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VCE Biology $\frac{3}{4}$
Recombinant Plasmids [0.8]
Workshop

Section A: Multiple Choice Questions (15 Marks)**Question 1 (1 mark)**

Recombinant plasmids are used to transfer nucleic acid between different organisms. To maintain the structural integrity of the plasmid, the phosphodiester bonds of the backbone must be reformed. This is catalysed by:

- A. RNA polymerase
- B. DNA polymerase
- C. Ligase
- D. ATP synthase

Question 2 (1 mark)

Human insulin can be harvested in bacterial cells through the use of recombinant plasmids. How would a laboratory determine that the transformation of the bacteria had been successful?

- A. The bacteria can grow colonies on agar.
- B. The bacteria replicate more rapidly.
- C. The bacteria can be observed under a fluorescent light.
- D. The bacteria grow in the presence of an antibiotic.

Question 3 (1 mark)

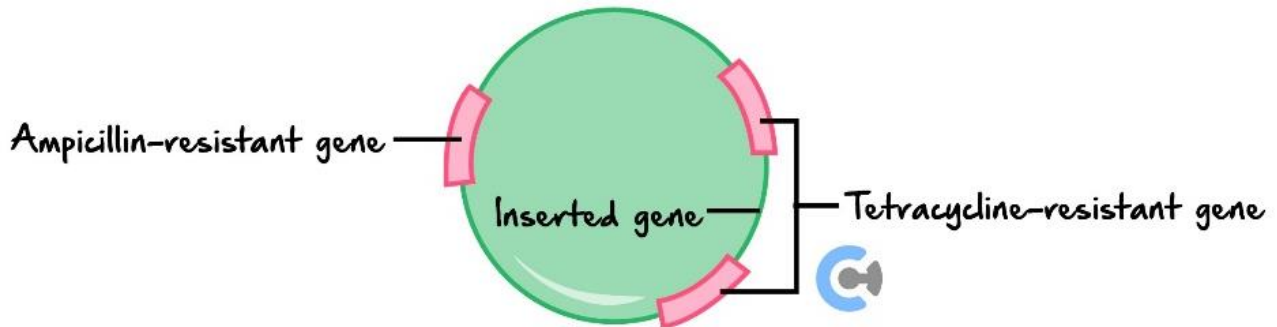
Which of the following steps would NOT occur in recombinant DNA technology?

- A. The gene of interest must be cut with a restriction enzyme to leave blunt ends.
- B. Antibiotic resistance genes are introduced into the plasmid to differentiate between bacteria that have taken up the plasmid and those that have not.
- C. Bacteria may be subjected to heat shock therapy to introduce the plasmid into the bacterial cells.
- D. Bacterial plasmids are cut with the same restriction enzyme as used for the gene of interest.

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Question 4 (1 mark)

The recombinant plasmid shown below was successfully integrated into a culture of bacteria. The inserted gene disrupted the tetracycline-resistant gene.



The transformed bacteria were smeared onto the following three Luria Broth (LB) nutrient agar plates:

- Plate 1 contains ampicillin.
- Plate 2 contains tetracycline.
- Plate 3 contains ampicillin and tetracycline.

Colonies of transformed bacteria would grow on:

- A. Plate 1 only.
- B. Plate 2 only.
- C. Plate 3 only.
- D. Plates 1 and 3 only.

Question 5 (1 mark)

When introducing a recombinant plasmid into bacterial cells, what factor is most crucial to ensure the plasmid is stably maintained in the host cell during cell division?

- A. The plasmid must contain a gene that confers resistance to a specific antibiotic.
- B. The plasmid must have a sequence that allows for replication independent of the bacterial chromosome.
- C. The plasmid should have a promoter sequence compatible with the host cell's transcription machinery.
- D. The plasmid should be linear rather than circular to facilitate easier integration into the host genome.

Question 6 (1 mark)

People with type 1 diabetes require regular injections of insulin to regulate their blood glucose concentration. Until 1978, insulin was obtained from cattle and pigs. However, this type of insulin caused allergic reactions in many patients. Currently, a significant percentage of insulin is produced by genetic engineering.

The gene for human insulin is inserted into a plasmid, which is then taken up by a bacterium. When that bacterium reproduces, all subsequent offspring will contain the plasmid as well. Which one of the following statements applies to the plasmid and the bacteria produced?

- A. The plasmid has been mutated, and the bacteria are vectors.
- B. The plasmid is amplified, and the bacteria are clonally selected.
- C. The plasmid is transformed, and the bacteria are selectively bred.
- D. The plasmid is recombinant, and the bacteria have been transformed.

Question 7 (1 mark)

A bacterial plasmid was modified by inserting a gene for an enzyme that provides resistance to the antibiotic ampicillin. A nutrient solution containing cells of the bacterium *Escherichia coli* was obtained. *E. coli* is naturally sensitive to the antibiotic ampicillin. The solution was divided into two equal volumes. The bacteria in one-half of the solution were left untreated. Plasmids were added to the other half of the solution, and the bacteria were treated to increase their chance of taking up the plasmids.

The next day, the bacterial cells were spread on agar plates as follows:

- Plate 1 - Untreated bacterial cells on nutrient agar.
- Plate 2 - Untreated bacterial cells on nutrient agar with ampicillin.
- Plate 3 - Treated bacterial cells on nutrient agar with ampicillin.
- Plate 4 - Treated bacterial cells on nutrient agar.

The plates were incubated overnight.

In order to collect only bacterial cells that had taken up the plasmid successfully, a sample should be taken from:

- A. Plate 1
- B. Plate 2
- C. Plate 3
- D. Plate 4

Question 8 (1 mark)

The process in which the bacterial cell takes up the plasmid is called:

- A. Translation
- B. Transcription
- C. Translocation
- D. Transformation

Question 9 (1 mark)

To produce a transgenic plasmid, a specific restriction enzyme was added to a mixture that contained two different DNA fragments: a plasmid and a small, linear section of DNA that carried a gene to be transferred. The restriction enzyme had one binding site on the plasmid and two binding sites on the linear DNA.

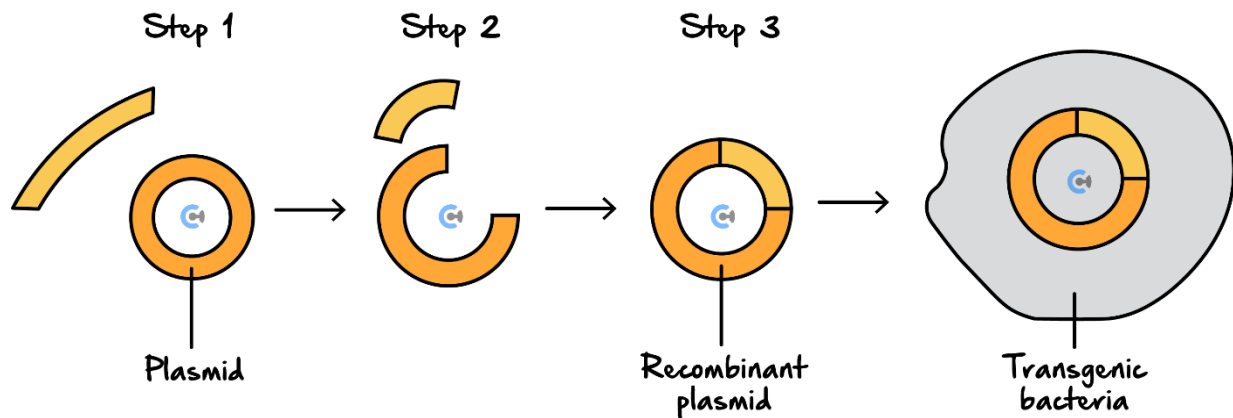
After the addition of the restriction enzyme, how many fragments of DNA would the mixture contain?

- A. 2
- B. 3
- C. 4
- D. 5

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The following information applies to the two questions that follow.

The diagram below shows the process that leads to the production of transgenic bacteria:



Question 10 (1 mark)

For the process shown in the diagram above to have a chance of successfully producing the transgenic bacteria:

- A. Ligase enzymes would need to be added in step 2.
- B. Restriction enzymes would need to be added in step 3.
- C. Shock treatment would need to be applied to the DNA and plasmid in step 1.
- D. All steps would need to be carried out at a high temperature.

Question 11 (1 mark)

Which one of the following is an example of a positive advancement that has been made using this type of technology?

- A. The production of insulin for individuals with type 1 diabetes.
- B. The insertion of a gene into the salivary gland of a pig.
- C. The production of antibiotic-resistant bacteria.
- D. The modification of a human ovum to form a superior genome.

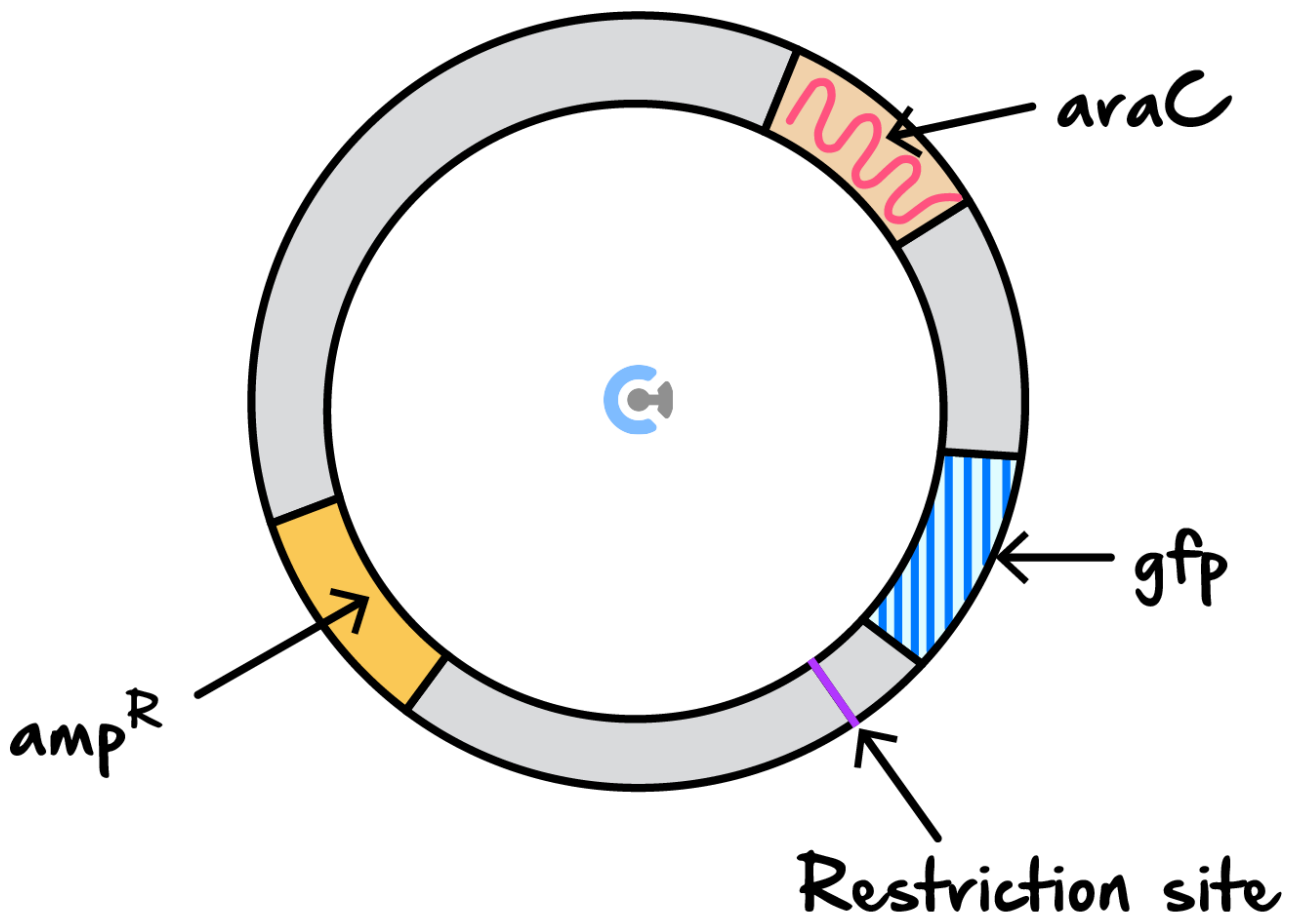
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The following information applies to the three questions that follow.

To clone a gene of interest, the following four steps are performed:

1. A plasmid is cut with a specific restriction enzyme.
2. The gene of interest is ligated into the plasmid.
3. Plasmids are transferred to bacteria.
4. Bacteria are grown on four nutrient agar plates (labelled W, X, Y, and Z) that are coated with or without ampicillin and arabinose.

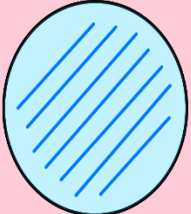
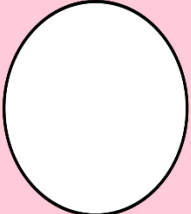
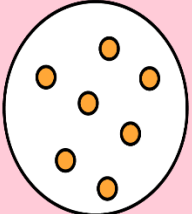
An example of a plasmid used in cloning is shown below:



This plasmid contains a restriction site and the following three genes:

- *amp^R*- confers resistance to the antibacterial agent ampicillin.
- *gfp*- encodes the green fluorescent protein (GFP), which fluoresces under UV light.
- *araC*- encodes a protein required to promote the expression of *gfp* when arabinose is present.

The results from a bacterial transformation experiment are shown in the table below:

Plate	W untransformed bacteria only	X untransformed bacteria only	Y transformed bacteria	Z transformed bacteria
Diagram of plate				
Added to plate	Nutrient agar only	Nutrient agar and ampicillin	Nutrient agar, ampicillin and arabinose	Nutrient agar and ampicillin
Description of result	Lawn of bacteria	No growth	Bacterial colonies present	Bacterial colonies present

Question 12 (1 mark)

Bacteria are used in gene cloning because they:

- A. Contain restriction enzymes that randomly cut chromosomes into fragments of varying size.
- B. Can replicate non-bacterial sequences of DNA in a short time.
- C. Replicate exponentially by undergoing mitotic divisions.
- D. Allow the entry of foreign DNA into their nuclei.

Question 13 (1 mark)

Which plate would contain bacteria that fluoresce under UV light?

- A. Plate W
- B. Plate X
- C. Plate Y
- D. Plate Z

Question 14 (1 mark)

Which one of the following statements is an accurate description for the purpose of plate W or X?

- A. Plate W shows that the plasmid was cut with a restriction enzyme.
- B. Plate W shows that the percentage of transformed bacteria was high.
- C. Plate X shows that the nutrient agar promoted the growth of viable bacteria.
- D. Plate X shows that ampicillin was effective in killing the untransformed bacteria.

Question 15 (1 mark)

Often, a human gene is added to a bacterial plasmid containing a gene for antibiotic resistance.

This is because:

- A. Only transformed bacteria will grow in a medium containing the antibiotic.
- B. The non-resistant bacteria can harm the scientists carrying out the procedure.
- C. The bacteria will be resistant to infection.
- D. It will prevent the emergence of a new disease.

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Section B: Short Answer Questions (59 Marks)**Question 16** (5 marks)

Diabetes can be an autoimmune condition where the body is activated to destroy the beta cells in the pancreas that produce insulin. Human insulin is a polypeptide hormone. Insulin consists of two polypeptide chains, chain *A* (21 amino acids) and chain *B* (30 amino acids), connected by two disulfide bridges. Human insulin can be made using recombinant plasmids.

Outline how human insulin is made using recombinant plasmids.

Question 17 (2 marks)

What are some benefits of utilising recombinant plasmids instead of other methods of extracting insulin?

Question 18 (4 marks)

Explain the significance of reporter genes and antibiotic resistance genes, and their purpose, in the transformation of bacteria for insulin production.

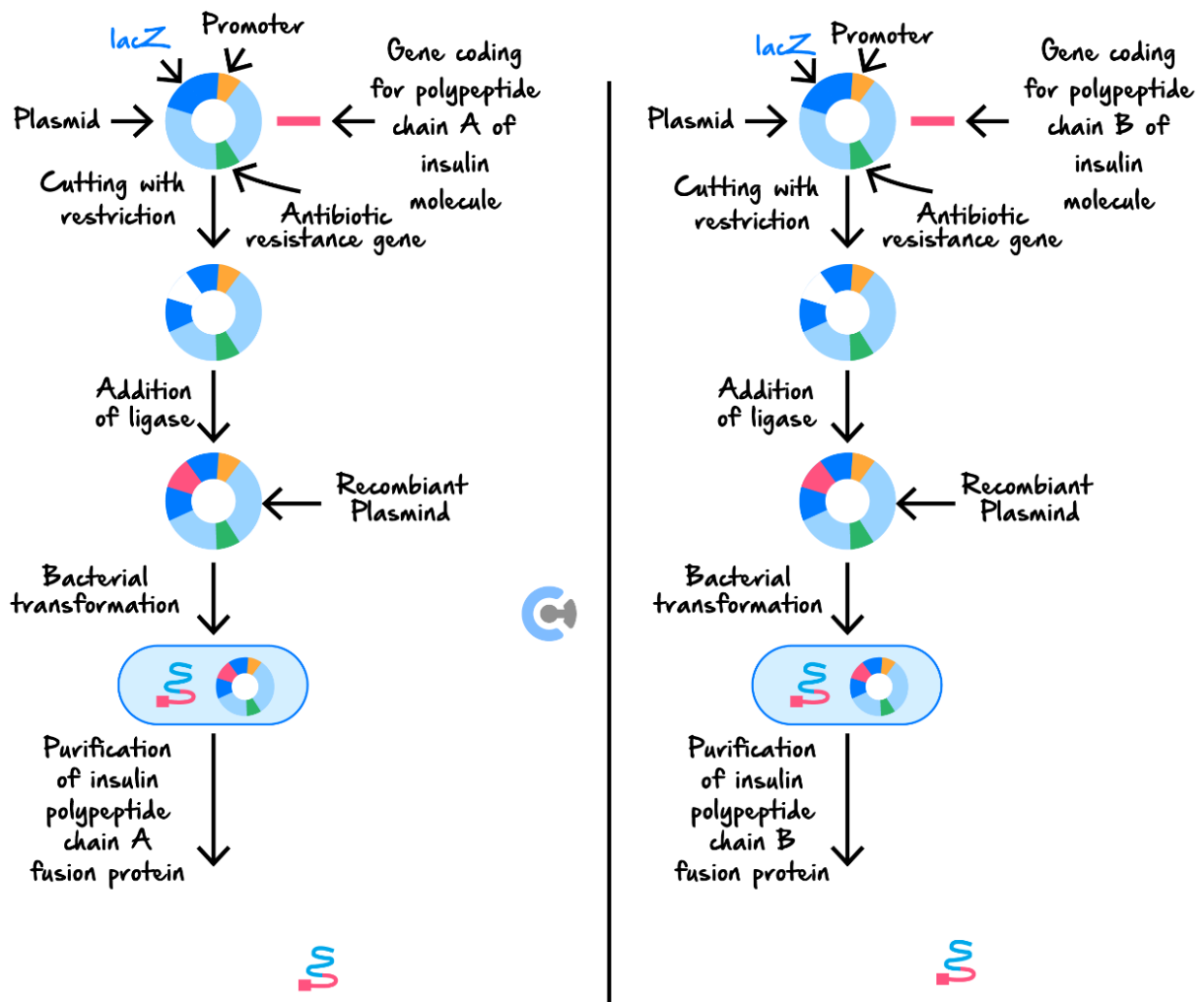
Question 19 (3 marks)

Describe what bacterial transformation is, and two methods that could be used to achieve it.

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Question 20 (7 marks)

Type 1 diabetics are unable to produce functional insulin, a protein consisting of two polypeptide chains (*A* and *B*) joined together. Initially, Type 1 diabetics relied on insulin injections from pigs to control their blood glucose levels. Pig insulin was isolated and purified and was the main source of pharmaceutical insulin. This use of pig insulin, however, came with some problems such as inducing allergic reactions in some Type 1 diabetic patients. Scientists are now able to identify the human insulin gene, isolate it and clone it using DNA manipulation technologies. A simplified diagram of this process is shown in the figure. Note: After purification of chain *A* and chain *B*, they are mixed to produce functional insulin.



- a. Name the part of the insulin genes that need to be removed before cloning. Explain why this step is necessary. (2 marks)

- b. Explain the function of restriction enzymes and DNA ligase in recombinant plasmid production outlined in the figure. (3 marks)

- c. Explain the purpose of the antibiotic-resistant gene in the recombinant plasmid. (2 marks)

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Question 21 (3 marks)

Describe the role of the b-gal protein in the production of human insulin. Will it be present in the final product given to diabetics?

Question 22 (18 marks)

One of the key treatments for Pompe disease is enzyme replacement therapy. The alpha-glucosidase is produced by recombinant Chinese hamster cells as shown in figure below:

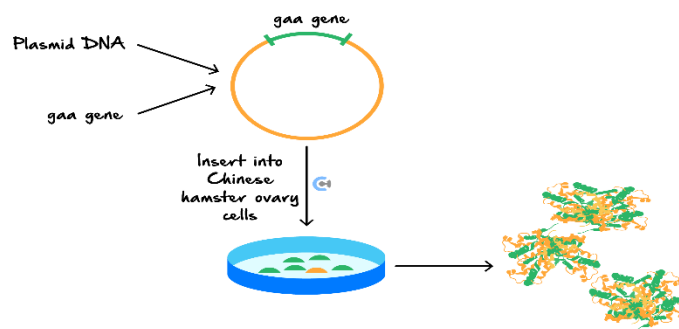


Figure 8: The process of producing alpha glucosidase

a. Define the term 'recombinant'. (1 mark)

b. Identify the enzyme that is used to join the GAA gene to the cut plasmid. (1 mark)

The recombinant alpha-glucosidase (rhGAA) is then purified and injected intravenously into patients with Pompe disease.

- c. Explain why the rhGAA needs to be injected into the bloodstream rather than ingested through the mouth. (4 marks)

One of the limitations of using rhGAA to treat Pompe disease is the large amounts that need to be injected every few weeks. Scientists are currently investigating the use of CRISPR-Cas9 to replace the malfunctioning gene in humans.

- d. Explain the original function of CRISPR-Cas9 in bacteria. (4 marks)

The single-celled organism *Chlamydomonas* is often used as a model to study the role of genes in photosynthesis. The CRISPR-Cas9 system is used to selectively disable genes so that their function can be determined. The CRISPR sequence requires a short section of DNA called spacers in order to function.

- e. Describe the role of a spacer sequence. (1 mark)

The spacer sequence binds to a nuclease called Cas9. The Cas9 enzyme will only cut the viral DNA if it is adjacent to a PAM sequence.

- f. Describe the role of the PAM sequence. (1 mark)

CRISPR-Cas9 has been used to disrupt the KRN2 gene in maize. When this gene is activated, the number of kernel rows in a cob of corn decreases.

- g. Describe how the disruption of the KRN2 gene could increase crop productivity. (2 marks)

- h. The scientists have sequenced the woolly mammoth's DNA. When creating the hybrid, explain if it would be more effective to use the DNA or the amino acid sequence of the woolly mammoth. (1 mark)

- i. What characteristics of CRISPR-Cas9 make it an effective tool in modifying the Asian elephant genome when compared to traditional endonucleases? (2 marks)

- j. Suggest a strategy that could be implemented to minimise the potential transfer of a novel disease that the woolly mammoth hybrid may carry to humans. (1 mark)

Question 23 (6 marks)

The term ‘genetically modified’ refers to organisms that have had a gene or genes transferred from another organism using a series of techniques such as cloning, cutting, and splicing DNA segments together, and inserting genes into cells.

The soil-dwelling bacterium *Agrobacterium tumefaciens* naturally transfers part of its DNA into plants. Genetic engineers use it as a vector to genetically modify crop plants such as cotton, corn, and canola so that they are disease and pesticide-resistant.

- a. Explain how a gene of interest is spliced into a plasmid. (3 marks)

Inserting recombinant plasmids into cells is an inefficient process, meaning that only a small percentage of cells will be transformed by the gene of interest. There are various strategies that can be used to identify transformed cells or tissues.

- b. After a recombinant plasmid is inserted into *Agrobacterium tumefaciens*, how are the transformed bacteria selected? (3 marks)

Question 24 (11 marks)

One of the first genes to be cloned for therapeutic purposes was the human insulin gene. In the late 1970s, scientists at Genentech chose the insulin gene as a target for cloning, because the insulin protein is very small (51 amino acids), and because mass production of synthetic insulin would prove to be of great benefit to society.

- a. Of what benefit to society is the mass production of synthetic insulin? (1 mark)

- b. Explain how it is possible for a gene that is different to the human insulin gene to encode an insulin molecule that is identical to natural human insulin. (2 marks)

- c. Suggest what advantage it might have been to scientists at Genentech to clone a very small gene rather than a much larger one? (1 mark)

- d. Even if the locus of the human insulin gene had been known to scientists in the 1970s, it would still not have been effective to cut the gene out of a human chromosome and insert it into a plasmid. Explain why not. (2 marks)

Preparing the synthetic insulin gene for insertion into a plasmid required cutting the ends of the insulin gene with two restriction enzymes and incubating the plasmids in a solution containing ligase.

- e. Explain whether these restriction enzymes would need to be cut with sticky ends or blunt ends. (1 mark)

- f. Explain what ligase is, and why it was required in this procedure. (1 mark)

Next, plasmids were mixed with *E. coli*, which had been treated with calcium chloride to make their cell membranes permeable to DNA. The *E. coli* were then subjected to a brief 'heat shock' which creates pores in their cell membrane, allowing plasmids to enter the cell. Nevertheless, only a small percentage of bacteria took up a plasmid.

- g. What name is given to a bacterium that has taken up a plasmid from its environment? (1 mark)

- h.** Explain how scientists at Genentech were able to distinguish between bacteria that had taken up a plasmid, and those which had not taken up a plasmid. (2 marks)

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