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VCE Biology ¾

Introduction to DNA Manipulation Techniques [1.5]

Test

41 Marks. 1 Minute Reading. 32 Minutes Writing.

Results:

Test	_____ / 35
Extension	_____ / 6

Section A: Test (35 Marks)

INSTRUCTION: 35 Marks. 1 Minute Reading. 28 Minutes Writing.

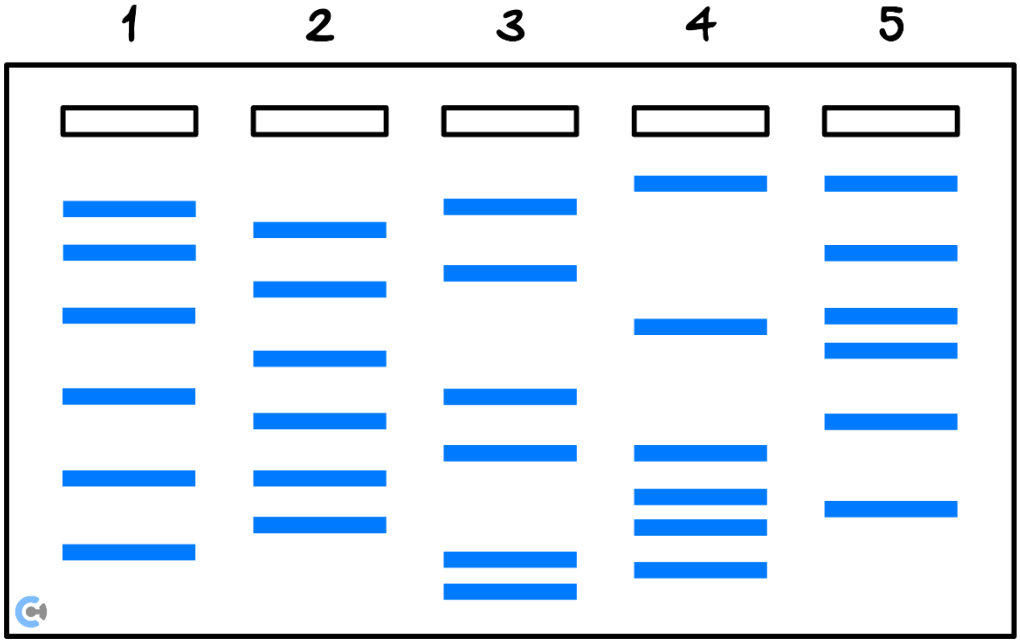
Question 1 (5 marks)

Tick whether the following statements are **true** or **false**.

	True	False
a. Polymerases are responsible for cutting DNA strands.		
b. Endonucleases can create both sticky ends and blunt ends when cutting DNA.		
c. Ligases are required to join DNA fragments together after they are cut.		
d. PCR uses a heat-stable enzyme called taq polymerase to replicate DNA.		
e. During PCR, the annealing step occurs at 90-95°C.		
f. Gel electrophoresis separates DNA fragments based solely on their sequence.		
g. Smaller DNA fragments move faster through the agarose gel than larger fragments.		
h. STRs (Short Tandem Repeats) are found in coding regions of DNA.		
i. In DNA profiling, using the same restriction enzyme on all samples ensures consistency in fragment sizes.		
j. DNA profiling involves the use of both PCR and gel electrophoresis.		

The following information applies to the two questions that follow.

Some gene loci vary considerably between individuals. To study this variation, three gene loci were amplified from five individuals to determine who amongst the individuals was most closely related. The gene loci vary in length and nucleotide sequence. Each individual has two copies of each gene locus in their body cells. The gel electrophoresis profiles of individuals 1-5 are illustrated in the diagram below.



Question 2 (1 mark)

The smallest DNA fragment in the gel belongs to the individual:

A. 1

B. 2

C. 3

D. 4

Question 3 (1 mark)

Which one of the following conclusions could be drawn from the gel electrophoresis profiles?

A. Individual 5 is more closely related to individual 3 than individual 1.

B. Individual 1 could be a parent of individual 3.

C. Individuals 2 and 4 are the least related individuals.

D. Individuals 1 and 2 could be the parents of individual 4.

Question 4 (1 mark)

The table below shows the recognition sequences of four restriction enzymes.

Enzyme	Recognition sequence	Cut site
EcoRI	5'GAATTA 3' CTTAAG	5'G AATTC 3' 3' CTTAA G5'
BamHI	5' GGATCC 3' CCTAGG	5' G GATCC 3' 3' CCTAG G5'
HindIII	5' AAGCTT 3' TTCGAA	5' A AGCTT 3' 3' TTCGA A 5'
Sau3A	5' GATC 3' CTAG	5' GA TC 3' 3' CT AG 5'

EcoRI is used to cut around a gene of interest for insertion into a plasmid.

Which one of the following correctly identifies the enzyme or enzymes that should be used to cut the plasmid open?

A. Only EcoRI should be used to cut the plasmid open.

B. Only Sau3A should be used to cut the plasmid open.

C. Any of the enzymes could be used to cut the plasmid open.

D. Only EcoRI, BamHI or HindIII should be used to cut the plasmid open.

Question 5 (1 mark)

When carrying out polymerase chain reactions (PCR), DNA engineers do not use human enzymes. Instead, they use a bacterial enzyme derived from a hot spring-dwelling microorganism.
Why is this?

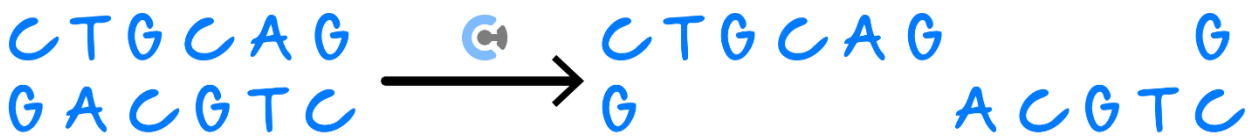
A. To assist in the transfer of insect-resistant genes to plants.

B. The production of insulin requires huge vats of microorganisms.

C. The PCR requires very hot temperatures.

D. The electrophoresis gel is very harsh.

Question 6 (1 mark)



The name of the molecule that carries out the process illustrated in the diagram above is a

A. ligase enzyme producing sticky ends.

B. ligase enzyme producing blunt ends.

C. restriction enzyme producing sticky ends.

D. restriction enzyme producing blunt ends.

Space for Personal Notes

Sub-Section: Short Answer Questions

Question 7 (5 marks)

The polymerase chain reaction (PCR) is a powerful technology that enables specific sections of DNA to be amplified into sufficient quantities for further studies. The cuvette that goes into the PCR machine contains sample DNA, nucleotides, primers, and taq polymerase.

a. Describe the importance of the primers and taq polymerase to the PCR process. (2 marks)

_____

_____

_____

_____

Once a sample is subjected to PCR, further studies may be conducted on the amplified section of DNA. One application is the diagnosis of genetic diseases such as sickle-cell anaemia. The gene causing the disease has two alleles (roughly the same size) and once the sample DNA has been subjected to PCR, restriction enzymes are used to cut the DNA once along the sickle allele, which liberates two unequally sized fragments (the non-sickle allele is not cut by the restriction enzyme). Gel electrophoresis can then be used to determine the genetic status of individuals.

b. Why would a restriction enzyme cut the sickle allele once but not the normal allele? (1 mark)

_____

_____

c. Draw a labelled diagram of the gel pattern of an individual that is heterozygous for the sickle-cell trait in the space below. (2 marks)

Question 8 (11 marks)

a. Polymerase chain reaction (PCR) is a technique used by researchers to make multiple copies of a piece of DNA. This is especially important if only a small sample of the DNA is available.
PCR uses an important enzyme called taq polymerase.

i. Why is taq polymerase used in PCR? (1 mark)

_____

_____

PCR requires a pool of spare nucleotides.

ii. Why are these needed? (1 mark)

_____

_____

Primers are also used during PCR.

iii. Describe the structure and function of a primer. (2 marks)

_____

_____

_____

_____

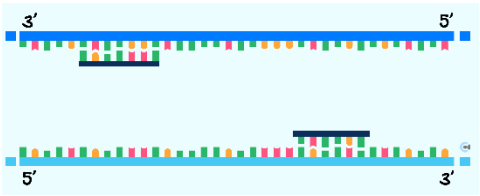
The process involves a cycle that is repeated again and again, each time doubling the sections of DNA.

iv. How many copies of a DNA segment will be present after 4 cycles? (1 mark)

_____

_____

v. Mark arrows on the diagram below to indicate in what direction new nucleotides be added to both strands of DNA. (1 mark)



b. Gel electrophoresis is a laboratory method used to separate mixtures of DNA, RNA, or proteins according to molecular size. In gel electrophoresis, the molecules to be separated are pushed by an electrical field through a gel that contains small pores.

Look at the image above depicting a gel used to separate DNA fragments.

i. Mark on the image, a + sign to indicate which end of the gel is attached to the positive terminal of the equipment. (1 mark)

ii. Mark on the image which fragment (A, B or C) would be the largest in size. (1 mark)

iii. Explain why there are two fragments shown in sample A. (2 marks)

_____

_____

_____

_____

iv. Explain why the fragment in sample C appears as a thicker band than the others. (1 mark)

_____

_____

Question 9 (9 marks)

a. Name the technique used to sort DNA fragments based on their size. (1 mark)

b. Describe the steps involved in sorting DNA fragments using the technique named in **part a.** Include an explanation of how this technique works to sort such fragments. (4 marks)

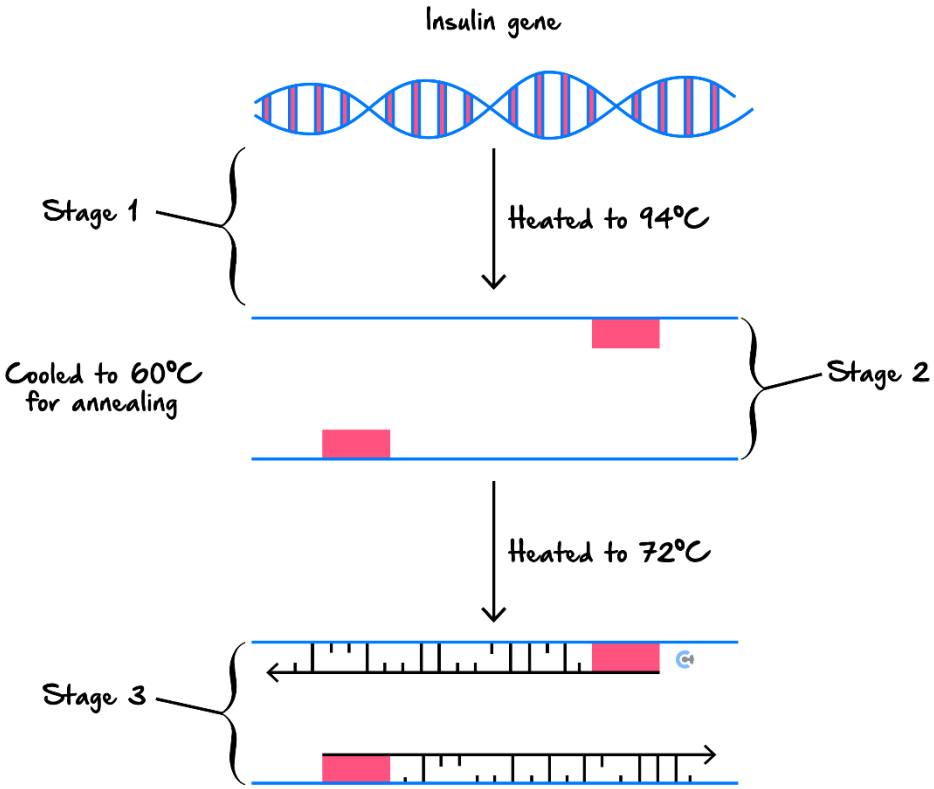
c. Outline how the technique named in **part a.** could be used by law enforcement officials in a case where there are multiple suspects and a blood sample from the crime scene. (4 marks)

Section B: Extension (6 Marks)

INSTRUCTION: 6 Marks. 4 Minutes Writing.

Question 10 (6 marks)

One of the steps outlined involves the amplification of the human insulin gene. The diagram below represents the amplification process.



a. Identify the labels for the missing stages in the above diagram and explain their importance for the overall process. (3 marks)

b. Explain how and why non-coding regions of DNA are used to profile individuals for crimes. (3 marks)

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