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VCE Biology ¾
Introduction to DNA Manipulation Techniques [1.5]
Test Solutions

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.

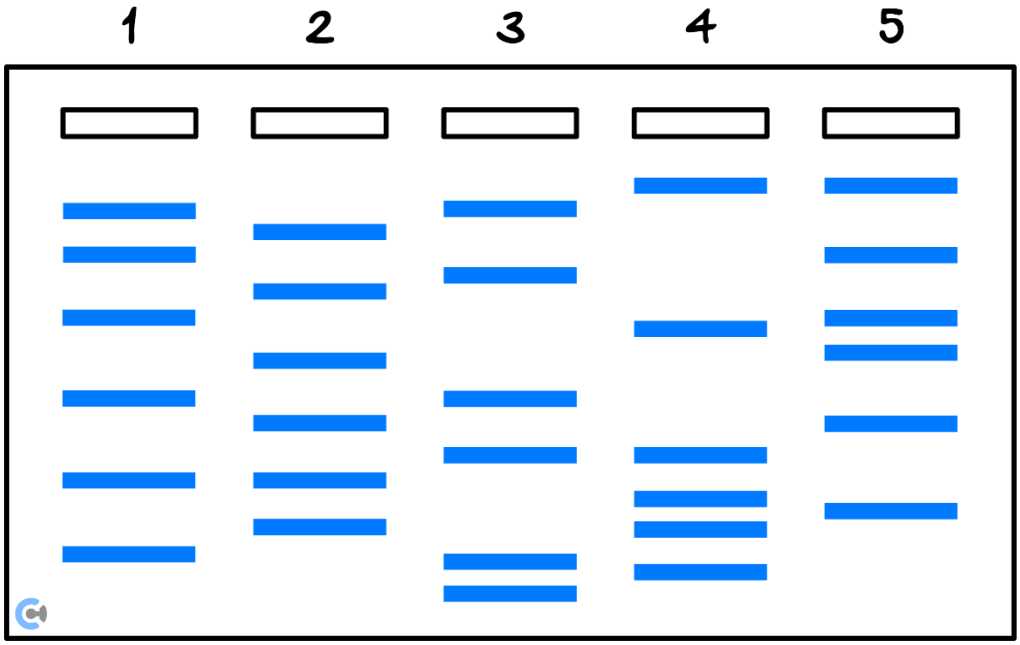
Question 1 (5 marks)

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

	True	False
a. Polymerase		✓
Polymerases synthesise DNA strands, while endonucleases cut DNA.		
	✓	
Endonucleases cut DNA at specific recognition sites, producing either blunt or sticky ends.		
c. Ligases are	✓	
Ligases form phosphodiester bonds to join DNA fragments.		
d. PCR use		
Taq polymerase is heat-resistant and used during the elongation phase of PCR.		
e. During PCR, the annealing step occurs at 90-95°C.		✓
Annealing occurs at 50–55°C, while denaturation occurs at 90–95°C		
f. Gel electrophoresis separates DNA fragments based solely on their sequence		✓
Gel electrophoresis separates DNA fragments based on size.		
g. Smaller DNA fragments move faster through the agarose gel than larger fragments.	✓	
Smaller fragments face less resistance and travel further through the gel.		
h. STRs (Short Tandem Repeats) are found in coding regions of DNA.		✓
STRs are found in non-coding regions of DNA		
i. In DNA profiling, using the same restriction enzyme on all samples ensures consistency in fragment sizes.	✓	
Using the same enzyme ensures DNA is cut at the same recognition sites.		
j. DNA profiling involves the use of both PCR and gel electrophoresis.	✓	
PCR amplifies DNA, and gel electrophoresis separates fragments to create a profile.		

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

C is correct. Smaller DNA fragments can move through the gel more easily and thus move further in the gel. The fragment that has moved the furthest in the gel, and therefore is the smallest, belongs to individual 3.

A, **B** and **D** are incorrect. These

B is correct. Individuals who are related would share similarities in their profiles. Individual 1 has three bands in common with individual 3; thus, they share 50% of their gene loci. This means individual 1 could be a parent of individual 3 or individual 3 could be a parent of individual 1.

A is incorrect. Individuals 3 and 5 share no common bands whereas individuals 1 and 5 share two common bands; therefore, individual 5 is more closely related to individual 1 than individual 3.

C is incorrect. Individuals 2 and 4 share one common band, so they may be somewhat related; individuals 3 and 5 share no common bands, making them the least related individuals.

D is incorrect. If individual 4 is the child of individuals 1 and 2, individual 4 would have inherited all of their DNA from individuals 1 and 2. However, individual 4 possesses a gel band that is not shared by individual 1 or individual 2, so they are not individual 4's parents.

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 b

B. Only Sau3A should

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.

Explanatory notes

The information provided states that EcoRI was used to cut around the gene of interest. Therefore, EcoRI is the only restriction enzyme that should be used to cut the plasmid open. It is necessary to use the same restriction enzyme to make both cuts so that complementary sticky ends are produced that enable the gene of interest to be annealed into the plasmid.

Question 5 (1 mark)

When carrying out polym ey use a bacterial enzyme der Why is this?

The polymerase enzyme comes from a bacterial species (*Thermus aquaticus*) that lives in hot springs. It is known as the *Taq* polymerase. It transfers genes from one species to another and the production of insulin requires restriction enzymes then ligase enzymes. The chemicals used in making a gel are hazardous but once set in the sugar base are relatively harmless to enzymes.

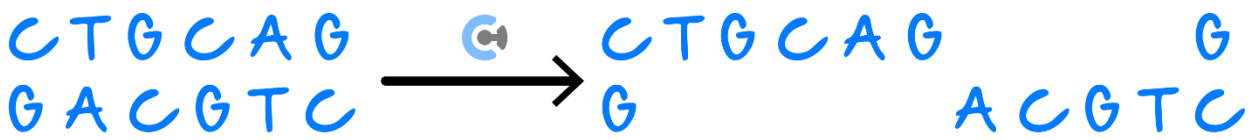
A. To assist in the transfer of insect-resistant genes to plants.

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.

Longer overhangs are called sticky ends. They are most often created by restriction endonucleases when they cut DNA at specific sites.

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)

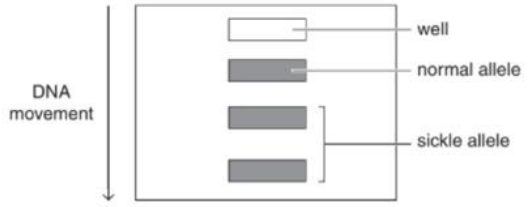
A **primer** is a sequence of single-stranded nucleotides complimentary to a target sequence on the DNA to be tested. The primers should anneal in two places with the target sequence in between.
Taq polymerase replicates DNA but is still functional after being exposed to high temperature, which is part of the PCR process.

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)

Restriction enzymes cut the DNA at specific sequences (2-6 nucleotides long). The sickle allele has the specific sequence once along the allele, allowing it to be cut into two fragments. The normal allele does not contain the specific sequence and is not cut.

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)



2 marks

The following points should be included in the diagram:

- *Clear diagram of a gel showing well position and the direction the DNA is moving.*
- *Three bands present.*
- *The two lower bands are the smaller sickle alleles, the higher band the normal allele.*

All three of the above points should be included for 2 marks; only two points will earn 1 mark.

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)

This enzyme can withstand high temperatures.

PCR requires a pool of spare nucleotides.

ii. Why are these needed? (1 mark)

The nucleotides are required to form the new strand on the exposed DNA nucleotides.

Primers are also used during PCR.

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

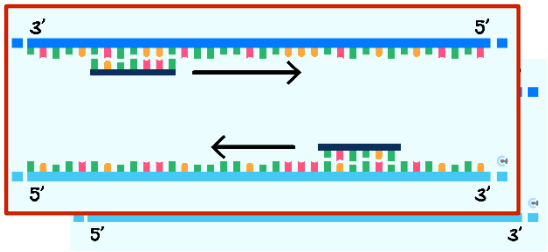
A primer is a short sequence of DNA nucleotides that is complimentary to an exposed sequence on each end of the DNA(1) and will mark where DNA polymerase will attach to and begin extension(1).

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

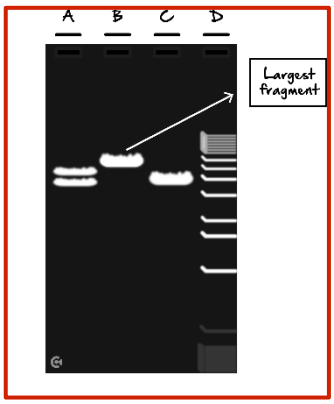
iv. How many copies of a DNA segment will be present after 4 cycles? (1 mark)

1 cycle = 2
2 cycles = 4
3 cycles = 8
4 cycles = 16 copies

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)

There are 2 bands shown in sample A because there are 2 fragments of different size in that sample.

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

The band in sample C appears thicker because there is more of that fragment present than the others.

Question 9 (9 marks)

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

The use of gel electrophoresis in sorting DNA fragments, including interpretation of gel runs	**Question 8a** (1 mark) Name the technique used to sort DNA fragments based on their size.	**Answer:** • *Gel electrophoresis.* **Marking protocol:** One mark for the above point.

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)

The use of gel electrophoresis in sorting DNA fragments, including interpretation of gel runs	**Question 8b** (4 marks) Describe the steps involved in sorting DNA fragments using the technique named in 8a. Include an explanation of how this technique works to sort such fragments.	**Answer:** • *DNA samples are placed into wells in one end of the slab of gel (the negative electrode end).* • *Electrodes are attached to each end of the gel and an electric current is passed through the gel from the negative end to positive end.* • *DNA is negatively charged, so it moves towards the positive end of the gel.* • *The smaller pieces are lighter so are able to travel further along the gel than the longer pieces. The size of each piece of DNA gives information about the number of bases present in each strand.* **Marking protocol:** One mark for each of the above points.

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)

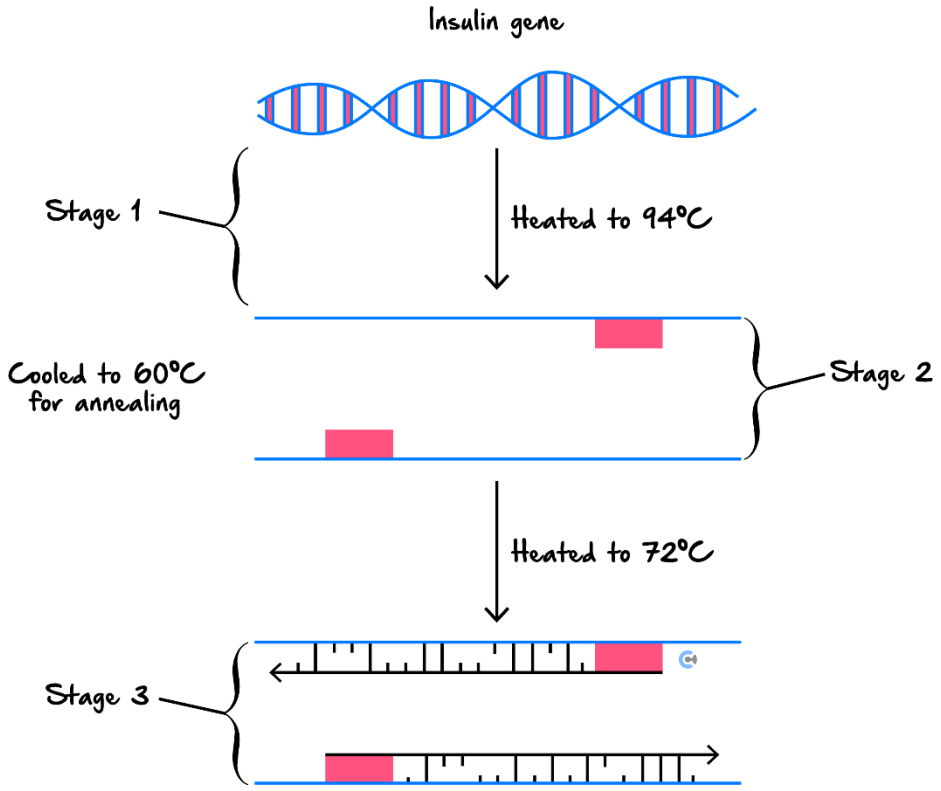
Techniques that apply DNA knowledge (specifically gene cloning, genetic screening and DNA profiling) including social and ethical implications and issues	**Question 8c** (4 marks) Outline how the technique named in 8a could be used by law enforcement officials in a case where there are multiple suspects and a blood sample from the crime scene.	**Answer:** • *A blood sample could be taken from the multiple suspects and the DNA isolated from each blood sample.* • *The same specific region of DNA from the suspects' blood samples (the same region as the blood sample from the crime scene) should be amplified via PCR and a restriction enzyme(s) used to digest the DNA into fragments.* • *The fragments should then be run in gel electrophoresis.* • *The DNA from the blood sample from the crime scene should also be run through gel electrophoresis, as outlined above, to determine if there is a match with one of the suspects.* **Marking protocol:** One mark for each of the above points.

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)

Stage 1: Denaturation of the DNA cooled to 60°C for annealing.	1 mark
Stage 2: Primer anneals to section of DNA complementary to the primer.	1 mark
Stage 3: Extension of the target DNA.	1 mark

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

- Non-coding regions such as satellite DNA are hypervariable.
- This means, that when cut with the same enzyme, fragments of differing lengths are created.
- These differences can be visualised on gel, as smaller fragments are separated from larger fragments to create unique band patterns for each individual.

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