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VCE Biology ¾
Recombinant Plasmids [0.8]
Workshop Solutions

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

DNA ligase restores the phosphodiester bonds between nucleotides. Polymerase catalyses the addition of free nucleotides, and ATP synthase catalyses the production of ATP.

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.

When bacteria are transformed, a gene for antibiotic resistance is added and the bacteria are cultured on a plate containing the antibiotic to identify which bacteria have successfully been transformed.

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

D. Bacterial plasmids are cut wit

Explanatory notes:
A is correct- gene of interest is cut to leave sticky ends not blunt ends.
B is incorrect- antibiotic resistance genes are introduced to distinguish bacteria that have taken up the plasmid from those that have not.
C is incorrect- heat therapy is used to introduce the plasmid to bacterial cells.
D is incorrect- the same restriction enzyme is used to cut bacterial plasmids and the gene of interest.

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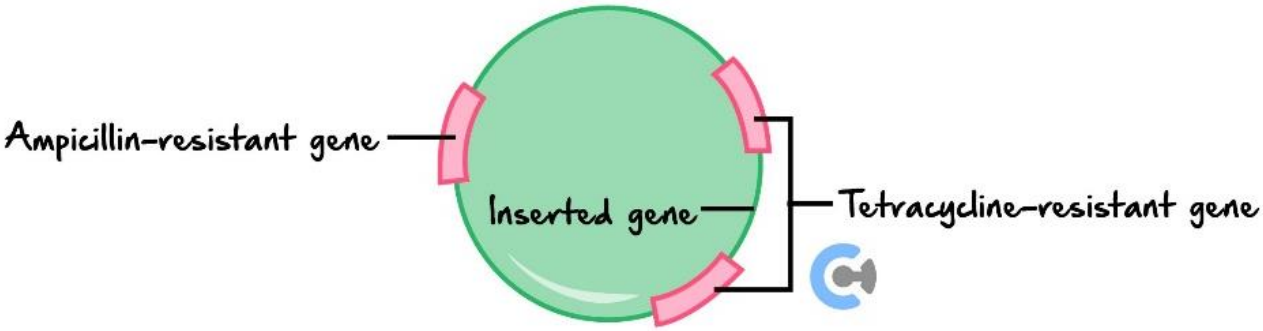
Question 4 (1 mark)

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

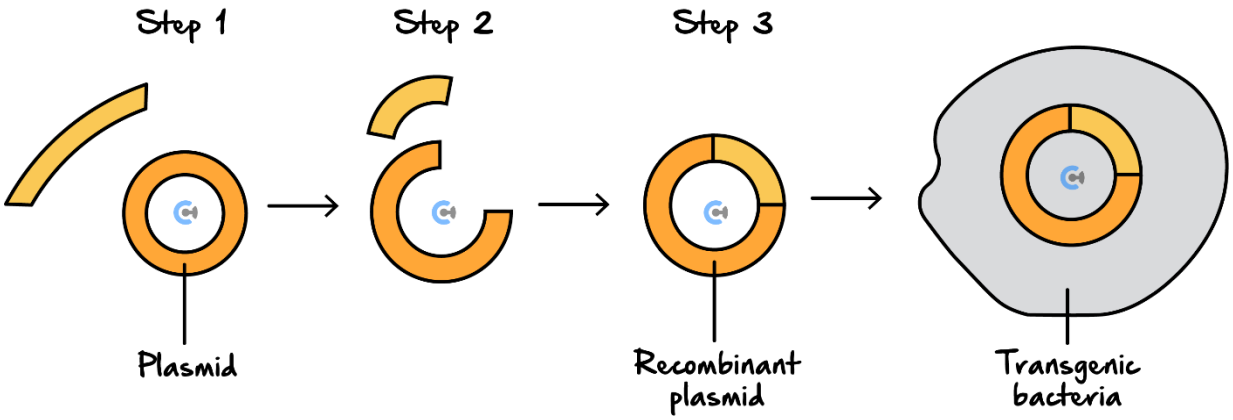
C. 4

D. 5

Space for Personal Notes

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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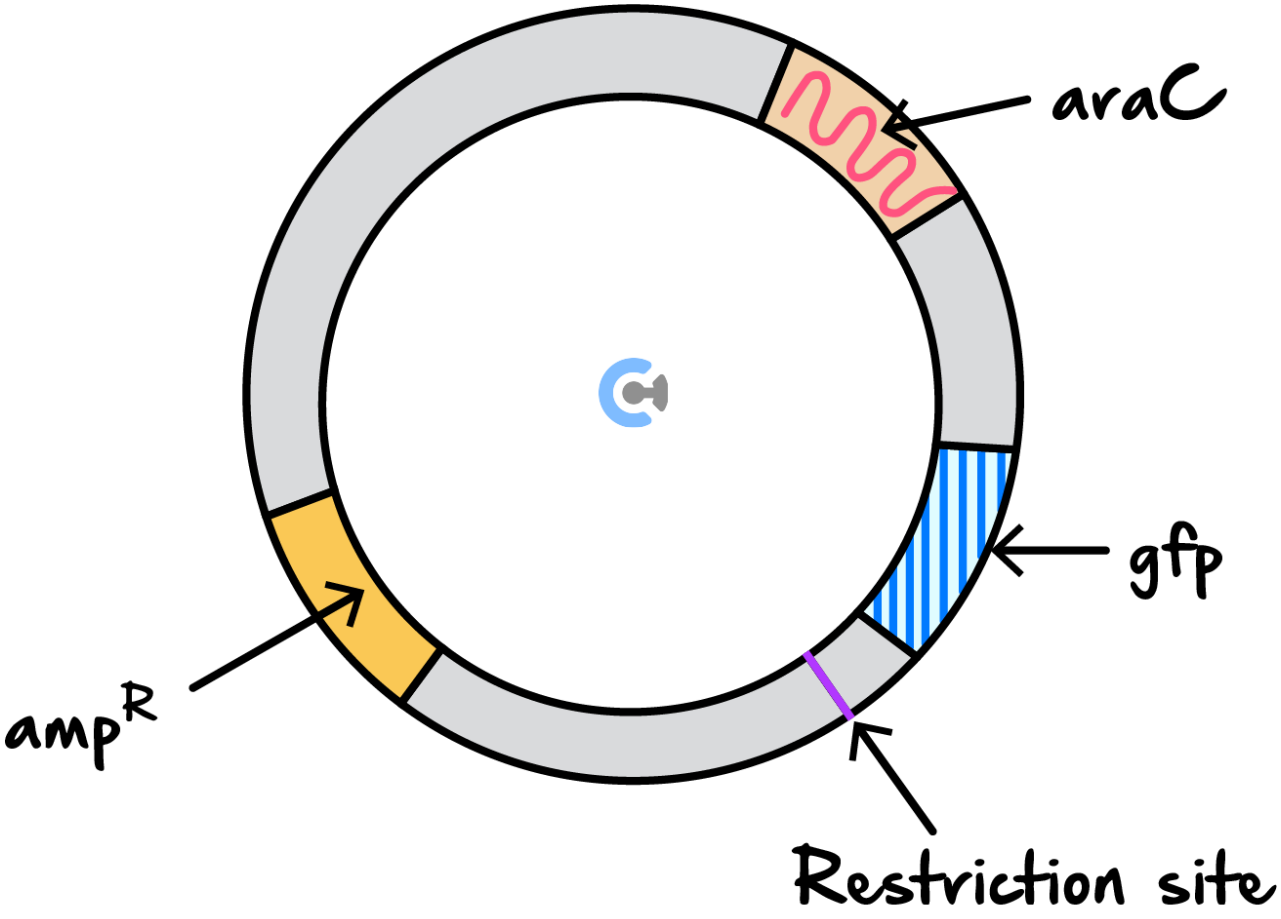
The following information applies to the three questions that follow.

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.

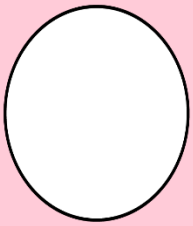
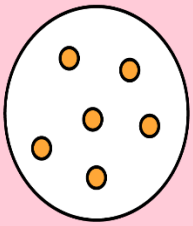
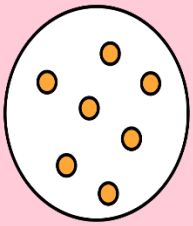
An example of a plasmid used in cloning is shown below:



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.

Question 40

Answer: A

Explanatory notes

Option A is correct. The use of antibiotic resistance allows the bacteria to be grown in a medium containing the antibiotic. Only bacteria with the antibiotic resistance gene, and therefore the transformed plasmid, will grow. These bacteria can then be collected.

Option B is incorrect. Other safety measures are taken to prevent infection by handling microbes.

Option C is incorrect. Bacteria are not infected by other bacteria. The antibiotics will kill any non-resistant bacteria in the medium.

Option D is incorrect. No new pathogenic strains are being created. The transfected plasmids are only used to produce a human protein.

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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.

Using same endonuclease, cleave plasmid containing antibiotic resistance gene and gene for b-gal, as well as a section of gene coding for chain A of insulin
chain A insulin added next to the gene for beta galactosidase and plasmid is ligated to form recombinant plasmid
Introduce into bacteria via heat shock or electroporation
Detection of bacteria containing recombinant plasmid can be achieved through applying antibiotic, and detecting beta galactosidase expression when plated on Xgal
once an appropriate colony is selected - it will express a fusion protein of b-gal + insulin chain A
This process is repeated for chain B
The fusion proteins are purified and then
& insulin chains A + B are mixed to form insulin

*Note that there is a lot of variation possible as VCAA has not exactly specified the depth!

Question 17 (2 marks)

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

- Avoiding animal cruelty, as the other methods involve it.
- Much less expensive to synthesise it this way compared to animals.
- Get a purer form of insulin generated compared to some animal derivatives, which might cause allergies.

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.

Transformation of bacteria involves delivering foreign DNA to bacteria to express, in order to obtain the desired protein product, most commonly via a recombinant plasmid.

In this process, recombinant plasmids contain both reporter and antibiotic resistance genes.

Antibiotic resistance genes allow us to select the bacteria that have actually taken up the desired plasmid, by applying that antibiotic to the transformed colony.

Only bacteria who have been transformed with the plasmid will survive.

The reporter gene is inactivated when the plasmid has the target gene inserted successfully, so it can be used to distinguish between the bacteria that have the recombinant plasmid and those who have a non-recombinant plasmid.

Hence, in combination, they can be used to isolate the desired bacteria- those that have been transformed using the recombinant plasmid.

Question 19 (3 marks)

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

Bacterial transformation describes bacteria that have taken up foreign DNA.

Heat Shock- involves the solution being included with Ca^{2+} ions, placed on ice. It is then heated to 37-42°C, and then placed back on ice to increase membrane permeability

Electroporation- involves passing an electric current through the solution containing bacteria and the recombinant plasmids to increase membrane permeability.

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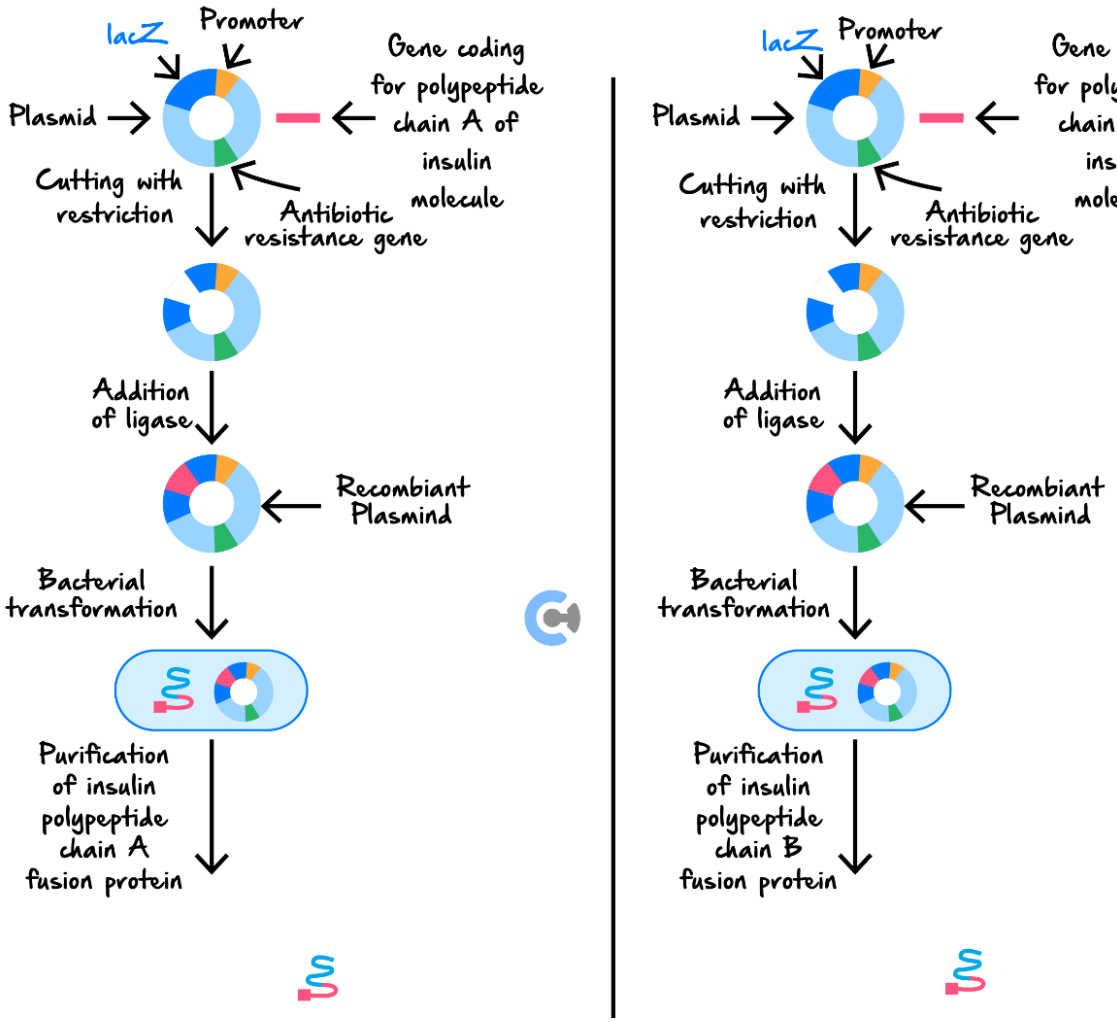
Question 20 (7 marks)

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)

Introns need to be removed from both insulin polypeptide *A* gene and insulin polypeptide *B* gene (1 mark) as they are non-coding regions of the genes (1 mark).

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

Restriction enzymes cut the human insulin polypeptide *A* and *B* gene sequences at specific recognition sites (1 mark). Restriction enzymes that cut the human insulin genes are used, and the same enzyme is used to cut the plasmid, leaving sticky ends (1 mark). DNA ligase will catalyse the joining of the human insulin polypeptide *A* and *B* gene sequences via phosphodiester bonds to the plasmid (1 mark).

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

The antibiotic-resistant gene enables identification of bacteria that have successfully taken up the recombinant plasmid (1 mark). The transformed bacteria will survive in the presence of the specific antibiotic on the plate culturing the bacteria. (1 mark).

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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?

The β-gal protein is used in the production of human insulin as part of a fusion protein. It enhances the expression and stability of the insulin protein when produced by bacteria and simplifies its isolation and purification. During the purification process, the β-gal protein is cleaved and removed.
No, the β-gal protein will not be present in the final insulin product given to diabetics, as only pure human insulin is provided to ensure safety and effectiveness.

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:

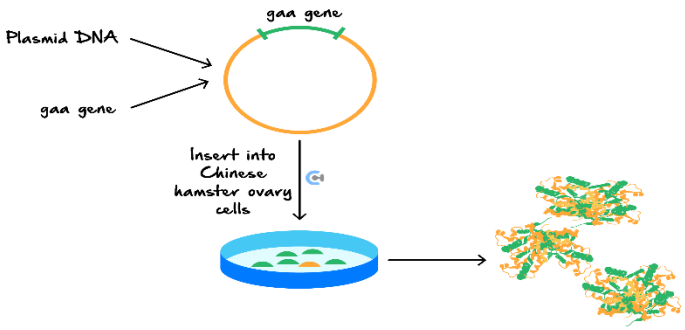

Figure 8: The process of producing alpha glucosidase

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

A cell that has genetic material introduced from another organism.

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

DNA ligase [NOTE: the DNA must be included for the 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)

The stomach has acid (1).
Acid changes the tertiary structure of a protein / enzyme (1), making it non-functioning (1).
Injecting it into the bloodstream does not have this problem (1).
Bacteria save copies of past viral DNA in their genome (1).

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)

Bacteria save copies of past viral DNA in their genome (1).
The CRISPR is the short complimentary sequences of past viral DNA (1).
It binds to the Cas9 enzyme (1).
The Cas9 enzyme cuts any DNA that binds to the CRISPR sequences (1).

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)

Spacer: segments of DNA cut from invading viruses used to recognise same virus in the future.

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)

The protospacer adjacent sequence is a nucleotide sequence between the spacers that is used to identify bacterial-DNA from non-self viral DNA.

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)

Decreasing KNR2 increases the number of kernel rows (1). This increases the number of kernels (1) increasing kernel productivity.

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)

Amino acid sequence as silent mutations will not have an effect on the proteins produced.

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

CRISPR Cas9 allows for sgDNA to be created that can cut at any sequence (1) whereas endonucleases have specific recognition sequences (1).

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)

Any of the following for 1 mark:
Do not allow humans to live in areas with direct contact with woolly mammoth hybrid
OR wash hands / sanitise following contact with animal
OR any other suitable response.

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)

Initially, the plasmid is mixed with a restriction enzyme that cuts it once. The DNA that contains the gene of interest is also mixed with the same restriction enzyme to isolate the gene of interest. 1 mark

The restriction enzyme leaves the plasmid and gene of interest with sticky ends, rather than blunt ends. 1 mark

Both the restricted plasmid and restricted gene of interest are mixed together with DNA ligase, which causes the complementary sticky ends to anneal and thus forms a recombinant plasmid. 1 mark

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)

To assist with the selection of transformed *Agrobacterium tumefaciens*, the recombinant plasmid will usually have an antibiotic-resistant gene alongside the gene of interest in the genome. 1 mark

The sample of bacteria that have been exposed to the recombinant plasmid are then grown on an agar plate that contains the antibiotic. 1 mark

As the recombinant plasmid carries the antibiotic-resistant gene and the gene of interest, any bacteria that grow in the presence of the antibiotic will also carry the gene of interest. 1 mark

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)

Many people in society are insulin-dependent diabetics. Providing synthetic human insulin on a mass scale makes it much more affordable for such people to take insulin.
Other answers were also possible.
For example, synthetic insulin is much safer than taking insulin from an animal because it is less likely to cause an allergic response. (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)

The genetic code is degenerate (1 mark); therefore, a single amino acid can usually be encoded by several different codons. For example, if scientists needed the amino acid Leucine (Leu), they could use any of six different codons (UUA, UUG, CUU, UUC, CUG, or CUA) that code for that amino acid. Therefore, the gene may have a very different nucleotide sequence, but the amino acid sequence can still be identical (1 mark). (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)

Since they were making the gene in a DNA synthesiser, a small gene is much easier to make than a big one. (Other reasonable answers may be awarded a mark too.) (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)

The gene on a human chromosome contains introns (1 mark). Bacteria such as *E. coli* do not contain introns and do not have the ability to splice them out as eukaryotic cells do, so a human gene, if inserted directly into a plasmid, would not work (1 mark). (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)

They would need to cut with sticky ends, to facilitate the insulin gene joining into the plasmid, which has been prepared with complementary sticky ends. (1 mark)

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

Ligase is an enzyme that creates strong sugar-phosphate (or phosphodiester) bonds between the deoxyribose of one nucleotide and the phosphate of the next on the 'backbone' of the DNA. This was needed in this case to complete the joining of the insulin gene and the plasmid. (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)

Transformed. (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)

The plasmid contained a gene for ampicillin resistance (1 mark). The *E. coli* bacteria were cultured on a medium containing ampicillin. Any *E. coli* which had not taken up a plasmid would be unable to grow on this medium. Therefore, any colonies that did grow on the medium must have been transformed (1 mark). (2 marks)

Note: If students answered with respect to the gene for tetracycline resistance, or with respect to the gene for β-galactosidase, they should not be awarded a mark, because each of these allow scientists to determine whether *E. coli* took up a recombinant plasmid (rather than a non-recombinant plasmid). The question, however, is asking how they distinguish bacteria which have taken up a plasmid from those which has not taken up any plasmid.

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