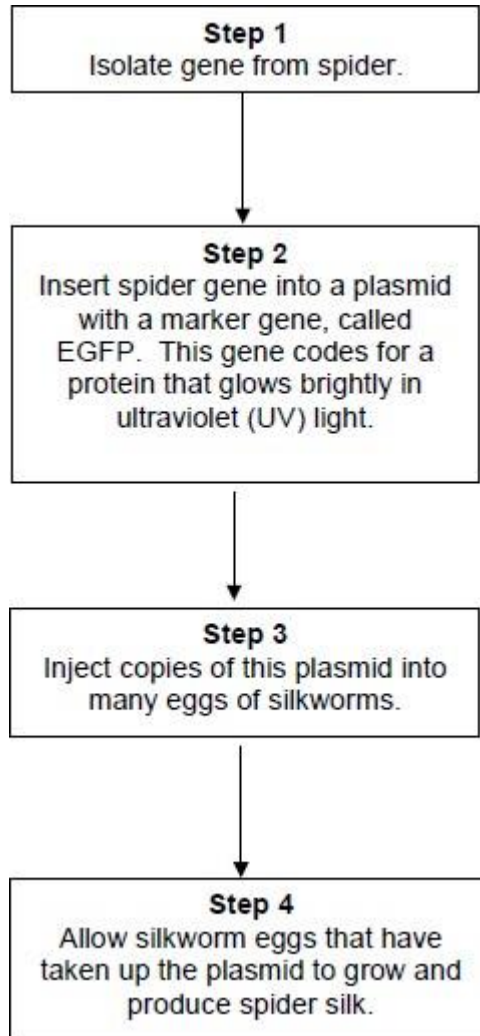


1

Scientists have produced genetically modified (GM) silkworms that contain a gene from a spider.

The GM silkworms secrete fibres made of spider web protein (spider silk), which is stronger than normal silk fibre protein.

The method the scientists used is shown in the figure below.



(a) Suggest why the plasmids were injected into the eggs of silkworms, rather than into the silkworms.



(2)

(b) Suggest why the scientists used a marker gene and why they used the EGFP gene.

(2)

The scientists ensured the spider gene was expressed only in cells within the silk glands.

(c) What would the scientists have inserted into the plasmid along with the spider gene to ensure that the spider gene was only expressed in the silk glands of the silkworms?

(1)

(d) Suggest **two** reasons why it was important that the spider gene was expressed only in the silk glands of the silkworms.

1. _____

2. _____

(2)

(Total 7 marks)

Some populations of flies are becoming resistant to insecticides intended to kill them.

2

Scientists developed a method for finding out whether a fly was carrying a recessive allele, **r**, that gives resistance to an insecticide. The dominant allele, **R**, of this gene does not give resistance.

The scientists:

- crossed flies with genotype **RR** with flies with genotype **rr**
- obtained DNA samples from the parents and offspring
- used the same restriction endonuclease enzymes on each sample, to obtain DNA fragments.



(a) Explain why the scientists used the same restriction endonuclease enzymes on each DNA sample.

(2)

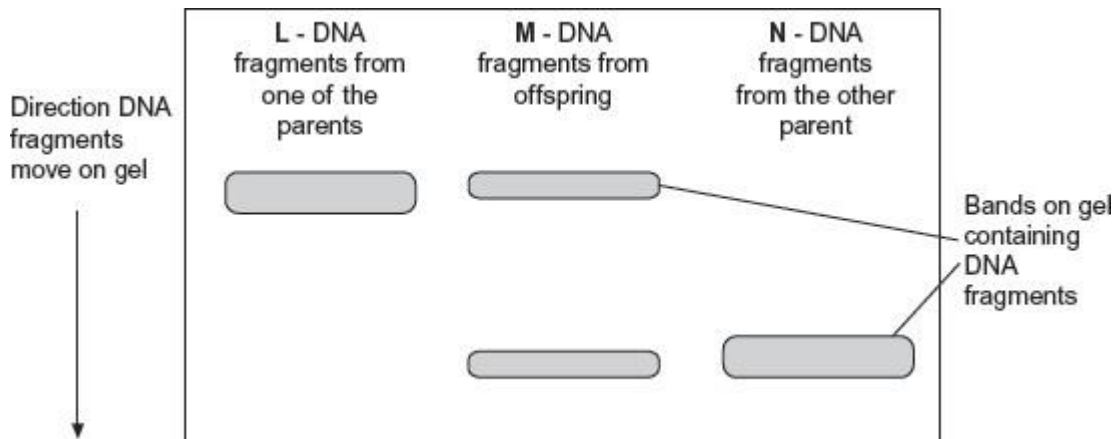
The scientists added two different primers to each sample of DNA fragments for the polymerase chain reaction (PCR).

- Primer A3 only binds to a 195 base-pair fragment from allele **r**.
- Primer A4 only binds to a 135 base-pair fragment from allele **R**.

The scientists separated the DNA fragments produced by the PCR on a gel where shorter fragments move further in a given time.

Their results are shown in **Figure 1**.

Figure 1



(b) Explain why primer A3 and primer A4 only bind to specific DNA fragments.

(2)

(c) Use all the information given to explain the results in **Figure 1**.

[Extra space] _____

(3)

(d) The scientists wanted to know on which chromosome the gene with alleles **R** and **r** was located. From the flies with genotype **RR**, they obtained cells that were in mitosis and added a labelled DNA probe specific for allele **R**. They then looked at the cells under an optical microscope.

Explain why they used cells that were in mitosis.

(2)

(e) Another group of scientists thought that pesticide resistance in some flies was related to increased activity of an enzyme called P450 monooxygenase (PM). This enzyme breaks down insecticides.

The scientists obtained large numbers of resistant and non-resistant flies. They then set up the following experiments.

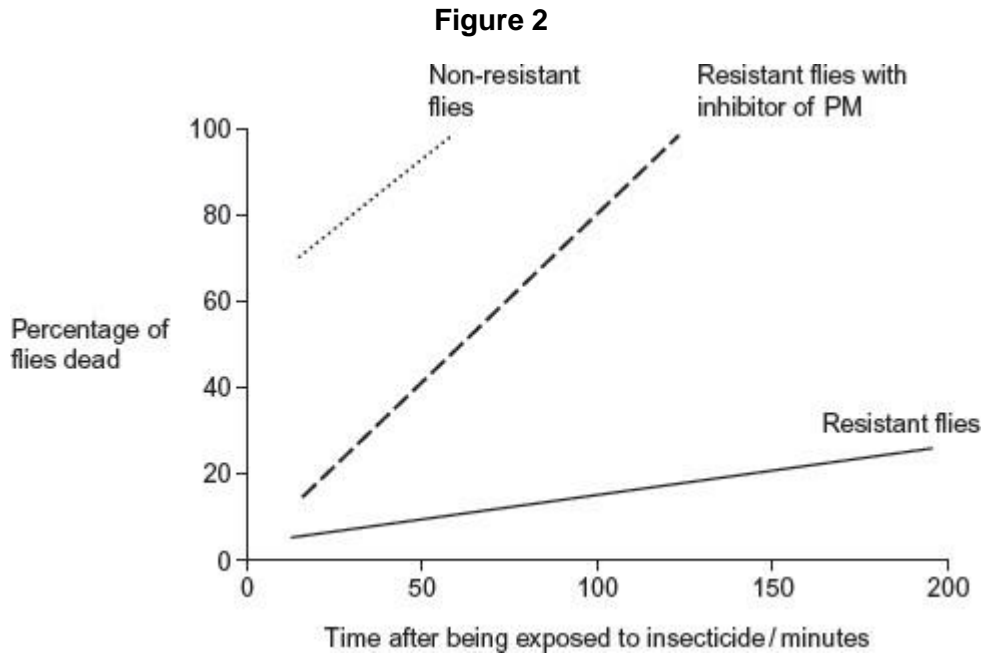
- Non-resistant flies exposed to insecticide.



- Resistant flies exposed to insecticide.
- Resistant flies treated with an inhibitor of PM and then exposed to insecticide.

They then determined the percentage of flies that were dead at different times after being exposed to insecticide.

Figure 2 shows their results.



- (i) Explain why the scientists carried out the control experiment with the non-resistant flies.

(2)

- (ii) The scientists concluded that the resistance of the flies to the insecticide is partly due to increased activity of PM but other factors are also involved.

Explain how these data support this conclusion.



[Extra space] _____

(4)
(Total 15 marks)



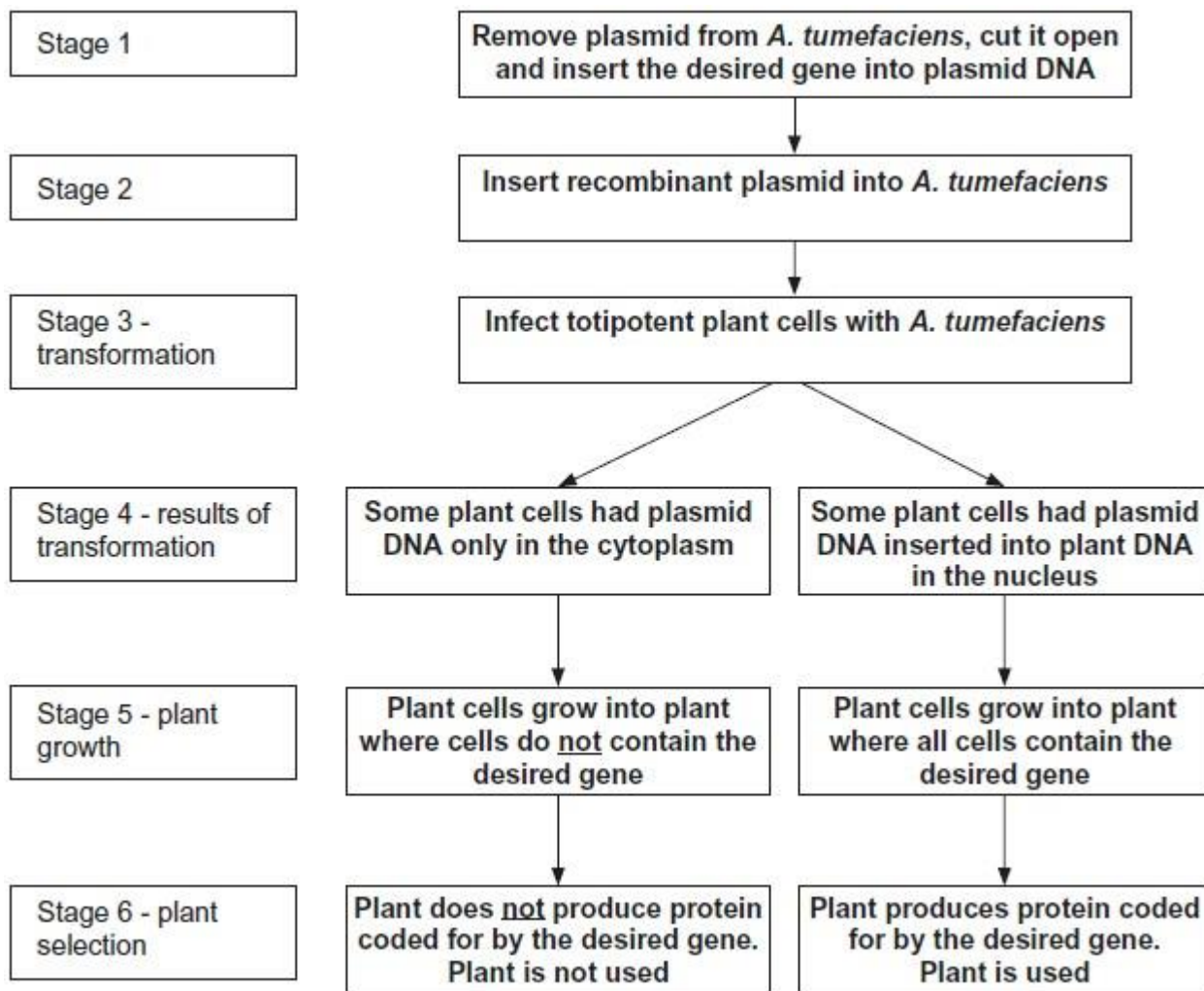
3

Agrobacterium tumefaciens is a bacterium that is often used in recombinant DNA technology to

produce transformed plants that benefit humans.

A. tumefaciens contains a plasmid which can be used as a vector to transfer a desired gene into plant cells. These plant cells may then develop into plants which produce the protein coded for by the desired gene.

The diagram outlines this process.



- (a) (i) In stage 1, an enzyme is used to cut open the plasmid. Name the type of enzyme used to cut open the plasmid.

(1)



- (ii) In stage 1, another enzyme is used to insert the desired gene into the plasmid DNA. Name the type of enzyme used to insert the gene into the plasmid.

(1)

- (b) In stage 4, some plant cells had plasmid DNA only in their cytoplasm. In other plant cells, the plasmid DNA had become inserted into plant DNA in the nucleus.

In stage 5, only cells with plasmid DNA inserted into the plant DNA in the nucleus grew into plants where all the cells contained the desired gene.

Explain why some of the plants in stage 5 contained the desired gene in all of their cells and others did not.

(3)

- (c) The **desired gene** in the diagram was from an insect. In stage 6, the plant containing this gene was able to use it to synthesise an insect protein.

The plant is able to synthesise the insect protein. Explain why this is possible.

(3)

(Total 8

marks) Scientists wanted to measure how much mRNA was transcribed from allele **A** of a gene in a

4

sample of cells. This gene exists in two forms, **A** and **a**.

The scientists isolated mRNA from the cells. They added an enzyme to mRNA to produce cDNA.

(a) Name the type of enzyme used to produce the cDNA.

(1)

The scientists used the polymerase chain reaction (PCR) to produce copies of the cDNA.

They added a DNA probe for allele **A** to the cDNA copies. This DNA probe had a dye attached to it. This dye glows with a green light **only** when the DNA probe is attached to its target cDNA.

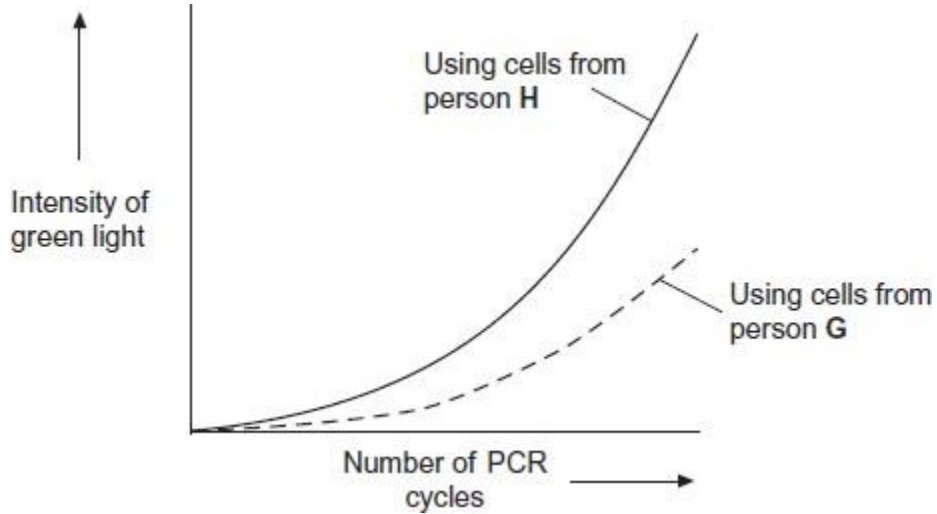
(b) Explain why this DNA probe will only detect allele **A**.

(2)



- (c) The scientists used this method with cells from two people, **H** and **G**. One person was homozygous, **AA**, and the other was heterozygous, **Aa**. The scientists used the PCR and the DNA probe specific for allele **A** on the cDNA from both people.

The figure shows the scientists' results.



- (i) Explain the curve for person **H**.

(Extra space) _____

(3)

- (ii) Which person, **H** or **G**, was heterozygous, **Aa**? Explain your answer.

(2)
(Total 8 marks)

5 (a) Adrenaline binds to receptors in the plasma membranes of liver cells. Explain how this causes the blood glucose concentration to increase.

(Extra space) _____

(2)

(b) Scientists made an artificial gene which codes for insulin. They put the gene into a virus which was then injected into rats with type I diabetes. The virus was harmless to the rats but carried the gene into the cells of the rats.

The treated rats produced insulin for up to 8 months and showed no side-effects. The scientists measured the blood glucose concentrations of the rats at regular intervals. While the rats were producing the insulin, their blood glucose concentrations were normal.

(i) The rats were not fed for at least 6 hours before their blood glucose concentration was measured. Explain why.

(1)

(ii) The rats used in the investigation had type I diabetes. This form of gene therapy maybe less effective in treating rats that have type II diabetes. Explain why.



(1)

(iii) Research workers have suggested that treating diabetes in humans by this method of gene therapy would be better than injecting insulin. Evaluate this suggestion.

(Extra space)

(4)

(Total 8 marks)

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

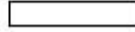
6

haemophilia. Sheep can be genetically engineered to produce Factor IX in the milk produced by their mammary glands. The diagram shows the stages involved in this process.



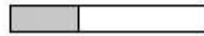
Stage 1

Gene for Factor IX is cut
from human DNA



Stage 2

Jellyfish gene is attached to
the human gene



Stage 3

Promoter DNA from
sheep is attached



Stage 4

Copies of this DNA are
inserted into the nuclei of
body cells from sheep



Stage 5

Each nucleus is
transplanted into a sheep
egg cell from which the
original nucleus has
been removed





The egg cells divide to form an embryo. Each embryo is implanted into the uterus of a different sheep

Stage 6

- (a) Name the type of enzyme that is used to cut the gene for Factor IX from human DNA(Stage 1) .

(1)

- (b) (i) The jellyfish gene attached to the human Factor IX gene (Stage 2) codes for a protein that glows green under fluorescent light. Explain the purpose of attaching this gene.

(2)

- (ii) The promoter DNA from sheep (Stage 3) causes transcription of genes coding for proteins found in sheep milk.

Suggest the advantage of using this promoter DNA.

(Extra space)_____

(2)

(c) Many attempts to produce transgenic animals have failed. Very few live births result from the many embryos that are implanted.

(i) Suggest **one** reason why very few live births result from the many embryos that are implanted.

(Extra space)

(2)

(ii) It is important that scientists still report the results from failed attempts to produce transgenic animals. Explain why.

(2)

(Total 9 marks)

7

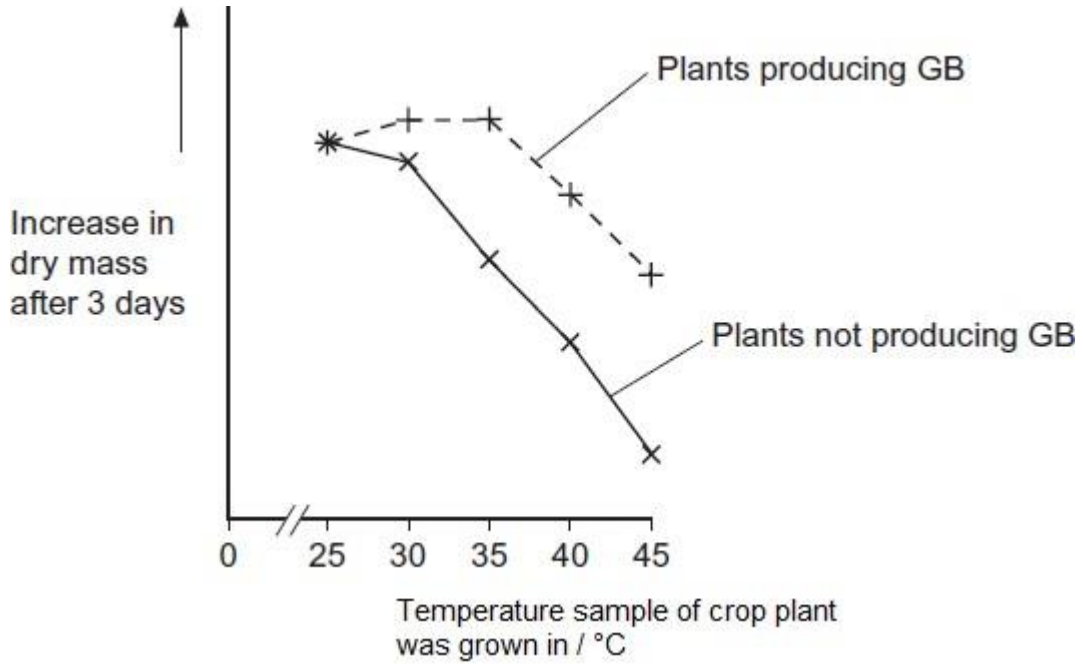
Some species of crop plant produce a substance called glycinebetaine (GB).

Scientists transferred the gene for GB into a species of crop plant that does not normally produce GB. These genetically modified plants then produced GB.

The scientists grew large numbers of the same crop plant with and without the gene at different temperatures. After 3 days, they found the increase in dry mass of the plants.

Figure 1 shows their results.

Figure 1



(a) Describe the effect on growth of transferring the gene for GB into this plant.

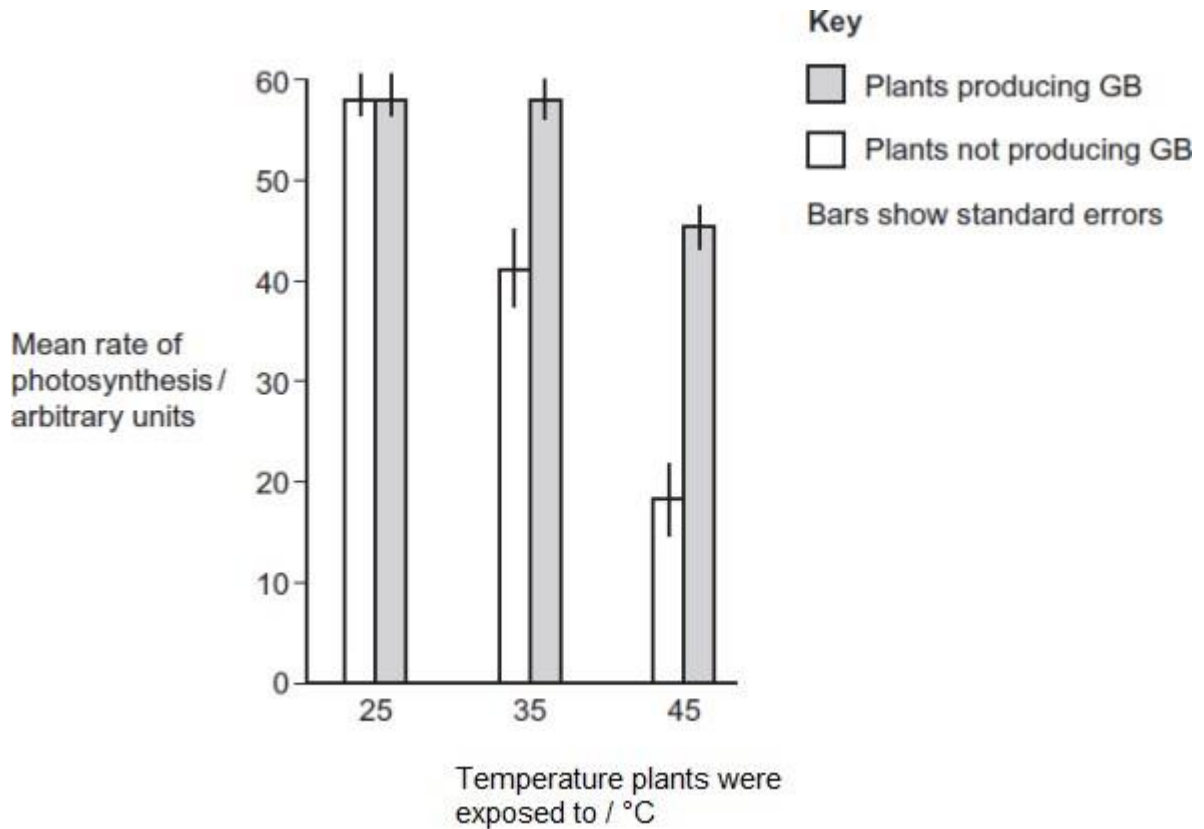
(2)

(b) The scientists measured the rate of photosynthesis in plants that produce GB and plants that do not produce GB at 25°C, 35°C and 45°C.

Figure 2 shows their results.



Figure 2



- (i) The scientists concluded that the production of GB protects photosynthesis from damage by high temperatures.

Use these data to support this conclusion.

(1)

- (ii) Use the data from **Figure 2** for plants that do not produce GB to explain the effect of temperature on changes in dry mass of the plants shown in **Figure 1**.



(Extra space)

(4)

Rubisco activase is an enzyme found in chloroplasts. It activates the light-independent reaction of photosynthesis.

The scientists discovered that, as temperature increased from 25°C to 45°C, rubisco activase began attaching to thylakoid membranes in chloroplasts and this stopped it working.

(c) Rubisco activase stops working when it attaches to a thylakoid.

Use your knowledge of protein structure to explain why.

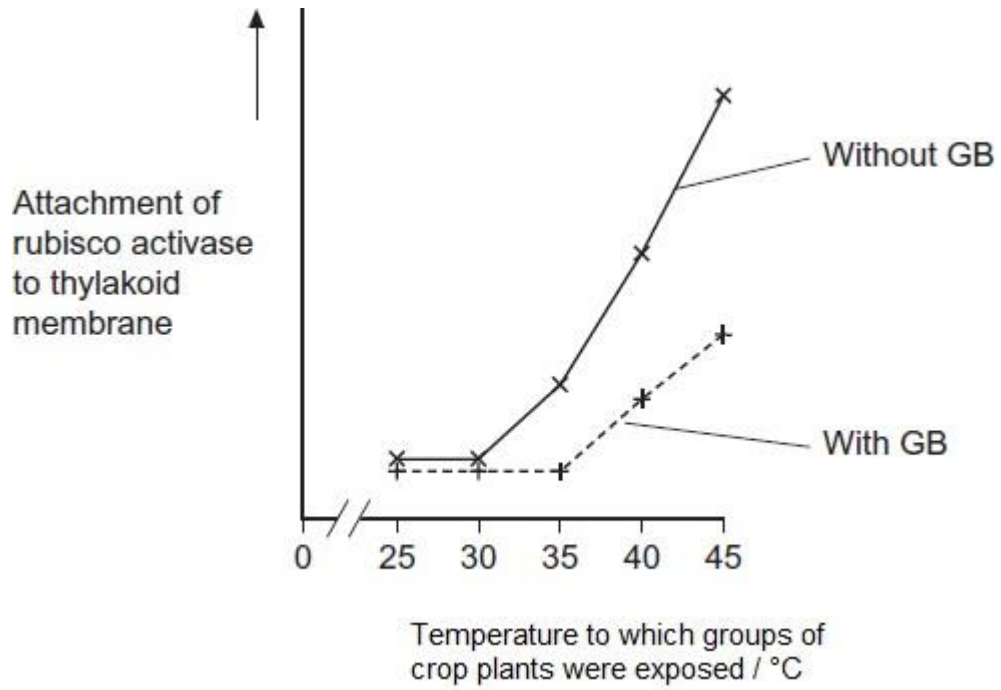
(2)

(d) The scientists investigated the effect of GB on attachment of rubisco activase to thylakoid membranes at different temperatures.

Figure 3 shows their results.



Figure 3



Use information from **Figure 2** and **Figure 3** to suggest how GB protects the crop plant from high temperatures.

(Extra space)



(4)

- (e) The scientists' hypothesis at the start of the investigation was that crop plants genetically engineered to produce GB would become more resistant to high environmental temperatures.

The scientists developed this hypothesis on the basis of previous research on crops that are grown in hot climates.

Suggest how the scientists arrived at their hypothesis.

(2)

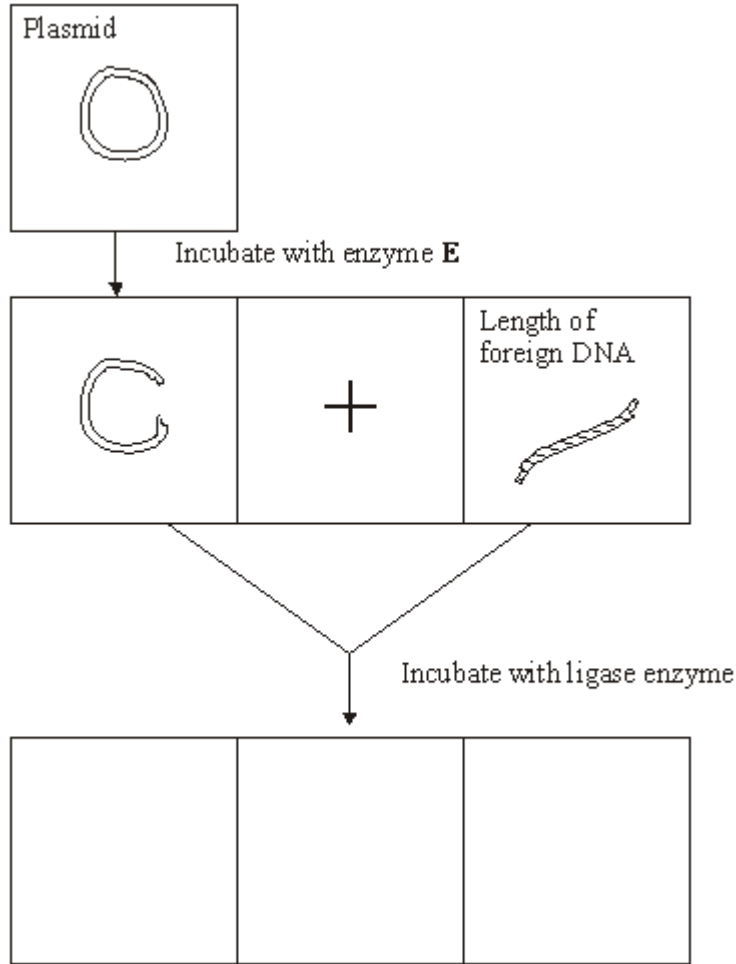
(Total 15 marks)



8

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**.

(1)

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



(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) (a) Scientists can use protein structure to investigate the evolutionary relationships between

9 different species. Explain why.

(2)

- (b) Comparing the base sequence of genes provides more evolutionary information than comparing the structure of proteins. Explain why.

(2)

(Total 4 marks)



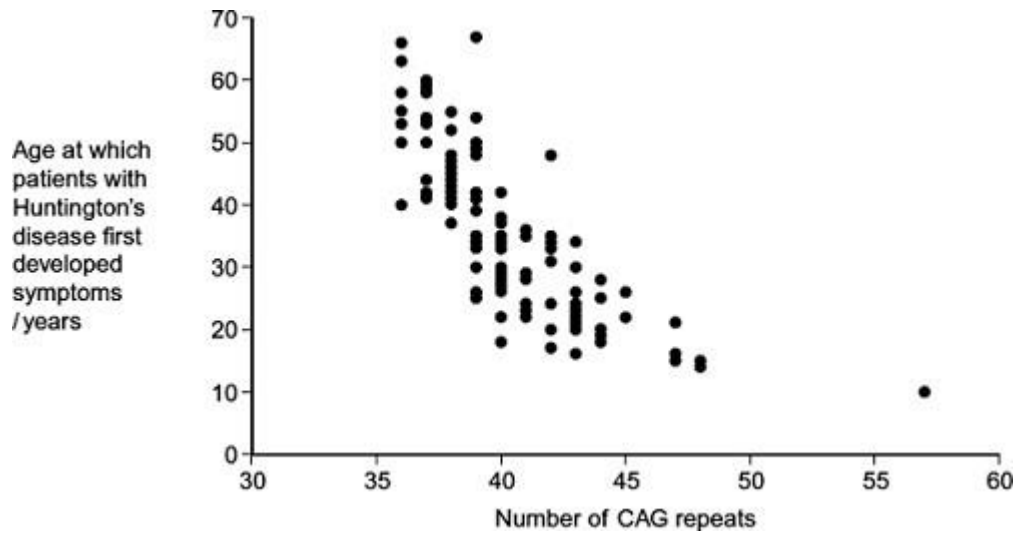
10

Huntington's disease is a genetic condition that leads to a loss in brain function. The gene

involved contains a section of DNA with many repeats of the base sequence CAG. The number of these repeats determines whether or not an allele of this gene will cause Huntington's disease.

- An allele with 40 or more CAG repeats will cause Huntington's disease.
- An allele with 36 – 39 CAG repeats may cause Huntington's disease.
- An allele with fewer than 36 CAG repeats will not cause Huntington's disease.

The graph shows the age at which a sample of patients with Huntington's disease first developed symptoms and the number of CAG repeats in the allele causing Huntington's disease in each patient.



- (a) (i) People can be tested to see whether they have an allele for this gene with more than 36 CAG repeats. Some doctors suggest that the results can be used to predict the age at which someone will develop Huntington's disease.

Use information in the graph to evaluate this suggestion.



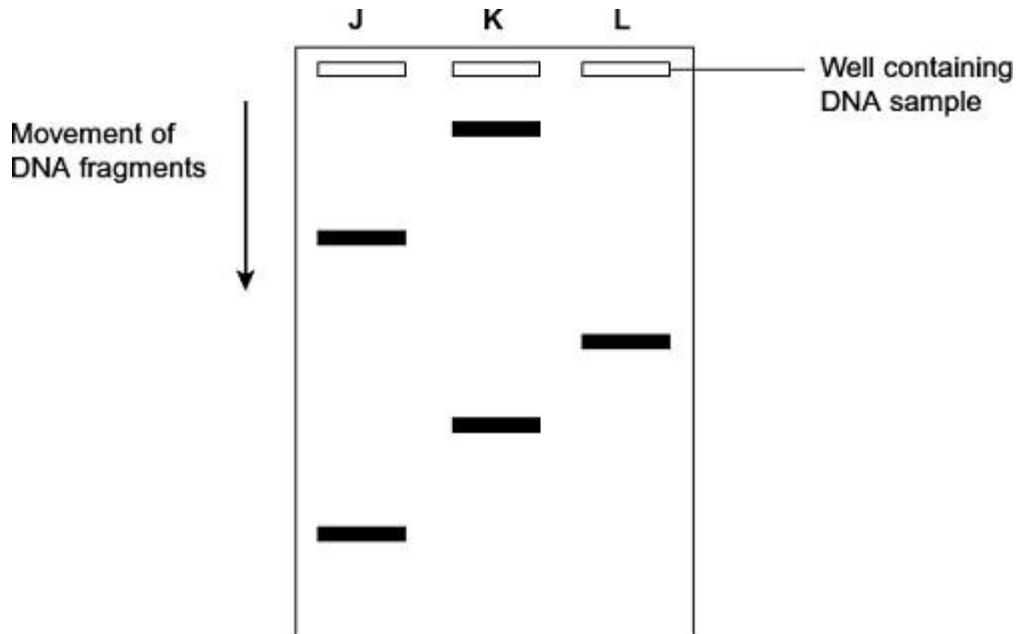
(Extra space)

(3)

- (ii) Huntington's disease is always fatal. Despite this, the allele is passed on in human populations. Use information in the graph to suggest why.

(2)

- (b) Scientists took DNA samples from three people, **J**, **K** and **L**. They used the polymerase chain reaction (PCR) to produce many copies of the piece of DNA containing the CAG repeats obtained from each person. They separated the DNA fragments by gel electrophoresis. A radioactively labelled probe was then used to detect the fragments. The diagram shows the appearance of part of the gel after an X-ray was taken. The bands show the DNA fragments that contain the CAG repeats.



- (i) Only one of these people tested positive for Huntington's disease. Which person was this? Explain your answer.

Person _____

Explanation _____

(2)

- (ii) The diagram only shows part of the gel. Suggest how the scientists found the number of CAG repeats in the bands shown on the gel.

(1)

- (iii) Two bands are usually seen for each person tested. Suggest why only one band was seen for Person L.

(1)
(Total 9 marks)

Essay

11

You should write your essay in continuous prose.

Your essay will be marked for its scientific accuracy.

It will also be marked for your selection of relevant material from different parts of the specification and for the quality of your written communication.

The maximum number of marks that can be awarded is

Scientific	16
Breadth of knowledge	3
Relevance	3
Quality of written communication	3

Write an essay on the following topic:

Using DNA in science and technology

(Total 25 marks)

Read the following passage.

12

Herpes viruses cause cold sores and, in some cases, genital warts. Scientists are well on the way to producing an antibody which will counteract herpes infection. This antibody works by sticking to the virus and blocking its entry into cells. It has proved very effective in animal tests.

- 5 One drawback with this approach, however, is that antibodies are at present produced using hamster ovary cells. This method is expensive and only produces limited amounts. A new technique is being developed to produce antibodies from plants. It involves introducing the DNA which codes for the required antibody into crop plants such as maize.

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

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(4)

- (c) Taking a course of these antibodies from plants to treat a herpes infection would not produce long-term protection against disease. Explain why.

(2)

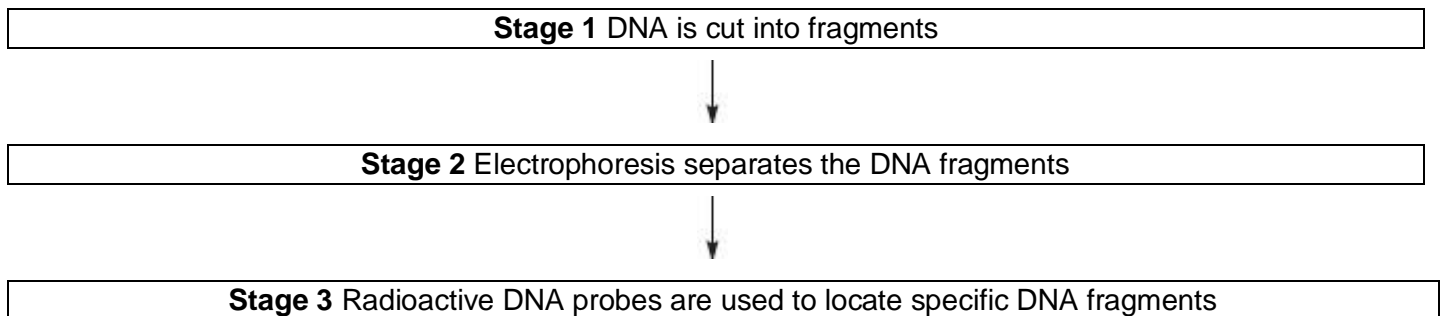
- (d) Explain **one** advantage of using antibodies from plants to treat a disease, rather than antibodies produced in an experimental animal (lines 5-6).

(1)

(Total 15 marks)

13

DNA probes may be used to identify the presence of specific genes associated with human diseases. The flow chart summarises the way in which they are used.





(a) Name the enzyme used in **Stage 1**.

(1)

(b) Explain how electrophoresis separates the fragments of DNA in **Stage 2**.

(2)

(c) (i) What is a *DNA probe*?

(2)

(ii) Explain why *radioactive* DNA probes are used to locate specific DNA fragments.

(2)

(Total 7 marks)

Scientists are working to produce a genetically modified bacterium to treat patients suffering from

14

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.

(1)

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

(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.

(1)

(Total 3

- marks) (a) (i) Some human DNA was cut into separate pieces using a restriction enzyme which

15 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.

(2)

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

(1)

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

(2)

(c) Molecular biologists often use plasmids which contain antibiotic resistance genes.

Explain the reason for this.

(2)

(Total 7 marks)

Read the following passage.

16

Shark-fin soup is an expensive delicacy. To provide the basic ingredient, fishermen catch the sharks, hack the fins off and throw the dead bodies back into the ocean. But sharks are slow to mature and produce only a few offspring at a time, so they are vulnerable to overfishing.

Monitoring the shark-fin trade is difficult, as once a fin has been cut off, it can be extremely difficult to work out precisely from which species it was taken.

The DNA from different species of sharks shows some differences in base sequence. This has enabled a new genetic fingerprinting technique to be developed. This technique would allow conservationists and fisheries managers to assess which of the 400 shark species are most threatened by the trade in shark fins.

10 An identification process has been developed using a range of “primers”. These are short pieces of single-stranded DNA that are complementary to a particular sequence of DNA. Each primer is specific to the DNA of one shark species.

15 The primers are added to DNA taken from a shark’s fin and the polymerase chain reaction is carried out. Only two primers, one at each end of a certain piece of DNA, will bind. The piece of DNA between the primers is replicated by the polymerase chain reaction. The primers that bind are specific to a particular species of shark and the length of the DNA fragment replicated differs for each species. When this DNA is run in an electrophoresis gel it produces a single band, enabling the researchers to identify which species of shark is involved.

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

(a) (i) Explain why the DNA for each species of shark shows differences in base sequence (line 6).

(2)

(ii) Each primer is specific to the DNA of one shark species (line 12).

Explain why a particular primer will only bind to the DNA of one species.



(2)

(iii) The length of the replicated DNA fragment is different for each species.

Explain why this is important in identifying the shark species involved.

(3)

(b) In conventional DNA fingerprinting, a series of bands is produced on the electrophoresis gel, resembling the rungs of a ladder. When the DNA in this new genetic fingerprinting technique is run in an electrophoresis gel it produces just one of these 'rungs'.

Explain the reason for the difference in the number of 'rungs' produced.

(2)

(c) Describe the polymerase chain reaction.



(6)
(Total 15 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.

(2)

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

(1)

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

(2)

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

(2)

(Total 7 marks)

18

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.

DNA samples extracted and cut into fragments using a restriction enzyme



Fragments separated from each other by electrophoresis



One region of the resulting gel was blotted with two pieces of filter paper. The first was soaked in a solution containing a radioactive DNA probe for the normal allele.
The second was soaked in a solution containing a radioactive DNA probe for the β -thalassaemia allele.

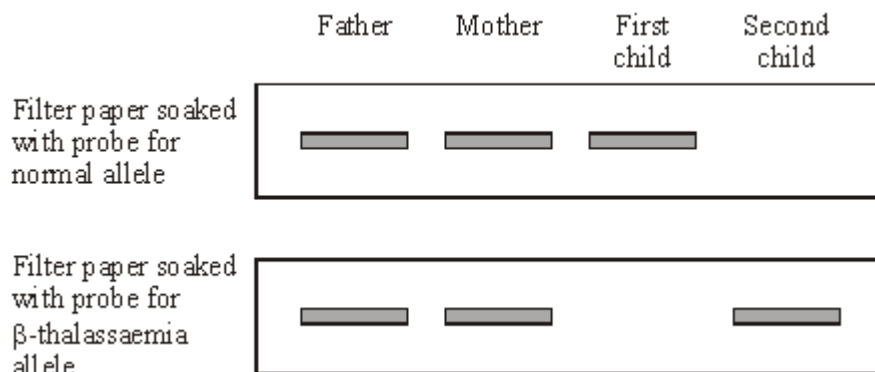


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Surplus probe washed off

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.

(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.

(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.

(2)

(Total 7 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**?

(1)

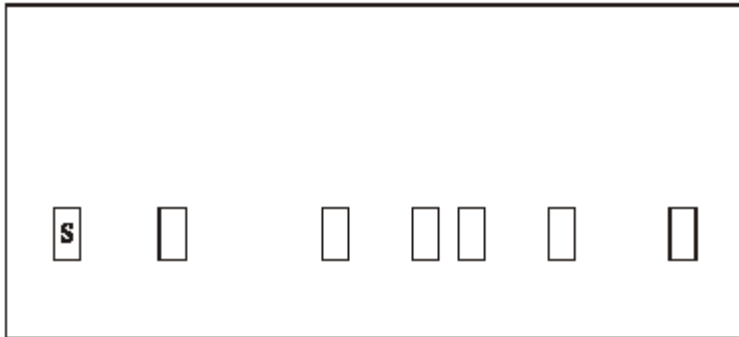
The table shows the number of base pairs present in the fragments.

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.



Direction of movement of fragments



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

(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.

(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)

Read the following passage.

(2)

(c) Explain why the nucleic acid on the test strip will only bind to altered DNA (lines 4-5).

(2)

(d) This test strip will allow cancers to be detected at a very early stage. Explain why cancer is more likely to be treated successfully if the disease is detected at a very early stage.

(2)

(e) Explain how examining mRNA (line 7) enables scientists to discover whether cancer is present.

(3)

(Total 15 marks)

Read the following passage.

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21

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.

5 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 10 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 15 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 20 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 25 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).

(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).

(2)

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

(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.

(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.



(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.

(2)

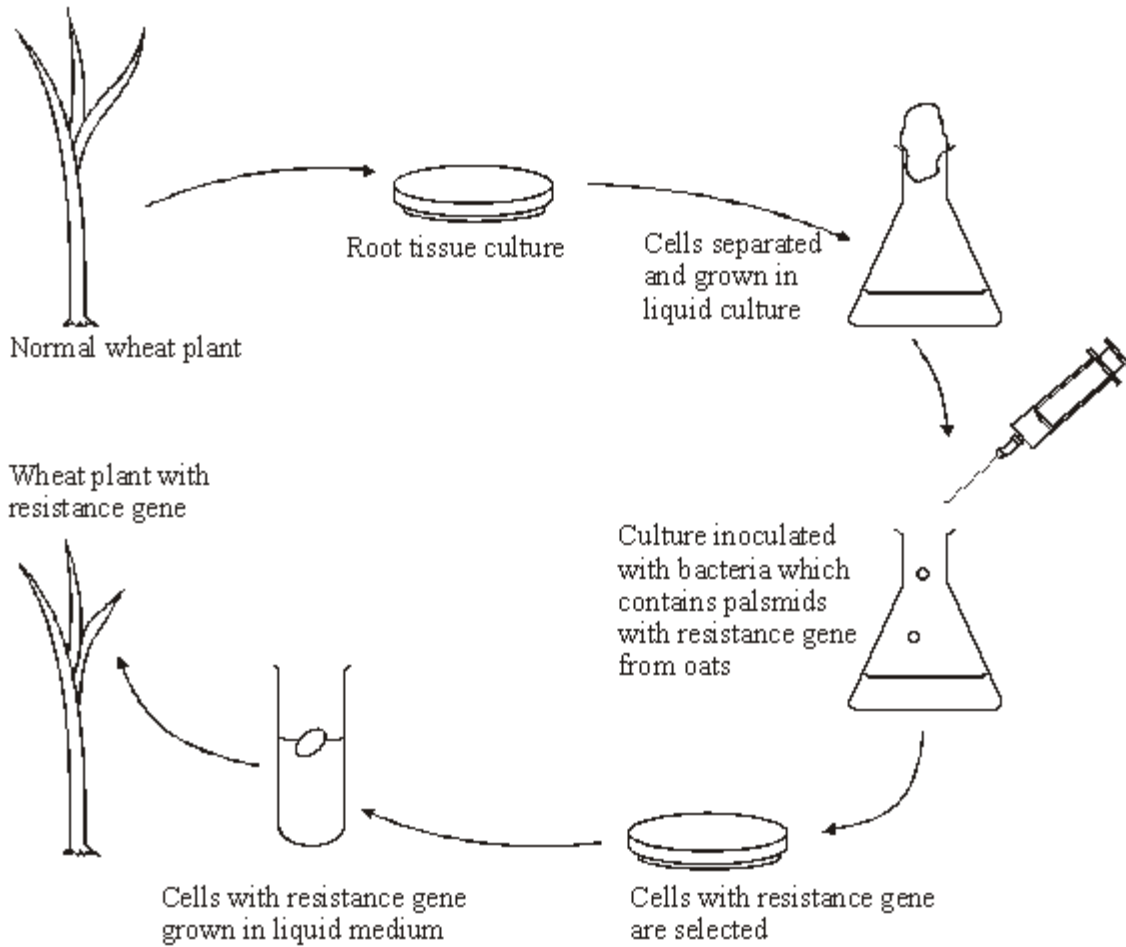
(Total 15 marks)

'Take-all' is a disease of wheat caused by a fungus. It can cause serious damage to the crop.

22

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*?

(1)

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

(1)

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

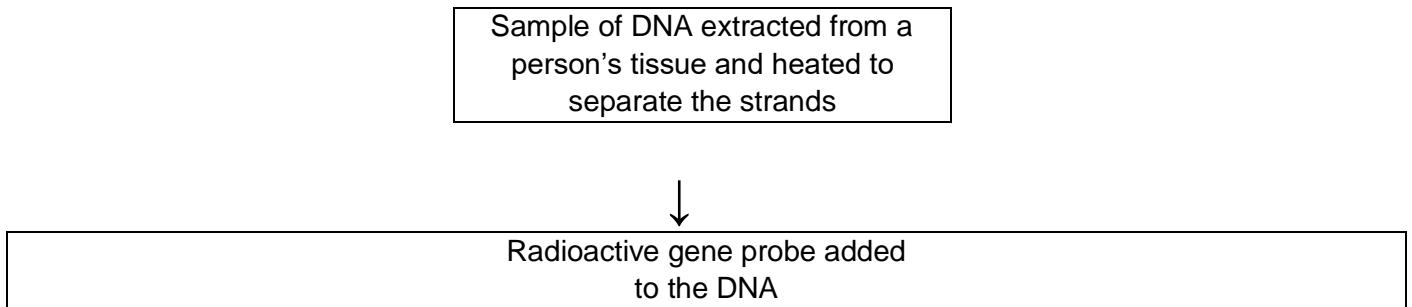
(4)

- S** (b) Hepatitis B vaccine contains a viral antigen produced by genetically modified bacteria. Describe how the isolated gene that codes for a protein in the virus's coat could be transferred to the bacterial cells.

(3)

(Total 7 marks)

- 24** (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.





Excess probe washed away



Sample tested for radioactivity

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.

(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

Against

(2)

(Total 6 marks)

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



25

(i) What is the role of a vector?

(1)

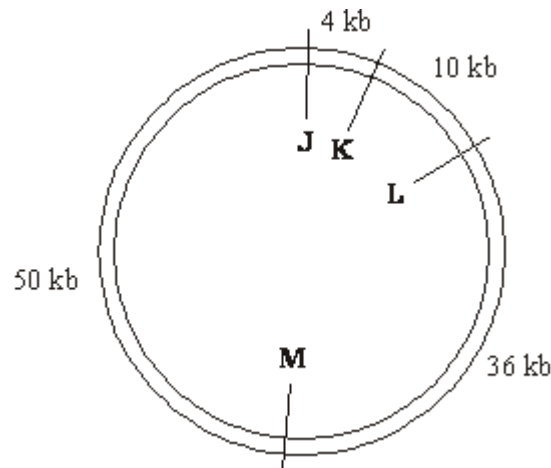
(ii) Describe the role of restriction endonucleases in the formation of plasmids that contain donor DNA.

(2)

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

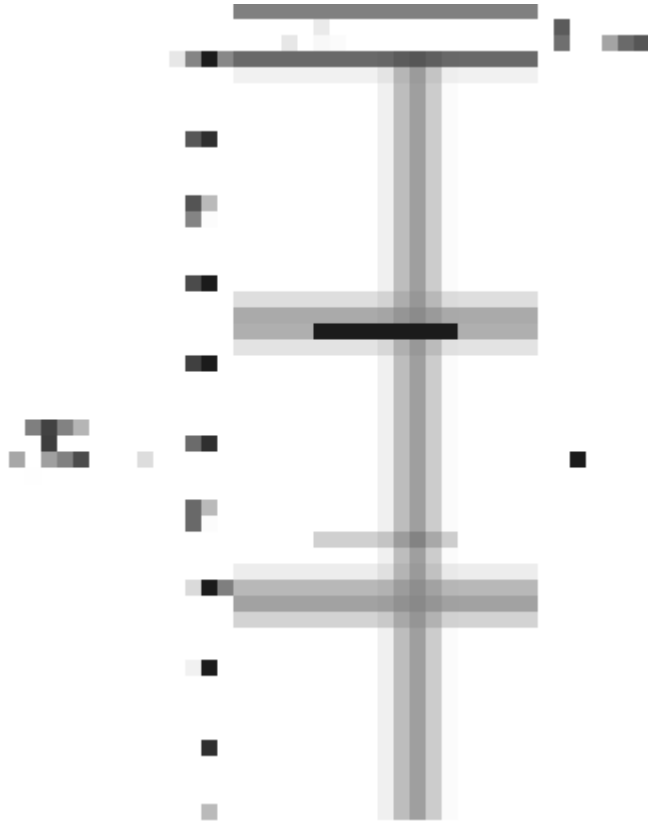
(1)

(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?

(1)

(ii) Explain your answer.

(1)

(Total 6

marks) A protein produced by a species of bacterium is toxic to caterpillars. The gene coding for this

26

protein was removed and transferred into a crop plant.

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

(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.

(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.

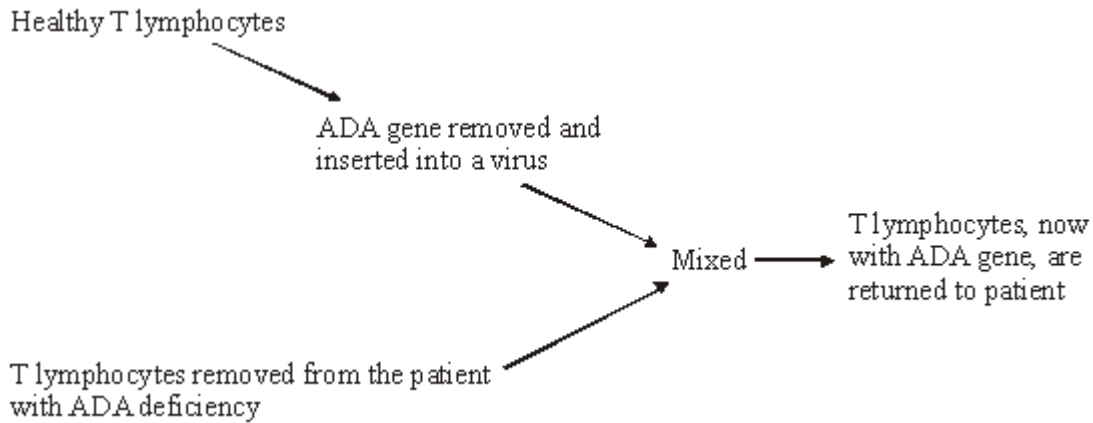
(3)

(Total 6 marks)

Gene therapy is used to treat the genetic disorder, ADA deficiency. Affected individuals are

27

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*?

(1)

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

1. _____

2. _____

(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.

(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.



(1)

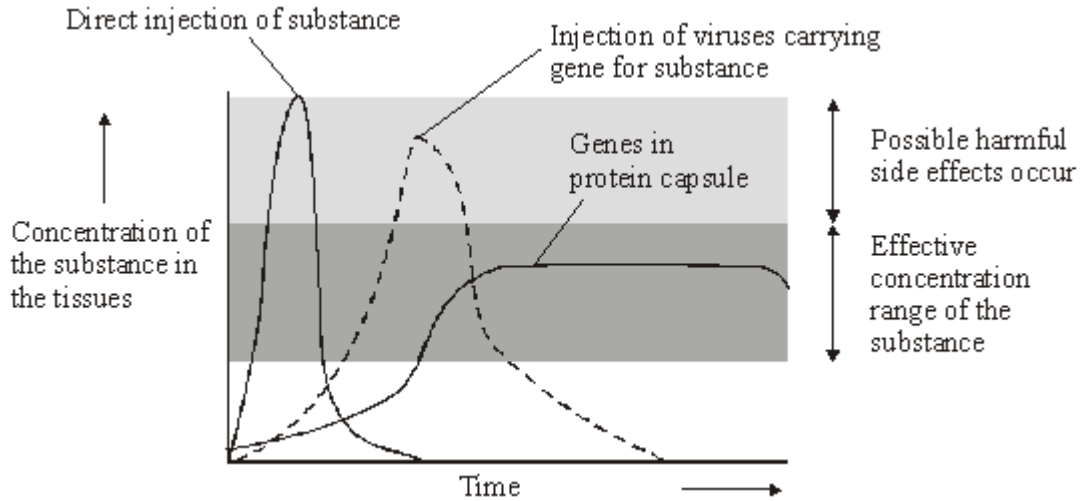
- (ii) Explain why treatment of ADA deficiency by gene therapy must be repeated at regular intervals, whereas a single bone marrow transplant can provide a permanent cure.

(2)

(Total 7 marks)



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.

(3)

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

Advantage _____

Disadvantage _____

(2)

(Total 11 marks)



30

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

(2)

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

(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.

(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.



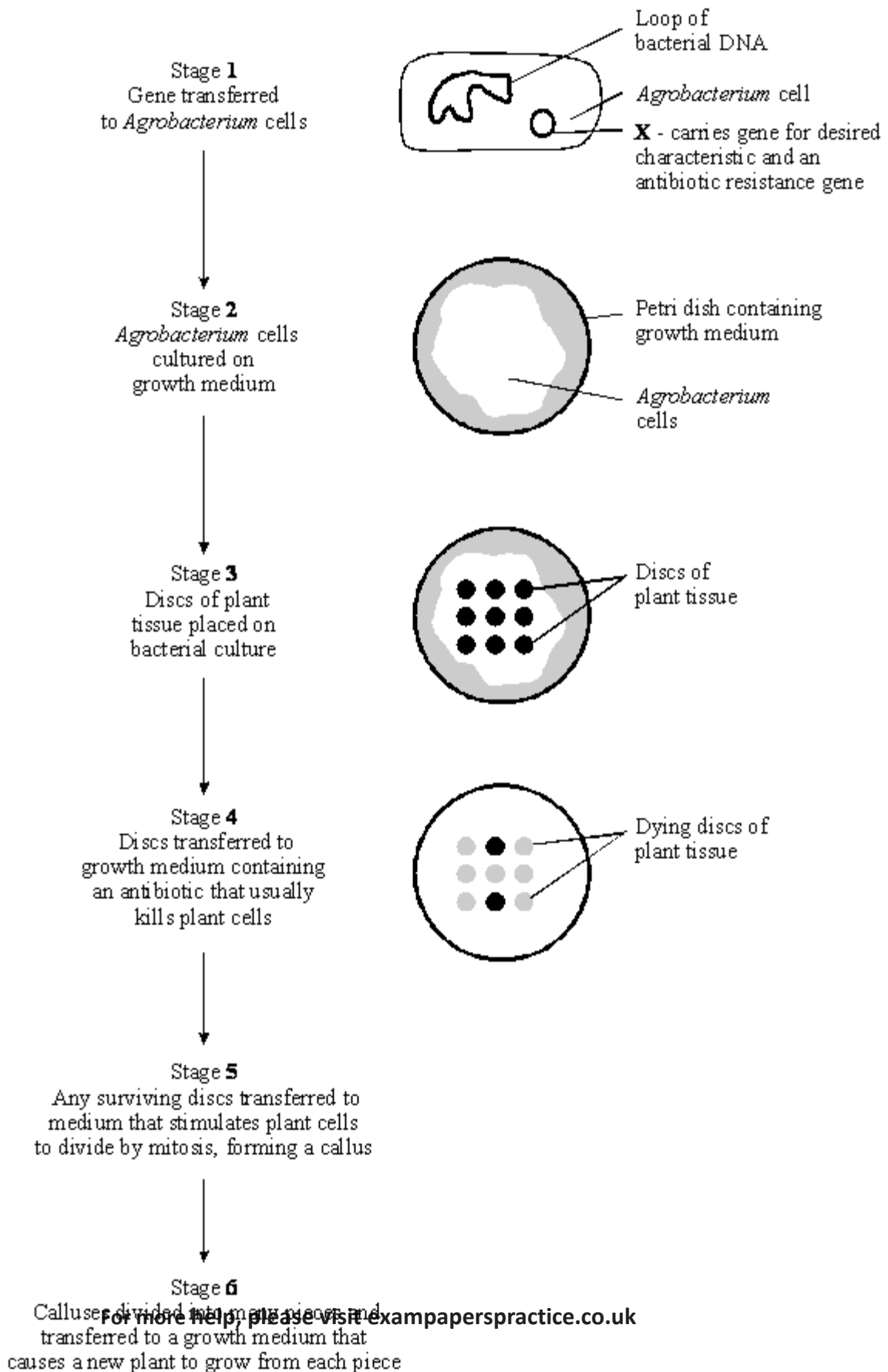
Explain why the enzyme is called *reverse transcriptase*.

(1)

(Total 9 marks)

(a) *Agrobacterium* is a bacterium used in genetic engineering of plants. The diagram shows

31 stages in the transfer of a gene into a plant.





(i) Name structure **X** in stage **1**.

(1)

(ii) In stage **2**, explain why the bacteria are cultured before the plant tissue is added.

(1)

(iii) In stage **4**, explain why the growth medium contains antibiotic.

(2)

(iv) Suggest why stages **5** and **6** are necessary for the commercial production of genetically engineered plants.

(2)

(b) (i) A toxin that kills insects can be sprayed directly onto the leaves of crop plants. A gene has now been transferred into crop plants that makes their leaves produce this toxin.

Explain **one** advantage to farmers of growing the genetically engineered crop plants, rather than spraying leaves with the toxin.

(1)



- (ii) Suggest **one** reason why some people are concerned that the toxin gene might get transferred to wild plants that are related to the crop plants.

(1)

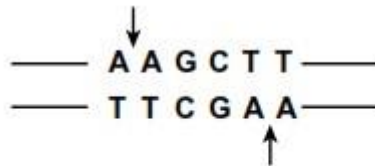
(Total 8 marks)

*Hind*III is an enzyme that cuts DNA into smaller fragments.

32

The enzyme cuts DNA at the specific base sequence shown in **Figure 1**.

Figure 1



↑ and ↓ = positions where DNA is cut

- (a) What general name is given to enzymes such as *Hind*III?

(1)

- (b) *Hind*III produces DNA fragments with sticky ends.

- (i) Use information from **Figure 1** to give the base sequence of one of these sticky ends.

(1)

- (ii) Sticky ends are useful in genetic engineering. Explain how.

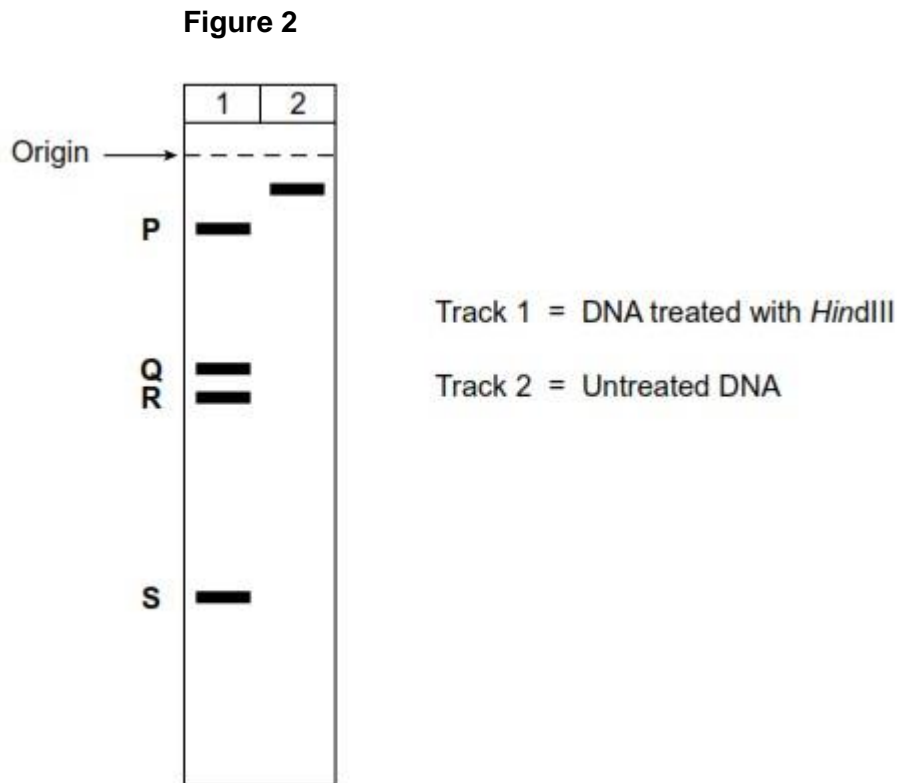
(2)



- (c) Scientists prepared a sample containing many identical molecules of DNA. The DNA molecules were linear (non-circular).

They divided the sample into two portions. They treated one portion with *Hind*III but did not treat the other portion. They then carried out gel electrophoresis on each portion.

The results are shown in **Figure 2**.



- (i) The lengths of the fragments produced from the DNA treated with *Hind*III were 287, 1232, 1550 and 4943 base pairs.
How many base pairs are there in fragment **P**?

P = _____ base pairs

(1)

- (ii) How many times did the base sequence, **AAGCTT** occur in the DNA?
TTCGAA

(1)

- (iii) In a certain genetic condition, **one** of these **AAGCTT** sequences is changed.

Predict what effect this would have on the appearance of the gel in Track 1 of Figure 2.

(2)

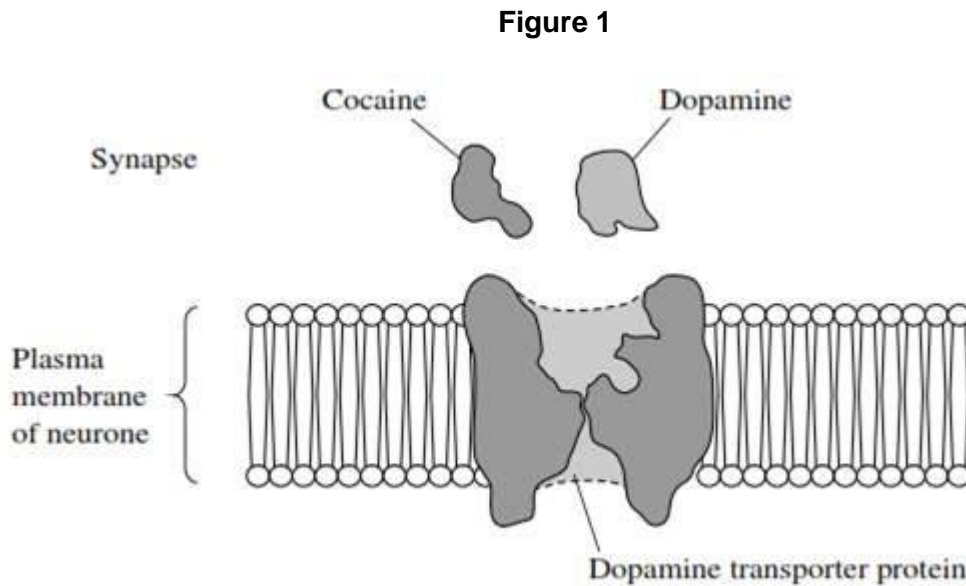
(Total 8 marks)

Cocaine is a highly addictive and illegal drug.

33

The release of the neurotransmitter dopamine in specific synapses in the brain leads to feelings of pleasure. Dopamine is removed from synapses by dopamine transporter proteins in the plasma membrane of neurones. Cocaine binds to the dopamine transporter protein.

Figure 1 shows a dopamine transporter protein and molecules of cocaine and dopamine.



(a) Using all of the information, suggest how cocaine leads to feelings of pleasure.

(Extra space) _____

(3)

- (b) (i) Scientists isolated a mutated gene for the dopamine transporter protein.

Name **one** method that the scientists could have used to produce many copies of the mutated gene in the laboratory.

(1)

- (ii) Copies of the gene were then inserted into early embryos of mice. When these mice were born, samples of their DNA were tested using DNA probes to make sure that the mutated gene was present in the mice.

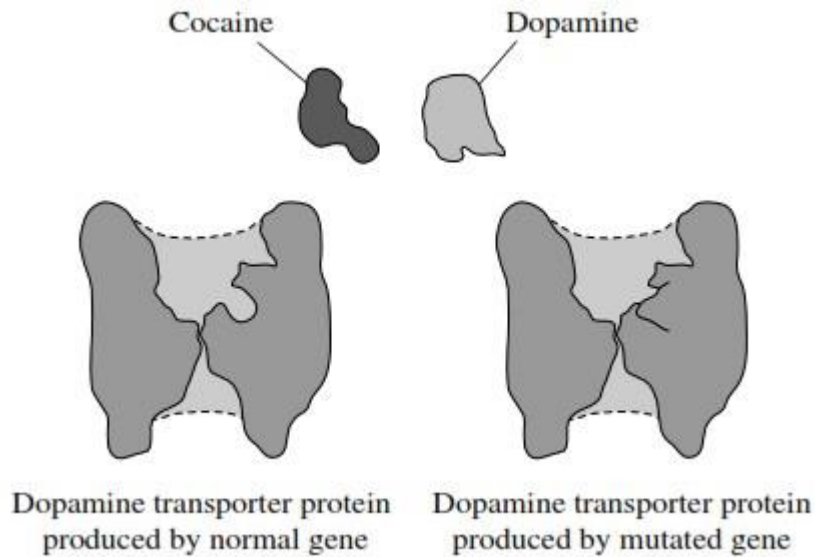
What is a DNA probe?

(2)

- (c) **Figure 2** shows dopamine transporter proteins produced from the normal gene and from the mutated gene.



Figure 2



Explain how the mutation leads to the production of a protein that transports dopamine but is **not** affected by cocaine.

(Extra space) _____

(3)
(Total 9 marks)

34

Scientists manufactured large quantities of human insulin using genetic engineering.

They started by isolating mRNA from pancreas cells. From this they produced DNA which coded for insulin.



- (a) (i) Suggest **two** reasons why it was better to start with mRNA from pancreas cells rather than with the DNA from these cells.

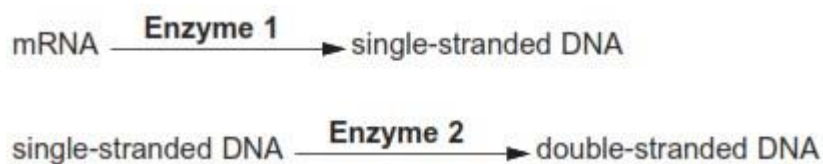
1. _____

2. _____

(2)

- (ii) The scientists used two enzymes, **Enzyme 1** and **Enzyme 2**, to produce DNA from mRNA.

The reactions catalysed by these enzymes are shown below.



Name enzymes **1** and **2**.

Enzyme 1 _____

Enzyme 2 _____

(2)

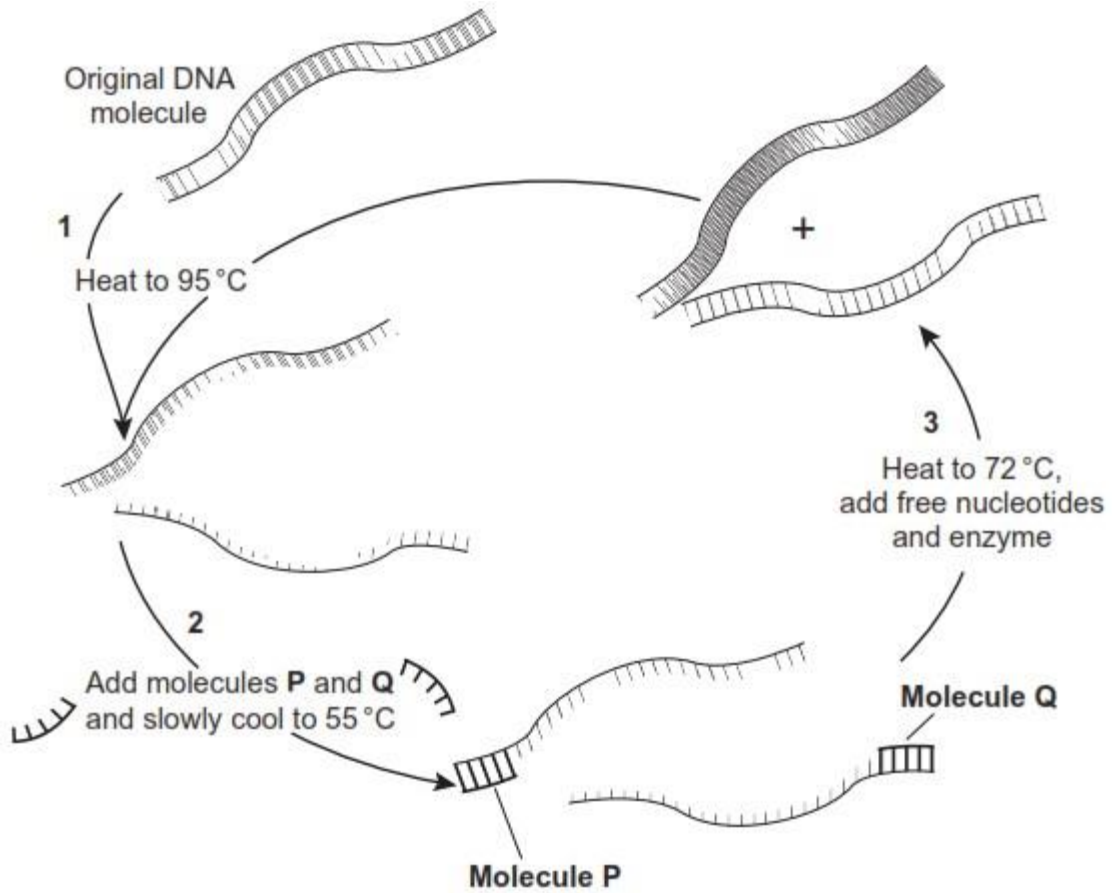
- (iii) In a double-stranded DNA molecule, the two strands are held together by weakbonds.

Name this type of bond.

(1)

- (b) The scientists used the polymerase chain reaction (PCR) to make copies of the DNA.

The diagram shows the stages of the PCR.



- (i) **P** and **Q** are short lengths of single-stranded DNA.

What name is given to molecules such as **P** and **Q**?

(1)

- (ii) The mixture is cooled from 95°C to 55°C at step 2.

Explain why.

(1)

- (iii) Explain the function of molecules **P** and **Q**.



(Extra space) _____

(2)

- (iv) How many copies of each original DNA molecule would be present after 5 cycles of PCR?

(1) (Total 10 marks)

Mark schemes

- 1**
- (a) 1. (If injected into egg), gene gets into all / most of cells of silkworm;
2. So gets into cells that make silk. 2
- (b) 1. Not all eggs will successfully take up the plasmid; 2. Silkworms that have taken up gene will glow. 2
- (c) Promoter (region / gene). 1
- (d) 1. So that protein can be harvested; 2. Fibres in other cells might cause harm. 2
- [7]** (a) 1. Cut (DNA) at same (base) sequence / (recognition) sequence;

2

- Accept: cut DNA at same place*
2. (So) get (fragments with gene) **R** / required gene.
Accept: 'allele' for 'gene' / same gene 2
- (b) 1. Each has / they have a specific base sequence; 2. That is complementary (to allele r or R). *Accept description of 'complementary'* 2
- (c) 1. Fragments L from parent rr, because all longer fragments / 195 base pair fragments;
Ignore: references to fragments that move further / less, require identification of longer / shorter or 195 / 135 *Accept: (homozygous) recessive*
2. Fragments N from parent RR, because all shorter fragments / 135 base pair fragments;
1 and 2 Accept: A3 for 195 and A4 for 135
2. *Accept: (homozygous) dominant*
3. (M from) offspring heterozygous / Rr / have both 195 and 135 base pair fragments.
Accept: have both bands / strips



Reject: primer longer / shorter

3

- (d) 1. (Cells in mitosis) chromosomes visible;
- 2. (So) can see which chromosome DNA probe attached to.

2

- (e) (i) 1. For comparison with resistant flies / other (two) experiments / groups;
Ignore: compare results / data / no other factors
- 2. To see death rate (in non-resistant) / to see effect of insecticide in non-resistant / normal flies. *Accept: 'pesticide' as 'insecticide'*
Accept to see that insecticide worked / to see effect of enzyme

2

- (ii) (PM must be involved because)
 - 1. Few resistant flies die (without inhibitor);
 - 2. More inhibited flies die than resistant flies;
 - 3. (PM) inhibited flies die faster (than resistant flies);
 (Other factors must be involved because)
 - 4. Some resistant flies die;
 - 5. But (with inhibitor) still have greater resistance / die slower than non-resistant flies.*Accept: (with inhibitor) die slower than non-resistant flies*

4 max

[15] (a) (i) Restriction endonuclease;

3

1

- (ii) (DNA) ligase;

1

- (b) (For those plants that contained the desired gene in the nucleus/plant DNA)

- 1. (DNA of desired gene) copied/replicated with host DNA/in nucleus;
- 2. Passed on by mitosis/plant grows by mitosis;
- 3. Produces genetically identical cells/clones;

Ignore references to protein synthesis or plasmids not taking up the gene

1. Accept DNA replication during mitosis

1. and 2. Accept converse for plants with the gene in the cytoplasm

3. Neutral 'identical unqualified'

3. Accept description, e.g., DNA is the same

3



- (c) 1. Genetic code is universal/triplets in DNA always code for same amino acid;
2. It/insect DNA can be transcribed;
3. Can be translated (process/mechanism same in all organisms/cells);
2. *Accept (basic) transcription (process/mechanism) same in all organisms/cells;*
2. *Accept descriptions of process*
3. *Accept descriptions of process*

3

[8]

- (a) Reverse transcriptase;

4

1

- (b) 1. Probe (base sequence) complementary (to DNA of allele A / where A is (and) binds by forming base pairs / hydrogen bonds; *Accept gene A*
2. So (only) this DNA labelled / has green dye / gives out (green) light; *Accept glows for green light*

2

- (c) (i) 1. More probe binding / more cDNA / mRNA / more allele / gene A means more light;
2. DNA (with **A**) doubles each (PCR) cycle;
3. So light (approximately) doubles / curve steepens more and more (each cycle) / curve goes up exponentially / increases even faster;

3

- (ii) (**G** because)

1. (Heterozygous) only has half the amount of probe for **A** attaching / only half the amount of DNA / allele A (to bind to); *Accept only one A to bind to*
2. (So,) only produced (about) half the light / glow / intensity (of **H**) (per cycle of PCR);
If reference to 'half' for point 1, allow 'less light' in 2.

2

- [8] (a) 1. Adenylate cyclase activated / cAMP produced / second messenger produced;

5

3. Activates enzyme(s) (in cell so) glycogenolysis / gluconeogenesis occurs / glycogenesis inhibited;



2. *Neutral: 'glucose produced' as given in the question stem*

Accept: correct descriptions of these terms

2

(b) (i) 1. Glucose / sugar in food would affect the results;

1. *Accept references to starch / carbohydrate Or*

2. Food / eating would affect blood glucose (level);

Or

3. (Allows time for) blood glucose (level) to return to normal;

3. Neutral: allows time for insulin to act

1 max

(ii) Type 2 diabetes is a failure to respond to insulin / still produces insulin / is not insulin-dependent;

1

(iii) (For) – 3 max

A maximum of three marks can be awarded for each side of the argument

1. Avoids injections / pain of injections;

2. Long(er) lasting / permanent / (new) cells will contain / express gene; *Ignore references to methodology e.g. sample size not known*

3. Less need to measure blood sugar / avoids the highs and lows in blood sugar;

4. Less restriction on diet;

(Against) – 3 max

5. Rats are different to humans;

6. May have side effects on humans;

6. *Accept: virus may be harmful / disrupt genes / cause cancer*

7. Long(er) term effects (of treatment) not known / may have caused effects after 8 months;

8. (Substitute) insulin may be rejected by the body;

4 max

[8] (a) Restriction / endonuclease;

6

Ignore specific names of restriction enzymes e.g. EcoR1

1

(b) (i) 1. (Acts as a) marker gene to show that the (human) gene has been taken up / expressed;

1. *Accept: gene marker*

2. (Only) implant cells / embryos that show fluorescence / contain the jellyfish gene;

2

(ii) 1. Factor IX present in / extracted from milk;

2. Gene only expressed in mammary glands / udder / gene not expressed elsewhere;

2. *Ignore references to milk*

The 'only' aspect is important here.

3. Do not need to kill sheep (to obtain Factor IX);

2 max

(c) (i) 1. Mutation / nucleus / chromosomes / DNA may be damaged / disrupts genes;

1. *Neutral: cell may be damaged*

2. May interfere with proteins (produced) / gene expression / translation; *Ignore references to hormone levels or time of implantation*

OR

3. Embryo / antigens foreign;

3. *Neutral: antigens change*

4. Embryo is rejected / attacked by immune system;

4. *sNeed idea that the immune system is involved if mark point 3 has not been given*

'Embryo foreign so rejected' = 2 marks

'Embryo rejected by immune system' = 1 mark

'Embryo is rejected' = 0 marks

2 max

(ii) 1. Saves time / money for others;

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2. Same work is not repeated / methods can be compared / improved / amended / same errors are not made;

2

[9]

7

- (a) 1. No effect at 25°C

The question only refers to plants with GB

1. *Reject same mass*

2. Keeps growing at 30°C and 35°C / up to 35°C (more than without GB);

3. Above 35°C, falls but grows more than plant without GB;

3. Accept at all temperatures above 25°C more growth than without GB

2 max

- (b) (i) Significantly different / SEs do not overlap ;

Accept converse without GB

1

- (ii) (As temperature increases,)

1. Enzyme activity reduced / (some) enzymes denatured;

2. Less photosynthesis, so fewer sugars formed;

3. Less respiration / less energy / ATP for growth;

4. Less energy for named function associated with growth

4. Eg mitosis, uptake of mineral ions

4

- (c) 1. (Rubisco activase attaches to thylakoid and) this changes shape / tertiary structure (of enzyme) / blocks active site / changes active site;

Note - question states enzyme stops working when it attaches to thylakoid, not before

1. *Accept rubisco in this context*

2. (This) prevents substrate / RuBP entering active site / binding;

2. Accept prevents ES complex forming

2. Accept no longer complementary to substrate / RuBP

2

- (d) 1. GB prevents / reduces binding of rubiscoactivase to (thylakoid membrane);

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1. *Accept enzyme instead of rubiscoactivase. Accept rubisco*
2. (Prevents it) up to 35°C;
3. (So) rubiscoactivase / enzyme remains active;
4. (So) photosynthesis / light-independent stage still happens;
 4. *Accept descriptions of light-independent stage*
5. Above 35°C, some binding still occurs but less than without GB, so less reduction in growth;

4 max

- (e) 1. Looked for information / journals, on crop plants that grow at high temperatures;
 1. *“other research” is minimum accepted*
1. *Accept previous experiments research with temperature resistant crops
 Ignore simple references to looking at previous studies / other plants - need to relate to this context*
 2. (Crop plants cited in this research) contain / make GB;
 3. So assumed making plants produce GB makes them resistant to high temperatures;

2 max

[15] (a) restriction (enzyme) / endonuclease / named example;

8 **1**

- (b) unpaired bases / sticky ends / staggered; complementary / explained;

2

- (c) *1 mark for each correct outcome* plasmid with foreign DNA joined in ring; ring with plasmid only; ring of foreign DNA only; *ignore linear structures*

3

[6] (a) 1. Closer the (amino acid) sequence the closer the relationship;

9 **1**

2. (Protein structure) related to (DNA) base / triplet sequence;
Amino acid sequence is related to (DNA) base / triplet sequence = two marks;

2



- (b) 1. Reference to base triplets / triplet code / more bases than amino acids / longer base sequence than amino acid sequence;
Different (base) triplets code for same amino acids = 2 marks;
Degeneracy of triplet code = 2 marks
2. Introns / non-coding DNA / degeneracy of code / more than one code for each amino acid;
Ignore reference to codon.

2

[4]

10

- (a) (i) 1. Negative correlation;
Accept: description for 'negative correlation'
Neutral: 'correlation'
Reject: positive correlation
2. Wide range;
3. Overlap;
4. (Graph suggests that) other factors may be involved (in age of onset);
2 / 3 Accept the use of figures from the graph
2 / 3 Can refer to age of onset or number of CAG repeats
Ignore references to methodology
3 max
- (ii) 1. Age of onset can be high / symptoms appear later in life;
Accept: 'gene' for 'allele'
2. (So) individuals have already had children / allele has been passed on;

OR

3. Individuals have passed on the allele / already had children;
4. Before symptoms occur;

2 max

- (b) (i) 1. Person **K**;
2. (As has) high(est) band / band that travelled a short(est) distance / (er) so has large(st) fragment / number of CAG repeats; *Must correctly link distance moved and fragment size*

- (ii) Run fragments of known length / CAG repeats (at the same time);

Accept: references to a DNA ladder / DNA markers

Do not accept DNA sequencing

1

- (iii) Homozygous / (CAG) fragments are the same length / size / mass;

Accept: small fragment has run off gel / travelled further

1

[9]

Essay Using DNA in science and technology

11

DNA and classification

2.2 Structure of DNA

2.3 Differences in DNA lead to genetic diversity

2.9 Comparison of DNA base sequences

Genetic engineering and making useful substances

2.5 Plasmids

5.8 The use of recombinant DNA to produce transformed organisms that benefit humans

Other uses of DNA

2.5 Cell cycle and treatment of cancer

5.8 Gene therapy;

Medical diagnosis and the treatment of human disease;

The use of DNA probes to screen patients for clinically important genes.

- (a) (i) protein / immunoglobulin;

12

specific to antigen;
idea of 'fit' / complementary shape;

2 max

- (ii) 1. virus contains antigen;

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- 2. virus engulfed by phagocyte / macrophage;
- 3. presents antigen to B-cell;
- 4. memory cells / B-cell becomes activated;
- 5. (divides to) form clones;
- 6. by mitosis;
- 7. plasma cells produce antibodies;
- 8. antibodies specific to antigen;
- 9. correct reference to T-cells / cytokines;

6 max

- (b) 1. antibody gene located using gene probe;
 2. cut using restriction enzyme;
 3. at specific base pairs;
 4. leaving sticky ends / unpaired bases;
 5. cut maize / DNA / vector using same restriction enzyme;
 6. join using DNA ligase;
 7. introduce vector into maize / crop / recombinant DNA into maize;

4 max

- (c) passive / person is not making own antibodies / antibodies not replaced; memory cells not produced;

2

- (d) fewer ethical difficulties / less risk of infection;

1

[15]

- (a) Restriction (enzyme / endonuclease);

13

1

- (b) Move towards anode / move because charged;

Different rates of movement related to charge / size;

2

- (c) (i) Piece of DNA;
 Single stranded;
 Complementary to / binds to known base sequence / gene;

max 2

- (ii) DNA invisible on gel / membrane;
 Allows detection;

2

[7] (a) (i) Reverse transcriptase;



14 **1**

(ii) Idea that mRNA is present in large amounts in cell making the protein / mRNA has been edited / does not contain introns / mRNA codes for single protein;

1

(b) (Ligase) splices / joins two pieces of DNA / “sticky ends”;

1

[3] (a) (i) Sticky ends / description;

15

Reference to complementary base-pairing

2

(ii) Ligase;

1

(b) Carrier of DNA / gene; (*context of foreign DNA*)
Into cell / other organism / host;

2

(c) Act as marker gene;
Allows detection of cells containing plasmid / DNA;

2

[7]

16

(a) (i) Different genes / characteristics / features;

Reference to mutations;
Or
Base sequence determines protein;
Different species have different protein sequences;

max 2

(ii) Primer has different DNA sequence;
DNA specific / complementary base-pairing;

2

(iii) Electrophoresis separates DNA;
(So they can be) identified by position on gel;
Smaller / shortest fragments travel furthest / quicker / or reverse argument;

3



(b) (*conventional*) Many lengths / all DNA / (*new*) one length;
Each rung is DNA of one / specific length;

2

(c) 1 Heat DNA;
2 Breaks hydrogen bonds / separates strands;
3 Add primers;
4 Add nucleotides;
5 Cool;
6 (to allow) binding of nucleotides / primers;
7 DNA polymerase;
8 Role of (DNA) polymerase;9 Repeat cycle many times;

max 6

[15] (a) (i) Sticky ends / description;

17

Reference to complementary base-pairing

2

(ii) Ligase;

1

(b) Carrier of DNA / gene; (*context of foreign DNA*)
Into cell / other organism / host;

2

(c) Act as marker gene;
Allows detection of cells containing plasmid / DNA;

2

[7] (a) Mother and father both heterozygotes / Tt / carriers;

18

Probability of thalassaemia 1/4 and female 1/2;
Probability of both 1/8;

3

(b) (i) Cut at same base sequence as same enzyme used;
Fragments are same length / size / have same charge;

2

(ii) Single base occurs many times;
Sequence of 20 unlikely to occur elsewhere;
Allow one mark for establishing the principle where neither marking point clearly made.

2



19

1

(b) DNA made of base pairs;
Each base pair is same length / occupies same distance
along backbone;

2

(c) (i) Second blank box from left labelled 6;

1

(ii) Distance moved depends on length / number of base pairs /
second longest fragment / second shortest distance identified;

1

(d) 5;

1

[6] (a) 1 (DNA altered by) mutation;

20

2 (mutation) changes base sequence;
3 of gene controlling cell growth / oncogene / that monitors cell division;
4 of tumour suppressor gene;
5 change protein structure / non-functional protein / protein not formed;
6 (tumour suppressor genes) produce proteins that inhibit cell division; 7 mitosis;
8 uncontrolled / rapid / abnormal (cell division);
9 malignant tumour;

max 6

(b) cancer cells die / break open; releasing DNA;

2

(c) normal DNA and changed DNA have different
sequences;
DNA only binds to complementary sequence;

2

(d) fewer abnormal / cancerous cells / smaller
tumours; less cell damage / less spread / fewer
locations to treat;

2

(e) mRNA base sequence has changed; gene / DNA
structure is different / has mutated; cancer gene active
/ tumour suppressor gene inactive;

3

[15]

- (a) Presence of resistant and non-resistant varieties / mutation produces resistant variety;

21

Resistant ones survive / non-resistant ones killed by treatment;
These will reproduce and produce more resistant parasites / pass on resistance allele;

3

- (b) Likelihood of being infected (by strain resistant to both drugs) is less;
 $1/500 \times 1/500 = 1/250\,000$;
Drug has longer effective life;

max 2

- (c) (i) As comparison / to show that nothing else in the treatment was responsible;

1

- (ii) Given injections of saline / injection without SPf66;
(otherwise) treated the same as experimental group;

2

- (d) (i) 100%;

1

- (ii) 10%;

1

- (e) (i) Different lengths of DNA have different base sequences / cut at specific sequence;
Results in different shape / different shape of active site;
Therefore (specific sequence) will only fit active site of enzyme;

3

- (ii) Recognition sites contain only AT pairs;
Which would occur very frequently;

2

[15] (a) (i) contains genes / nucleotides / sections of DNA / artificial

22

DNA from two species / 2 types of organisms;

1

- (ii) carries gene / DNA (into the other organism / gene carrier);

1

- (iii) expose cells to the fungus; non-resistant ones die, resistant ones survive;

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OR identify by adding marker gene / gene probe / (qualified) marker probe; description of positive result e.g. radioactivity / fluorescence / complementary base pairing;

2

- (b) EITHER 1 cut desired gene (from DNA) of oat plant;
 2 using restriction endonuclease / restriction enzyme;
 OR 1 use mRNA from oat which will code for resistance;
 2 and use reverse transcriptase to form desired DNA;
 OR 1 make artificial DNA with correct sequence of bases;
 2 using DNA polymerase;
 3 cut plasmid open;
 4 with (same) restriction endonuclease / restriction enzyme;
 5 ref. sticky ends / unpaired bases attached;
 6 use (DNA) ligase to join / ref. ligation;
 7 return plasmid to (bacterial) cells;
 8 use of Ca²⁺ / calcium salts / electric shock; (if ref. to 'insulin' allow 5 max.)

max 6

[10] (a) 1 macrophages present antigens to B lymphocytes;

23

- 2 antigen binds to / is complementary to receptors on lymphocyte;
 3 binds to a specific lymphocyte;
 4 lymphocytes become competent / sensitised;
 5 (B) lymphocytes reproduce by mitosis / (B) lymphocytes cloned; 6 plasma cells secrete antibodies;

4 max

- (b) 1 restriction enzyme / endonuclease;
 2 to cut plasmid / to form sticky ends in plasmid;
 3 (use) ligase(to join) gene to plasmid;
 4 culture bacteria with (in medium containing) plasmids
 5 to allow uptake of plasmids / transformation;
 6 use of cold shock / chemical treatment (to enhance uptake) / heat shock;
(ignore bullets / electroporation / microinjection)

3 max

[7] (a) probe will attach (to mutant allele);

24

attaches to one DNA strand; as a result of complementary base pairing; radioactivity detected on film / X-ray / by autoradiography (if mutant allele present);

4



- (b) for gene is only active in mammary cells / only affects milk / easy to obtain product / product produced in large amounts / gene passed to offspring;

1

against long term effects not known / qualified reference to animal exploitation e.g. use of embryos / effect of inserted gene on other sheep tissues / genes;

1

[6] (a) (i) transfer / carry genes from one organism to another / into

25 bacteria / cells;

1

- (ii) cut open plasmid; cut donor DNA, to remove gene / length of DNA; cut donor DNA and plasmid with the same enzyme / enzyme that cuts at the same base sequence; sticky ends / (overhanging) ends with, single strand / bases exposed; association / attachment / pairing of complementary strand;

2 max

- (iii) annealing / splicing / backbones joined / phosphodiester bonds;

1

- (b) (i) L and M;

1

- (ii) fragments 64 and 36(kilobases obtained)

1

[6] (a) (i) restriction (endonuclease) enzyme;

26

cuts DNA at specific / restriction points / after specific base sequence;

2

- (ii) PCR / polymerase chain reaction;

1

- (b) isolated cells divide by mitosis; can get many plants (producing toxin) / rapid production of (toxin producing) plants; all cells (in the new plant / clone) will produce the toxin;

3

[6]

- (a) introduction of healthy gene / 'replacement' of defective gene;

**27****1**

- (b) can enter cells / infect cells / inject DNA into cells; targets specific cells; replicates (in cells); **2**
- (c) reproductive cells / gamete cells do not contain ADA allele / gene; **1**
- (d) (i) to 'prevent' rejection / immune response; **1**
- (ii) T lymphocytes have a limited life span / die off / do not reproduce;
bone marrow provides continual supply of T lymphocytes /
(ADA) gene enzyme; **2**

[7] (a) (cut out gene using an) endonuclease / restriction enzyme;

28

reference to specificity / recognition site; sticky ends; use the same enzyme to cut; plasmid / virus / potato DNA; fixed by ligase; method of introducing vector e.g. micropipette / virus injects DNA / remove plant cell wall;

6 max

- (b) different genes are expressed;
producing different enzymes / proteins;

2

[8] (a) isolate wanted gene / DNA from another organism / mRNA from cell / organism;

29

using restriction endonuclease / restriction enzyme / reverse transcriptase to get DNA and produce sticky ends; use ligase to join wanted gene to plasmid; also include marker gene e.g. antibiotic resistance; add plasmid to bacteria to grow (colonies) then (replica) plate onto medium where the marker gene is expressed; bacteria / colonies not killed have antibiotic resistance gene and (probably) the wanted gene;

6

- (b) (i) injection, rapid rise and fall; virus, slower rise and longer in effective / harmful range; capsule slowest rise, longest in effective / harmful range; injection and virus give harmful concentrations but capsule does not;

3 max

- (ii) advantage e.g.:



substance never reaches harmful levels / no side effect / less likely to harm the organism, longer relief from symptoms / less frequent treatment needed / longer effective range / longer but without harmful side effects;

1 max

disadvantage e.g.: takes longer to take effect;

1

[11] (a) use restriction enzyme / endonuclease / named, e.g. Bam / Eco;

30 to cut DNA in specific place / base sequence;

2

(b) heat DNA to 90 – 95 °C;strands separate; add primers; and nucleotides; cool so that primers bind to DNA; (DNA) polymerase forms new strands / joins nucleotides;

4 max

(c) (i) virus is inhaled / sprayed into the lungs;gets into cells, inserting the healthy gene;

2

(ii) makes DNA from RNA
rather than other way round

1

[9] (a) (i) plasmid;

31

1

(ii) the bacteria divide / grow, producing many copies of desiredgene / plasmid;
OR
the bacteria divide / grow to cover the agar;

1

(iii) plant tissue that has antibiotic resistance survives;identifies plant tissue which has desired gene / plasmid;

2

(iv) to clone plants / produce genetically identical plants with gene / characteristic;
and produce large numbers / quickly;

2



- (b) (i) (*one reasonable suggestion*),
e.g. toxin present all the time; save costs
of buying / application of spray; no spray
drift onto other fields / insects; 1 max
- (ii) (*one reasonable suggestion*),
e.g. killing of harmless / useful insects that feed on wild plants;
damage to food chains starting with wild plants; 1 max

[8] (a) Restriction enzyme / restriction endonuclease;

32

- (b) (i) A-G-C-T / T-C-G-A;
Allow A-G-C-T-T / T-T-C-G-A 1
- (ii) Joining two pieces of DNA;
By complementary binding/complementary base-pairing; 2
- (c) (i) 4943; 1
- (ii) 3; 1
- (iii) 2 bands disappear / only 3 bands;
New band formed at heavier position/nearer to origin/higher up; 2

[8] (a) Cocaine (binding) changes shape of transporter/prevents dopamine binding;

33

Reject references to active site

Transporter cannot move (bound) dopamine (through membrane / protein /
into cell);

Dopamine remains / builds up in synapses (leading to feelings of pleasure);

3

- (b) (i) Polymerase chain reaction / PCR; 1

(ii) Single-stranded DNA;

Reject reference to a single strand of DNA

Bases / sequence complementary to DNA / gene to be identified;

(Radioactively / fluorescent) labelled so that it can be detected;

2 max

(c) Mutation changes base sequence of gene / DNA;

Accept references to active site

(Thus) changing amino acid sequence;

Changes tertiary structure / shape of protein/transporter;

Cocaine binding site changes/cocaine cannot bind;

Dopamine can still bind (and be transported);

3 max

[9] (a) (i) Amount of mRNA > amount of DNA / multiple copies of mRNA;

34

Insulin mRNA/the specific mRNA is found in pancreas cells;

Introns / non-coding information present in DNA / these removed
in mRNA / corr. ref. post-transcriptional modification;

2 max

(ii) Enzyme 1 = reverse transcriptase;

Enzyme 2 = (DNA)-polymerase;

2

(iii) Hydrogen (bonds) / H-(bonds);

1

(b) (i) Primers;

1

(ii) To allow H-bond re-formation / to allow joining of
primers/P(and Q) to (single-stranded) DNA / converse re. high
temp. breaks H-bonds / prevents joining;

1

(iii) To mark region of DNA to be 'copied' / to show enzyme
whereto start;

(Enzyme) needs starting strand onto which to attach nucleotides;

Allow idea of extending pre-existing chain

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(iv) 32;

2

1

[10]