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VCE Biology ¾
Recombinant Plasmids [1.7]
Test Solutions

42 Marks. 1 Minute Reading. 33 Minutes Writing

Results:

Test	_____ / 35
Extension	_____ / 7

Section A: Test (35 Marks)

Question 1 (10 marks)

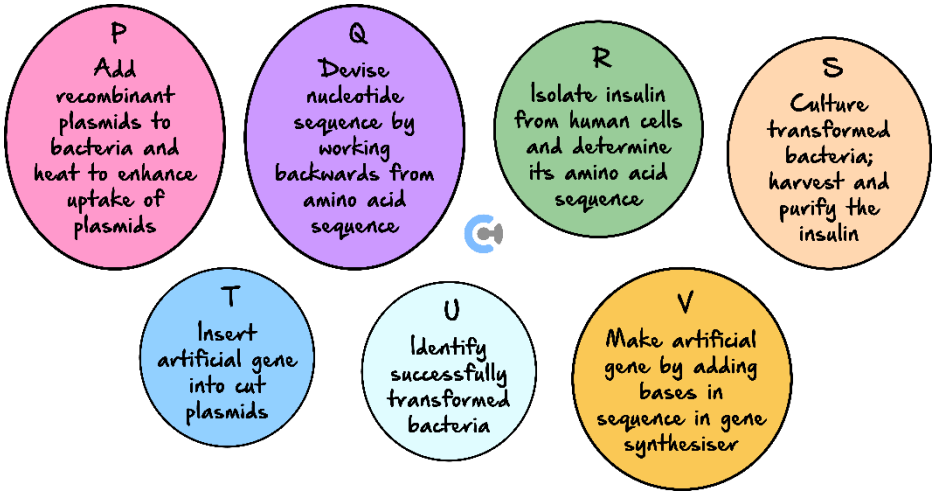
Tick whether the following statements are **True** or **False**.

	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 insulin gene are cut with the same restriction enzyme to create compatible sticky ends.)		✓
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 bacter… (Some bacteria may contain non-recombinant plasmids.) …tain recombina…		✓
f. Beta-galactosidase acts as a screening marker to confirm successful gene insertion into plasmids.	✓	
g. (The *A* and *B* chains are produced by separate strains of bacteria with different recombinant plasmids.)		✓
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 distinguish between transformed and non-transformed bacteria, while reporter genes like beta-galactosidase indicate recombinant plasmids.)		✓

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.

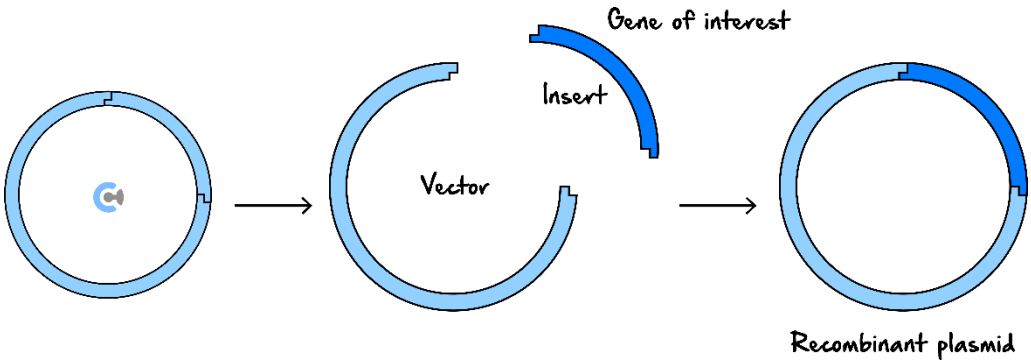
B. Universal.

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.

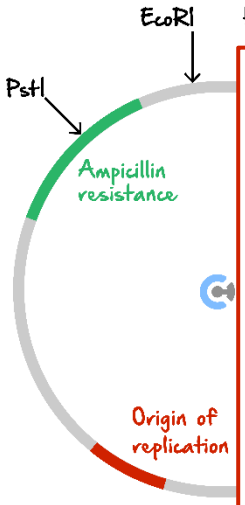
Question 28 **A**

To produce a recombinant plasmid, biotechnologists would need to start with a sample of unmanipulated plasmids that restriction enzymes would be added to. The same would be done with the target DNA so as to generate complementary sticky ends. Both the plasmid and target DNA would then be added together with ligase so that the recombinant plasmid would be formed. The biotechnologists would then need to purify it.

Space

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.



Solution: B

A is incorrect because those 2 restriction enzymes have 1 cutting site each on the plasmid, which would liberate 2 fragments (not 3). C is incorrect because cutting at 2 sites would liberate 2 strands with different annealing combinations, making it difficult to insert a gene of interest. D is incorrect because the recombinant plasmid, being bigger would move less distance in a gel rather than further. B is correct because the plasmid would need to be in a bacterium prior to the gene being expressed, so on its own the plasmid would not be susceptible to the tetracycline.

Study Design Reference:

The use of enzymes including endonucleases (restriction enzymes), ligases and polymerases. The use of gel electrophoresis in sorting DNA fragments, including interpretation of gel runs. The use of recombinant plasmids as vectors to transform bacterial cells.

Web Link

http://www.phschool.com/science/biology_place/biocoach/red/intro.html

The following conclusion can be made about the information provided:

A. If HindIII and Pvull 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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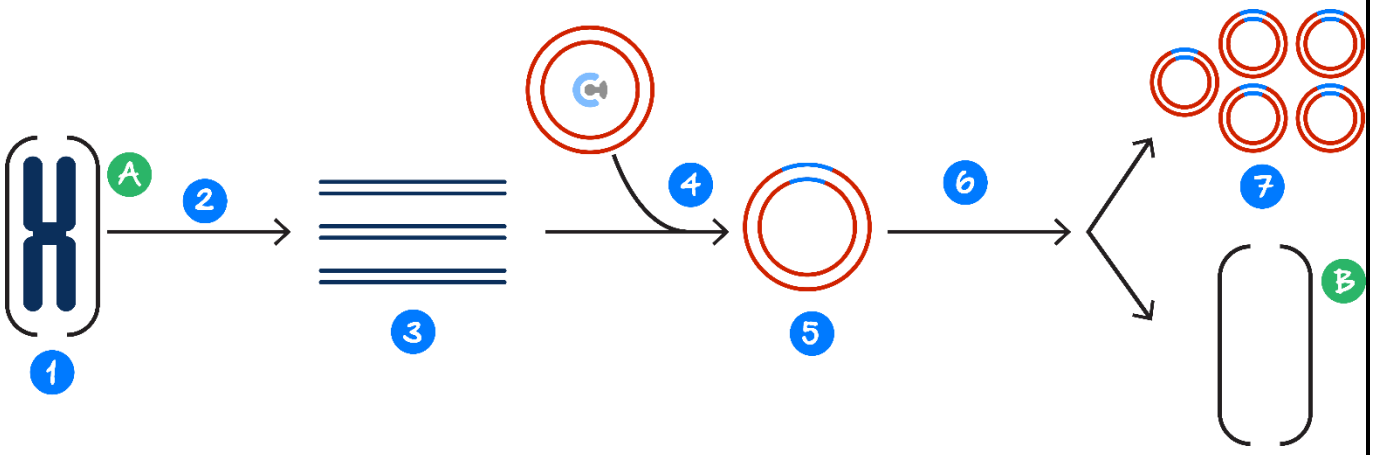
Question 10 (1 mark)

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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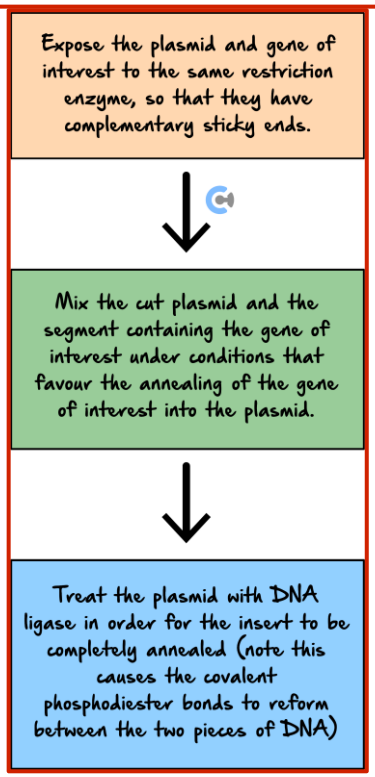
Question 11 (8 marks)

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)



Note: This is an example of an accepted answer. Determining the validity of a student's answer is at the discretion of the assessor.

Mark allocation: 3 marks

- 1 mark for stating that the plasmid and gene have to be exposed to the same restriction enzyme
- 1 mark for referring to the gene and plasmid annealing (binding together at the complementary sticky ends)
- 1 mark for referring to the use of DNA ligase to seal the gene insert into the plasmid

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

Worked solution

gel electrophoresis

Mark allocation: 1 mark

- 1 mark for identifying gel electrophoresis as an appropriate technique

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)

Worked solution

The plasmid is a means of delivering the gene of interest into a bacterial cell.

Mark allocation: 1 mark

- 1 mark for explaining what a vector is in the context of gene technology

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)

Worked solution

Grow all of the bacteria exposed to the recombinant plasmid in agar that contains ampicillin.

The bacteria that survive this can only do so because they have the ampicillin resistance gene incorporated into the plasmid. This means that they must be the transformed bacteria because all bacteria that were not transformed would have been killed by exposure to ampicillin.

Mark allocation: 2 marks

- 1 mark for identifying that the potentially transformed bacteria should be cultured onto agar that also contains ampicillin (or tetracycline)
- 1 mark for explaining that the transformed bacteria will survive this process because they have the antibiotic resistance gene from the recombinant plasmid. Bacteria that did not transform will not survive this process.

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

Worked solution

The biggest concern with the use of antibiotic resistance genes in gene technology is that these genes will spread to other bacteria, conferring resistance, and reducing the efficiency of antibiotics as a means of treating bacterial infections. This could lead to the development of superbugs – strains of bacteria that have become resistant to antibiotics.

Mark allocation: 1 mark

- 1 mark for explaining that there is a concern that the use of antibiotic resistance genes in the production of transgenic bacteria will result in a decrease in the efficiency of antibiotics against bacterial infections

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)

In order to form complementary sticky ends so that the DNA pieces will stick together they need to be cut with the same restriction enzyme.

c. Name the enzyme used to attach the foreign DNA into the plasmid. *DNA ligase* (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)

Bacteria after treatment are plated on a medium that contains both ampicillin and Xgal (1). Bacteria that have taken up a plasmid will be able to grow as they have the ampicillin resistant gene (1).
Colonies that are blue can produce β galactosidase but they do not contain the foreign DNA (1).
White colonies are not able to produce β galactosidase to break down Xgal to a blue colour so they must contain the foreign DNA as it is inserted within the LacZgene thus preventing production of β galactosidase (1).

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

One use of recombinant bacterial plasmids is to use them to carry a gene into bacteria. These bacteria will express this gene and produce the protein of interest.

Section B: Extension (7 Marks)

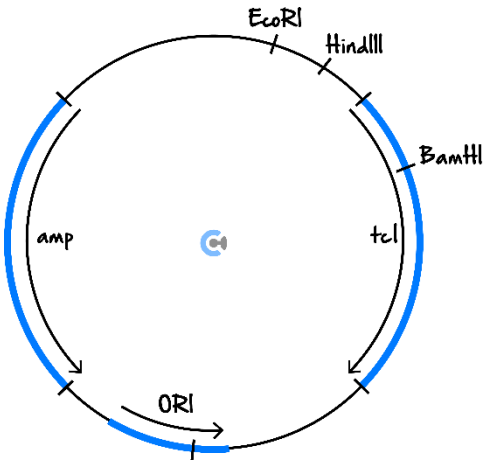
Question 13 (7 marks)

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)

A means of transferring/transporting foreign/desired DNA into a cell/organism

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.



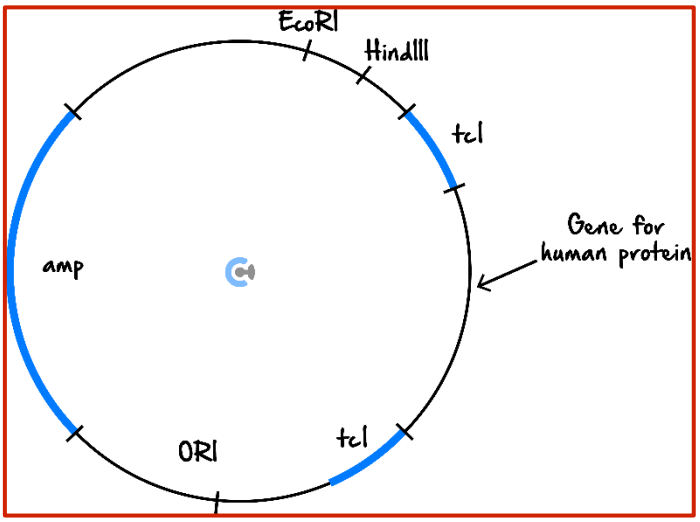
One purpose of using recombinant bacterial plasmids is to produce bacteria capable of synthesising human protein.

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)

The restriction enzymes are used to cut the ends of the plasmid and the desired gene so that the gene will insert into the plasmid.

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)

Grow the bacteria on agar containing ampicillin.

Those bacteria that will grow on agar containing ampicillin have the plasmid with the human gene included.

If plasmid is not taken up, those bacteria are killed by ampicillin.

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