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VCE Biology ¾
Recombinant Plasmids [1.7]
Test

42 Marks. 1 Minute Reading. 33 Minutes Writing

Results:

Test	_____ / 35
Extension	_____ / 7

Section A: Test (35 Marks)

Question 1 (10 marks)

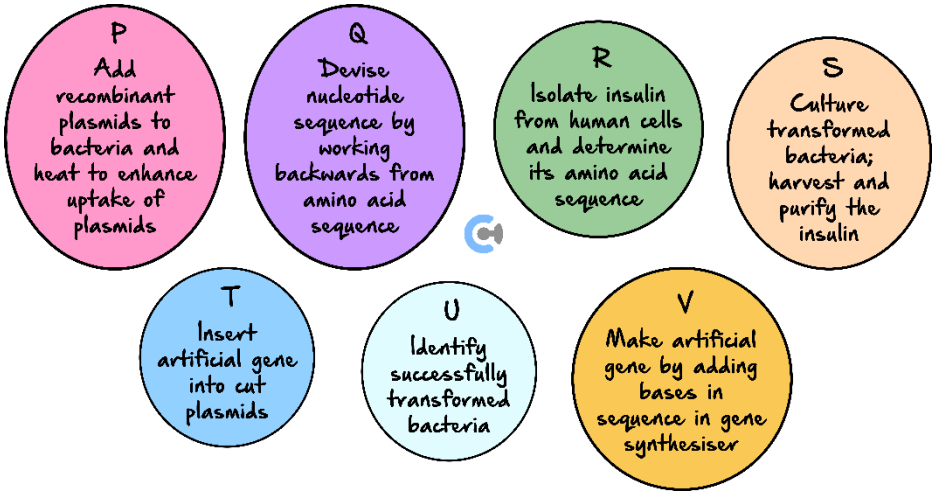
Tick whether the following statements are **True** or **False**.

	True	False
a. Reverse transcription is used to create complementary DNA (cDNA) from insulin mRNA to ensure the absence of introns.		
b. The plasmid and the insulin gene are cut using different restriction enzymes to ensure variety in the DNA fragments produced.		
c. DNA ligase is required to join the insulin gene with the plasmid to create recombinant DNA.		
d. Heat shock and electroporation are two methods used to introduce recombinant plasmids into bacteria.		
e. All bacteria that survive antibiotic agar plates after transformation contain recombinant plasmids.		
f. Beta-galactosidase acts as a screening marker to confirm successful gene insertion into plasmids.		
g. Insulin *A* and *B* chains are produced by the same strain of bacteria after transformation.		
h. Fusion proteins consist of insulin chains attached to beta-galactosidase, which are later cleaved to isolate the insulin polypeptides.		
i. Disulfide bonds form between insulin *A* and *B* chains to create functional human insulin after purification.		
j. Antibiotic resistance genes in plasmids allow researchers to distinguish between recombinant and non-recombinant bacteria.		

Space for Personal Notes

The following information applies to the two questions that follow.

Bacteria can be transformed with an artificial insulin gene and cultured to make insulin in commercial quantities. The steps taken to produce genetically engineered insulin are summarised below. The order of the steps has been mixed up.



Question 2 (1 mark)

The correct sequence of steps when producing insulin is:

A. V, P, T, S, U, R, Q

B. V, T, P, U, S, Q, R

C. R, Q, V, T, P, U, S

D. R, V, Q, T, P, S, U

Question 3 (1 mark)

The tool used for joining the artificial gene to plasmid DNA at step T is:

A. A primer.

B. DNA ligase.

C. DNA polymerase.

D. Gel electrophoresis.

Question 4 (1 mark)

The artificial insulin gene may have a different nucleotide sequence from the human insulin gene.
This is because the DNA code is:

A. Redundant.

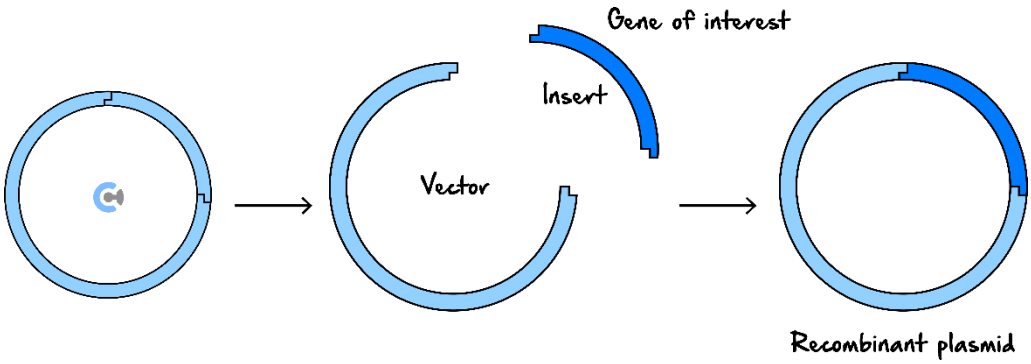
B. Universal.

C. Mutated by heat shock.

D. Contaminated by bacterial DNA.

Question 5 (1 mark)

The following diagram illustrates the steps involved in producing a recombinant plasmid.



Various chemicals need to be mixed at certain stages in the transformation process.
The correct order of chemical use would be:

	Step 1	Step 2
A.	Restriction enzymes are added to the plasmid.	The cut plasmid, gene of interest and ligase are added together.
B.	Ligase is added to some of the restriction enzymes.	Both are then mixed with the plasmid and the gene of interest.
C.	Mix the plasmid and the gene of interest with ligase.	Followed by restriction enzymes soon after.
D.	The gene of interest and the original plasmid are added.	The restriction enzyme and ligase are added.

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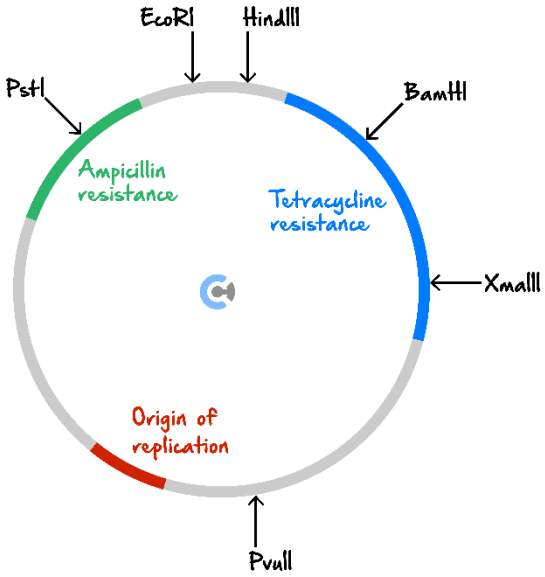
Question 6 (1 mark)

The diagram below is of a plasmid from *S.aureus* showing 2 genes conferring antibiotic resistance and 6 restriction enzyme cutting sites.



The following conclusion can be made about the information provided:

A. If HindIII and PvuII were mixed with this plasmid, 3 strands of DNA would be formed.

B. A *S. aureus* bacterium containing this plasmid would not be destroyed in the presence of tetracycline and/or ampicillin.

C. An effective method of forming a recombinant plasmid would be to use both HindIII and EcoRI so the foreign DNA could be inserted between these cuts.

D. If a gene of interest was added to the plasmid it would move further in a gel that underwent electrophoresis.

Question 7 (1 mark)

Which of the following properties have **not** been introduced into plants via genetic engineering?

A. Resistance to pests.

B. Resistance to competitors.

C. Greater nutritional value.

D. Delayed ripening time.

Question 8 (1 mark)

Genetic modification always involves:

A. A change to an organism's DNA.

B. Gel electrophoresis.

C. A plasmid vector.

D. A new gene inserted into an organism.

Question 9 (1 mark)

In the process of genetic transformation, a reason for growing potentially transformed bacteria on a culture containing an antibiotic could be for:

A. Showing that the bacteria was infected with a pathogen.

B. Showing the presence of viable transformed bacteria.

C. Showing the effectiveness of an antibiotic in killing untreated bacteria.

D. To provide a food source for the bacteria.

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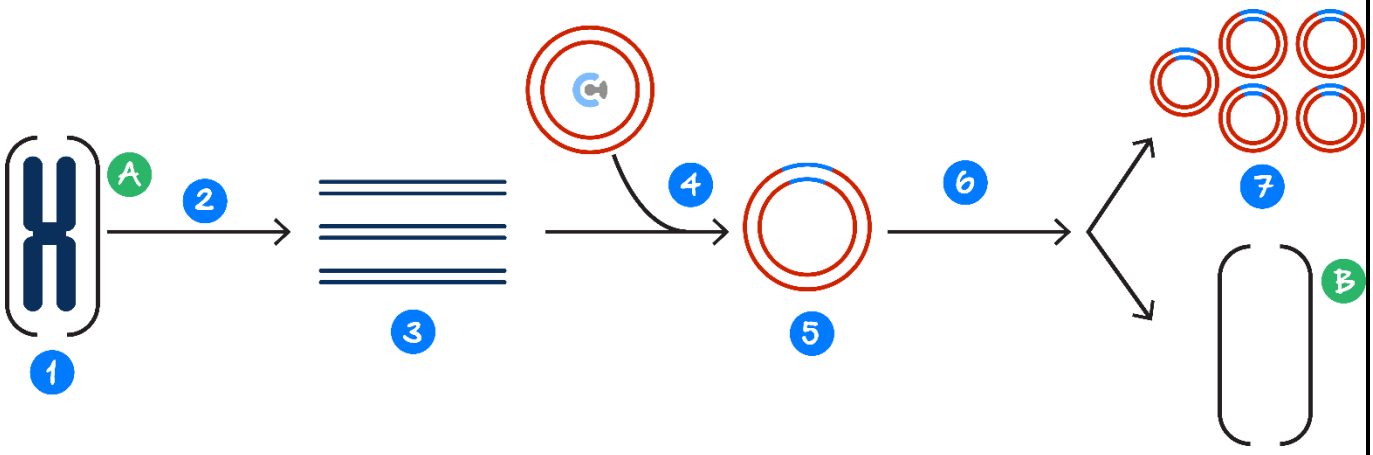
Question 10 (1 mark)

Bacteria can be transformed with a human insulin gene and cultured to make this insulin in commercial quantities.



The correct terms corresponding with the numbers in the diagram below are:

A. 1 is PCR; 3 is Isolation of gene from human cell; 6 is Insertion of Plasmid into Human cell.

B. 1 is Isolation of gene from human cell; 3 is PCR; 7 is Multiple expression of insulin gene in Bacterial cell.

C. 2 is Insertion of gene into the plasmid; 5 is PCR; 6 is Insertion of plasmid into Human cell.

D. 2 is Insertion of gene into the plasmid; 5 is PCR; 7 is Multiple expression of insulin gene in Bacterial cell.

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Question 11 (8 marks)

The production of synthetic human growth hormone (HGH) is accomplished using a strain of *Escherichia coli* (E. coli) bacteria as a host. A laboratory plasmid that already contained two antibiotic resistance genes - one for tetracycline and one for ampicillin - was further modified by having the gene for HGH inserted. Copies of the vector plasmid produced were then exposed to the bacteria, and those that transformed were subsequently used to produce HGH.

a. Draw a flow chart identifying at least three stages in the production of the vector plasmid. (3 marks)

b. Identify a technique that could be used to separate vector plasmids from plasmids that did not contain the HGH gene. (1 mark)

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The *E. coli* that took up the vector plasmid are referred to as being transformed.

c. Why is the plasmid referred to as a vector? (1 mark)

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d. Identify a procedure that could be carried out to isolate the transformed bacteria. How might this procedure enable the transformed bacteria to be identified? (2 marks)

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e. Why is antibiotic resistance a risk in gene technology? (1 mark)

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Question 12 (8 marks)

A particular bacterial plasmid containing the lacZ gene and an ampicillin-resistant gene can be cut by the restriction enzyme EcoR1 at the following recognition site within the lacZ gene. A foreign piece of DNA can then be inserted into the plasmid at that site. EcoR1 cuts at the site shown below to give sticky ends.

a. Draw the restriction cut on the complementary strand of DNA. (1 mark)

b. Explain why the foreign piece of DNA to be inserted needs to be cut by the restriction enzyme EcoR1. (1 mark)

_____

_____

c. Name the enzyme used to attach the foreign DNA into the plasmid. _____ (1 mark)

The recombinant plasmids are mixed with a culture of bacteria and treated so that some bacteria will take up the recombinant plasmids. Some bacteria may also take up plasmids that do not contain the foreign gene. β galactosidase is the product of the lacZ gene. Xgal is a molecule, that when present in the media, can be broken down by the enzyme β galactosidase making the colonies of bacteria blue. If the bacteria cannot produce β galactosidase then the colonies are white.

d. Explain how scientists use the presence of the ampicillin-resistant gene and the presence of Xgal in the media to identify the colonies that have been successfully transformed. (4 marks)

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e. Suggest the use of recombinant bacterial plasmids. (1 mark)

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Section B: Extension (7 Marks)

Question 13 (7 marks)

Scientists use recombinant bacterial plasmids as vectors to transform bacteria for a range of purposes in research and biotechnology.

a. What is meant by the term 'vector' in the context given? (1 mark)

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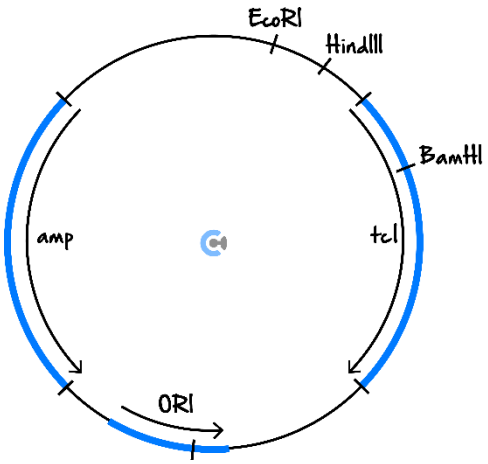
A particular bacterial plasmid contains recognition sites for the restriction enzymes EcoRI, HindIII and BamHI, along with two antibiotic-resistant genes, ampicillin resistance (amp) and tetracycline resistance (tel), and an origin of replication (ORI).
The diagram below shows the positions of these recognition sites and antibiotic-resistant genes as well as the position of the origin of replication within this plasmid.



One purpose of using recombinant bacterial plasmids is to produce bacteria capable of synthesising human protein.

b. The restriction enzyme BamHI was used to help insert a gene coding for a human protein into this plasmid.

i. Describe how restriction enzymes such as BamHI are used to help insert a gene coding for a human protein into this plasmid. (2 marks)

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ii. Draw and label a diagram in the space below to show the position of the human gene in this plasmid when BamHI is used. Include the position of the recognition sites for the restriction enzymes EcoRI, HindIII, and BamHI on the plasmid. (1 mark)

c. After the scientists had carried out the steps required to make plasmids with the inserted human gene, these plasmids were mixed with a culture of bacteria. This mixture was treated so that these plasmids would move into the bacterial cells. Not all bacteria took up these plasmids.
Explain how scientists use antibiotics to identify which of the bacterial cells have been successfully transformed with plasmids carrying the human gene. (3 marks)

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