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VCE Biology ¾

Introduction to DNA Manipulation Techniques [0.6]

Workshop

Section A: Multiple Choice Questions (18 Marks)

Question 1 (1 mark)

Genetic engineers use a variety of tools to manipulate DNA. One of the tools used to join pieces of DNA together is:

A. DNA polymerase

B. DNA ligase

C. Endonuclease

D. DNA primer

Question 2 (1 mark)

The specific enzyme responsible for catalysing the joining of free nucleotides during the polymerase chain reaction (PCR) is:

A. DNA polymerase

B. DNA ligase

C. Taq polymerase

D. Taq ligase

Question 3 (1 mark)

One method of biotechnology uses a particular enzyme to break the phosphodiester bonds holding nucleotides together in a single polynucleotide chain. This enzyme is called:

A. Ligase

B. Polymerase

C. Endonuclease

D. Reverse transcriptase

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Question 4 (1 mark)

Which one of the following statements about polymerase chain reaction (PCR) is correct?

A. The denaturation stage is carried out at 60°C and breaks the hydrogen bonds to form DNA strands that are complementary to the template strand.

B. The denaturation stage occurs after the extension stage.

C. The annealing stage is carried out at 95°C and enables primers to join with complementary sequences on either side of the target section of DNA.

D. The extension stage is carried out at 72°C and allows the Taq polymerase to bind to the primers and form DNA strands that are complementary to the template strand.

Question 5 (1 mark)

The process known as polymerase chain reaction (PCR) involves repeated cycles made up of several steps. During PCR, the:

A. First step in each cycle is to anneal primers to the DNA at a low temperature.

B. Temperature must be lowered to 37°C before the beginning of each cycle.

C. Second step in each cycle is to heat the DNA to a high temperature.

D. Final step of each cycle involves the use of DNA polymerase.

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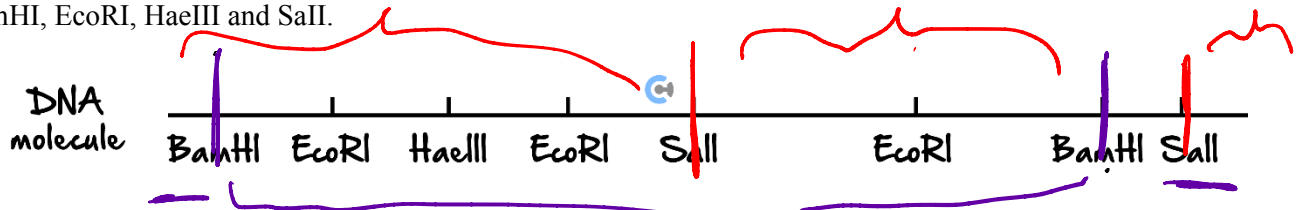
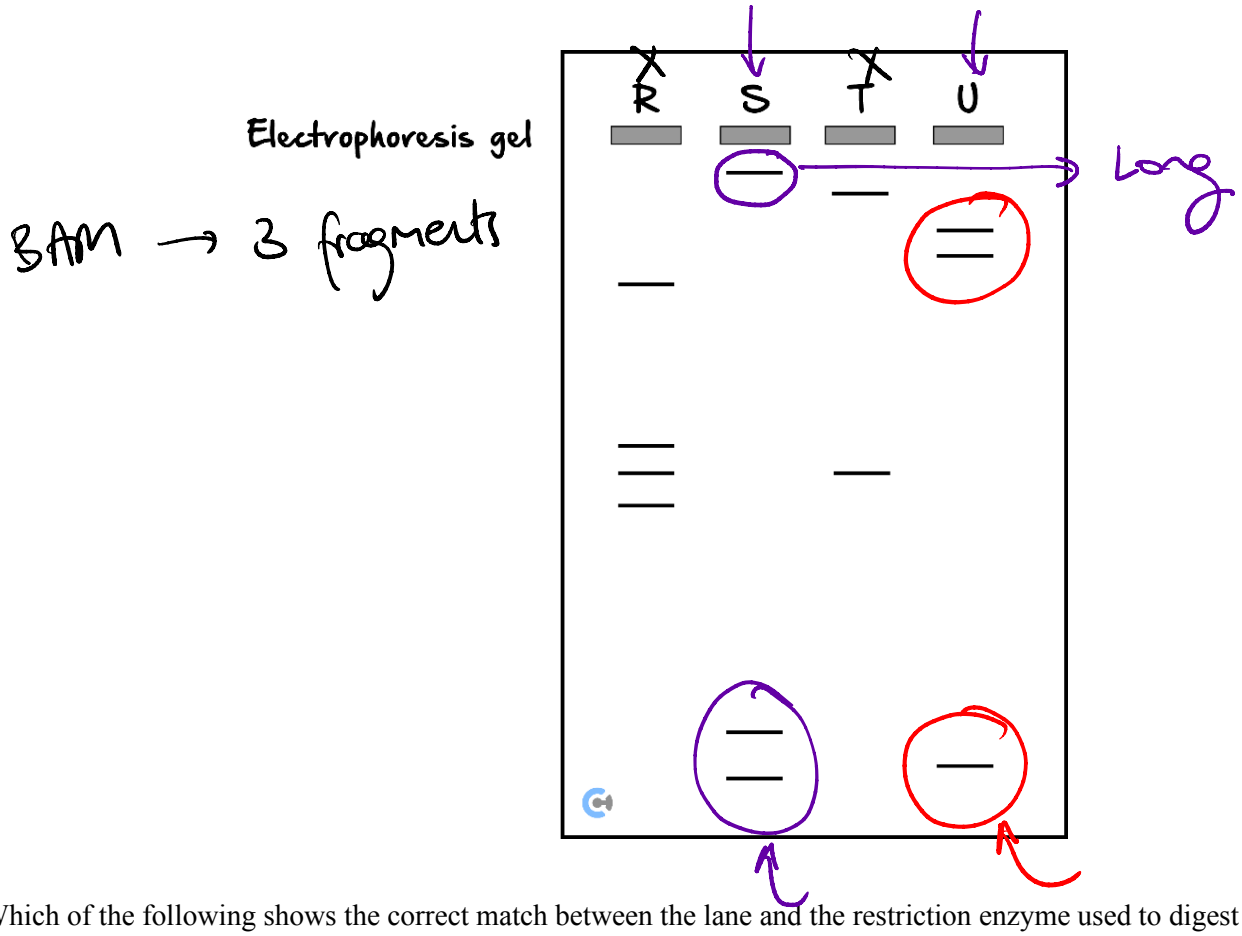
Question 6 (1 mark)

The diagram below represents a DNA molecule and the position of the recognition sites for the restriction enzymes BamHI, EcoRI, HaeIII and SaII.



Also shown is a diagram of an electrophoresis gel in which the lanes R, S, T and U show the separation of DNA segments resulting from the digestion of molecules with one of the restriction enzymes.



Which of the following shows the correct match between the lane and the restriction enzyme used to digest the DNA molecule?

	R	S	T	U
A.	SaII	EcoRI	HaeIII	BamHI
B.	EcoRI	BamHI	HaeIII	SaII
C.	EcoRI	BamHI	SaII	HaeIII
D.	HaeIII	SaII	BamHI	EcoRI

Question 7 (1 mark)

DNA profiling, using short tandem repeats (STR) within a person's DNA, helps to determine the genetic relationship between individuals.

DNA profiles based on four STRs for five individuals are shown below. The results of a gender identifier are also shown.

STR	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5
CSFIPO	7,14	7,11	8,13	7,14	7,14
TPOX	6,10	10,12	6,9	10,12	10,10
D21S11	27,30	29,32	27,27	29,30	27,28
D8S1179	9,11	12,13	17,17	11,12	9,11

	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5
Gender Identifier	Male	Female	Female	Male	Male

Which one of the following conclusions can be made using the information given?

A. Individual 4 is the father of Individual 5.

B. Individual 3 is the mother of Individual 4.

C. Individual 5 could be the child of Individual 1 and Individual 3.

D. The parents of Individual 4 could be Individual 1 and Individual 2.

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Question 8 (1 mark)

The restriction endonuclease EcoR1 cuts the DNA molecule between G and A in the recognition sequence G A A T T C.

In the diagrams below the cut site is shown as a line. The diagram that best corresponds to the cutting pattern of EcoR1 would be:

A. 5′ G | A A T T C 3′

B. 5′ G | A A T T C 3′
3′ C T T A A | G 5′

C. 5′ G | A A T T C 3′
3′ G | A A T T C 5′

D. 5′ G | A A T T | C 3′
3′ C | T T A A | G 5′

Question 9 (1 mark)

When trying to identify bodies after mass disasters such as a burning building, the best technique to help with the identification of the unknown persons would be:

A. DNA hybridisation

B. DNA profiling

C. Genetic screening

D. Gene cloning

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Question 10 (1 mark)

Plasmids of bacteria are used to transfer selected genes from one species to another.
The process can be represented as follows.

Bacterial Plasmid Cut → Foreign Gene and Plasmid Mixed → Plasmid with Inserted Foreign Gene

Enzymes are used to facilitate several of these steps.
Which one of the following shows the enzymes required for the first and last steps of the process?

	Cuts Plasmid	Inserts Genes
A.	Restriction enzyme	DNA ligase
B.	Restriction enzyme	DNA polymerase
C.	DNA ligase	DNA polymerase
D.	DNA polymerase	DNA ligase

Question 11 (1 mark)

Why are the primers that are used in the process of polymerase chain reaction (PCR) about 20 nucleotides long?

A. The Taq polymerase needs to bind to a strand that is 20 nucleotides long. ✗

B. The chance of the target nucleotide strand appearing in the genome more than once is reduced.

C. The primers provide a higher concentration of nucleotides for the extension stage.

D. When the PCR process is complete, the primers are easier to purify and recycle.

Question 12 (1 mark)

The PCR process is applied to five strands of DNA. How many resultant strands would be formed after four complete PCR cycles?

A. 16

B. 32

C. 40

D. 80

$2^4 = 16 \times 5$

$= 80$

Question 13 (1 mark)

Small amounts of DNA can be collected from items such as cigarette butts and hair follicles that are found at crime scenes. This DNA can be replicated for analysis and used as evidence in court cases.

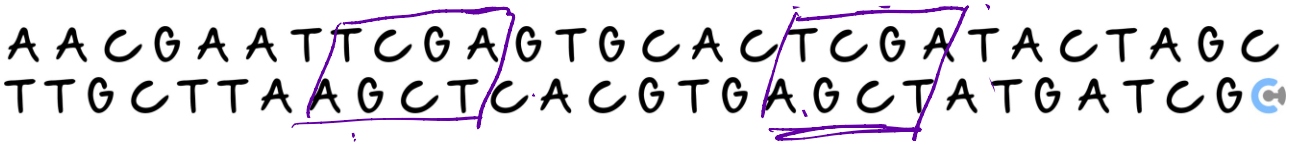
Which of the following identifies the method of DNA replication and the enzyme used?

	Method	Enzyme Used
A.	Polymerase chain reaction	Taq polymerase
B.	DNA profiling	DNA ligase
C.	Polymerase chain reaction	DNA ligase
D.	DNA profiling	Taq polymerase

Question 14 (1 mark)

The restriction enzyme Taq1 has the recognition sequence:

If the following DNA sequence were cut with Taq1, how many fragments would result?



A. 1

B. 2

C. 3

D. 4

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Question 15 (1 mark)

Which of the following statements is correct for the DNA fragments involved in gel electrophoresis?

A. Due to their size and molecular weight, larger fragments of DNA move more slowly through the gel than smaller fragments.

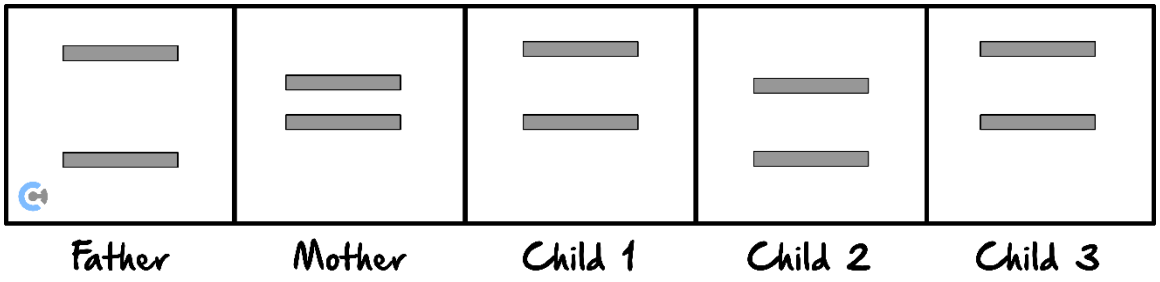
B. As larger fragments have more negative charge than smaller fragments, they move faster through the gel.

C. As DNA fragments have different amounts of negative charge, they move at different rates through the gel.

D. As smaller fragments have less negative charge, they move faster through the gel towards the positive end than larger fragments.

Question 16 (1 mark)

A couple has a set of triplets and notices that Child 2 looks very different from Child 1 and Child 3, so they decide that the entire family should undergo testing. The DNA profiles of the family are shown below.



Based on the information given, it would be reasonable to conclude that:

A. Child 1 and Child 3 must be identical twins.

B. Child 2 is the only biological child of the parents.

C. Child 2 has no DNA in common with the other two children.

D. All of the children inherited 50% of their DNA from each parent.

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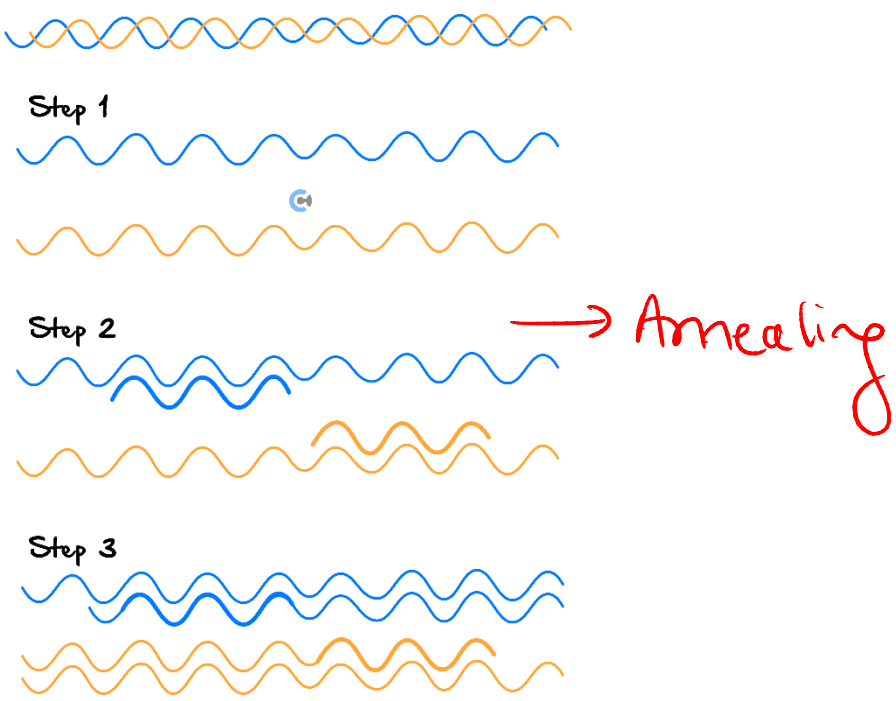
Question 17 (1 mark)

PCR has been used to amplify specific sections of DNA from small samples. It has uses in DNA sequencing, forensic analysis and genetic testing for diseases.

The diagram below illustrates the steps involved in each replication cycle.



A temperature of 50°C is needed for:

A. Step 1 only

B. Step 2 only

C. Step 3 only

D. Step 2 and 3 only

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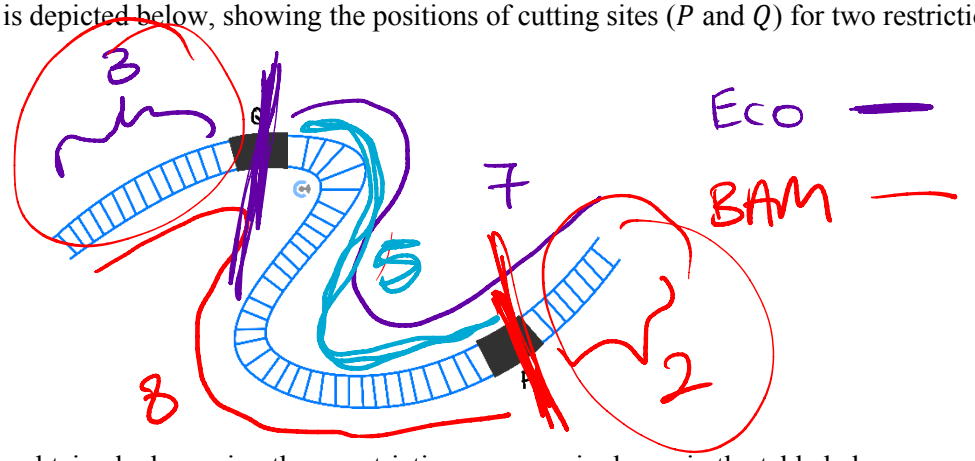
Question 18 (1 mark)

The genome of a small virus is depicted below, showing the positions of cutting sites (P and Q) for two restriction enzymes.



The length of DNA fragments obtained when using these restriction enzymes is shown in the table below.

Cutting Site	Restriction Enzyme Used	Length of DNA Fragments Obtained (kb)
Q	EcoR1	3,7 = 10
P	BamH1	8,2 = 10

If both EcoR1 and BamH1 are used together on this viral DNA, the length of fragments obtained would be:

A. 3, 8, 5, 2

B. 7, 2,1

C. 3, 5, 2

D. 3, 7, 8, 2

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Section B: Short Answer Questions (46 Marks)

Question 19 (3 marks)

Describe the function and process of endonucleases, and explain which type of cut is most useful in DNA manipulation.

Question 20 (7 marks)

Throughout the COVID pandemic, PCR testing became a crucial tool in providing an accurate method to determine whether someone was positive or not.

These tests checked to see if anyone had COVID, by collecting genetic material via a swab and attempting to amplify it. COVID is caused by an RNA virus, so the steps taken to perform a test were a modified version of the PCR studied in VCE, where reverse transcriptase is applied to convert it to DNA.

a. Name the enzyme responsible for DNA replication. (1 mark)

b. Briefly describe the process of PCR. (3 marks)

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_____

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_____

_____

c. What is the significance of using reverse transcriptase? (1 mark)

_____

_____

Forward and reverse primers are required to target a certain sequence.

d. Why are 2 different types of primers required, and what role do they play in PCR? (2 marks)

_____

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Question 21 (11 marks)

A student wanted to investigate the effect of two different endonucleases (restriction enzymes) on a linear DNA fragment.

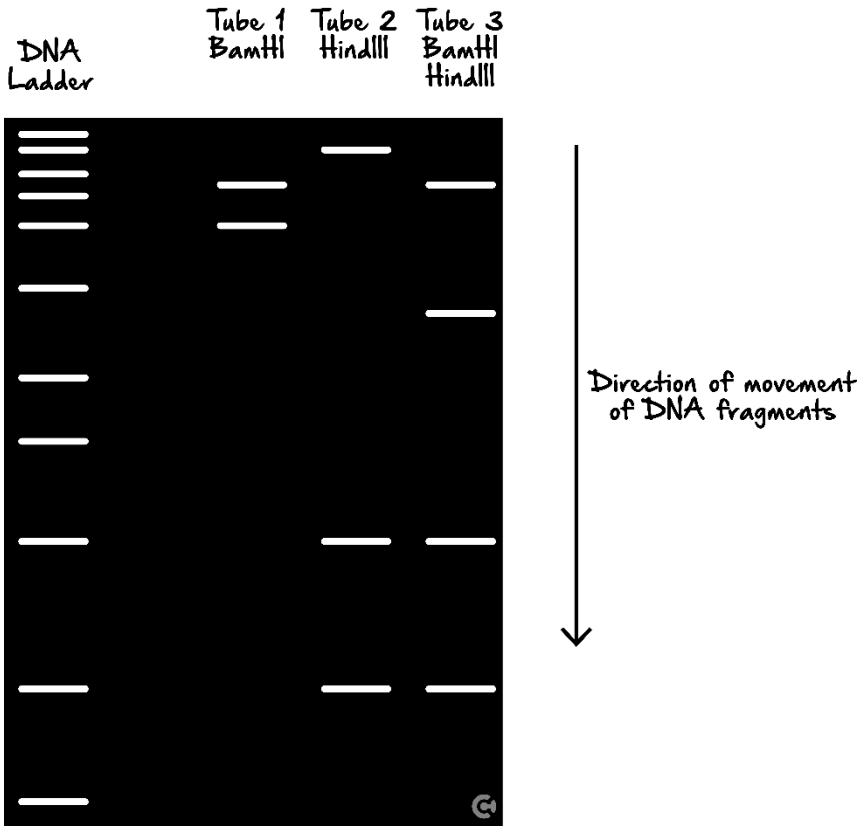
The student used three tubes containing a buffered solution of linear DNA fragments, each fragment being 9500 base pairs in length.

Two different endonucleases were available: BamHI and HindIII.

The student followed the steps below.

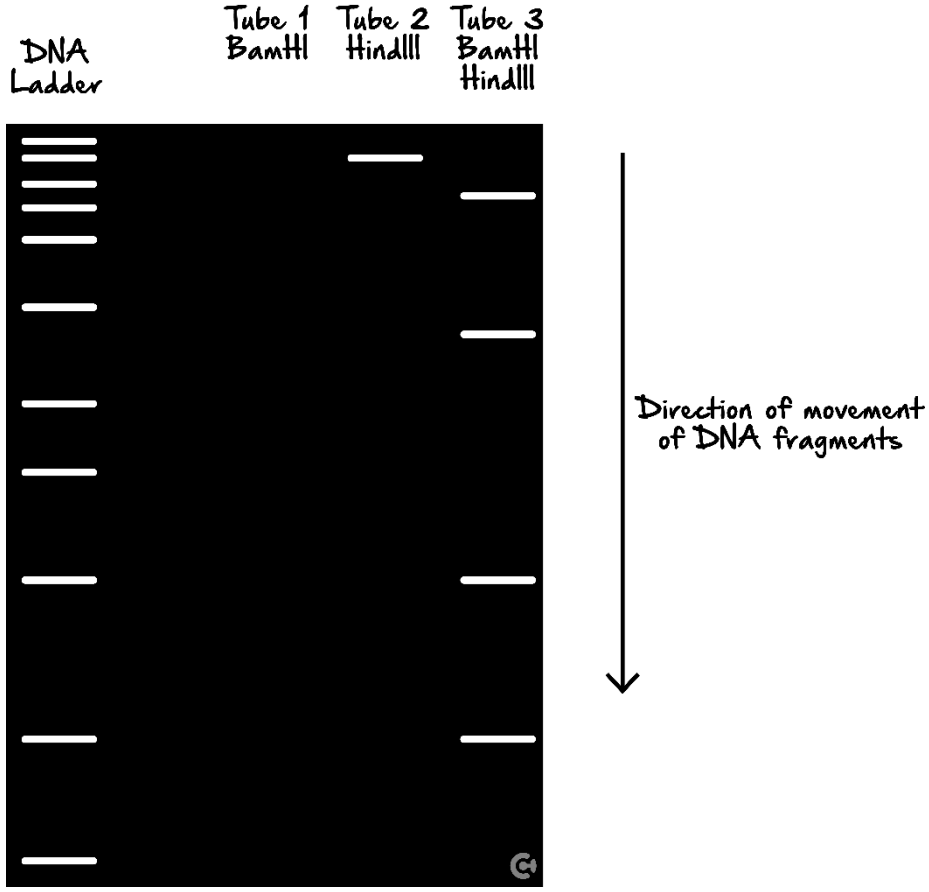
Step 1 – 2 μL of BamHI was added to the sample in Tube 1.
Step 2 – 2 μL of HindIII was added to the sample in Tube 2.
Step 3 – 2 μL of HindIII and 2 μL of BamHI were added to the sample in Tube 3.
Step 4 – All three tubes were incubated for one hour at a constant temperature of 37°C.
Step 5 – A 1% agarose gel was placed into an electrophoresis chamber and the gel was covered with a buffer solution.
Step 6 – 40 μL of a DNA ladder with fragments of known sizes was added to the first well of the 1% agarose gel. The known sizes of the fragments were 10000 bp, 8000 bp, 6000 bp, 5000 bp, 4000 bp, 3000 bp, 2000 bp, 1500 bp, 1000 bp, 500 bp and 250 bp.
Step 7 – 40 μL of the contents of each of the tubes were loaded into three separate wells of the 1% agarose gel.
Step 8 – An electric current of 100 V was run through the gel for 45 minutes.

After 45 minutes the student obtained the results below.



a. Analyse the results of the experiment. (5 marks)

The student repeated the experiment the next day and obtained the following results.



b. Identify one difference between the new results and the previous results, and suggest a possible reason for this difference. (2 marks)

_____

_____

_____

_____

c. State two factors that impact the movement of the fragments through the agarose gel. (2 marks)

_____

_____

_____

_____

d. Outline two safety guidelines that should have been followed by the student. (2 marks)

_____

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_____

_____

Space for Personal Notes

Question 22 (11 marks)

Oh no! There has been a murder at the Contour Education™ HQ! Ramodh was busy working late as usual, coming up with another genius plan to master the UCAT, until suddenly he was taken out from behind.

Police suspect that the nefarious UCAT ANZ Consortium is behind this, with Ramodh getting too close to the real truth behind the UCAT.

Unfortunately, such suspicions won't cut it in court, and they need hard evidence. Luckily, the killer was a bit stupid and left his DNA all over the place, confirming that this was not the work of a Contour Education™ student (they don't make silly mistakes).

The police have isolated a small sample of DNA from the crime scene, including that of the poor victim (RIP Ramodh).

a. What process could they take to increase the amount of DNA available? Describe its steps. (3 marks)

Polymer

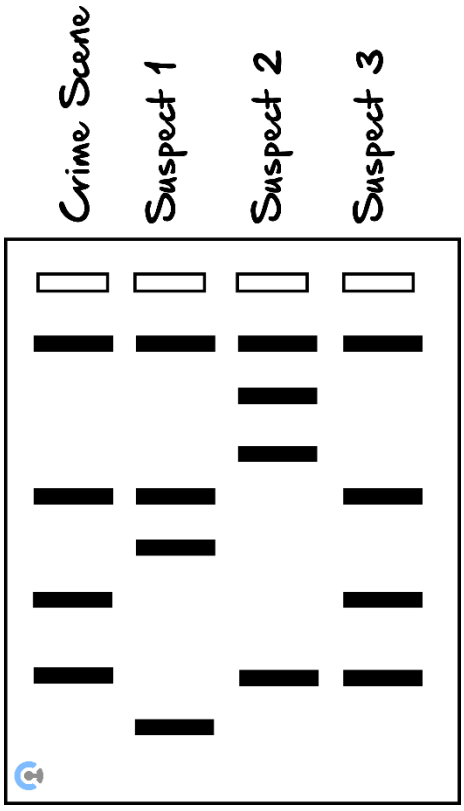
The police now have a large sample of DNA, from the victims, the killer, and a bunch of suspects they think could be the killer. They are planning to cut the STR DNA of each using the same endonuclease and run that through gel electrophoresis.

b. What are STRS? (1 mark)

c. Describe the steps of gel electrophoresis. (3 marks)

d. Why will this allow us to be able to tell each sample apart? (2 marks)

These are the results of the experiment.



e. Who is the killer? Explain. (2 marks)

Question 23 (8 marks)

The identity of a child's father was disputed and a DNA paternity test was carried out. Samples of DNA were voluntarily taken from the mother, the child and the two potential fathers. The polymerase chain reaction (PCR) was used on each sample, and short tandem repeat 1 (STR 1) was extracted from each sample. STR 1 is a section of DNA that shows some variability in Size. As each individual inherits two copies of STR 1, it can be used as a tool to determine individuality.

a. Describe the steps of PCR that enable multiple copies of a specific section of the genome to be amplified. (3 marks)

Once amplified, the sections of DNA can be compared to each other through a process of genetic profiling. STR 1 has four variations in size, as shown in the table below.

Variation	Size	Size of Sample (simplified)
1	850	0.8 kb
2	923	0.9 kb
3	1026	1.0 kb
4	1580	1.6 kb

The size of the STR 1 alleles of the mother, the child and the two potential fathers was determined by using gel electrophoresis on their DNA samples.

b. Explain why the process is called gel electrophoresis. (2 marks)

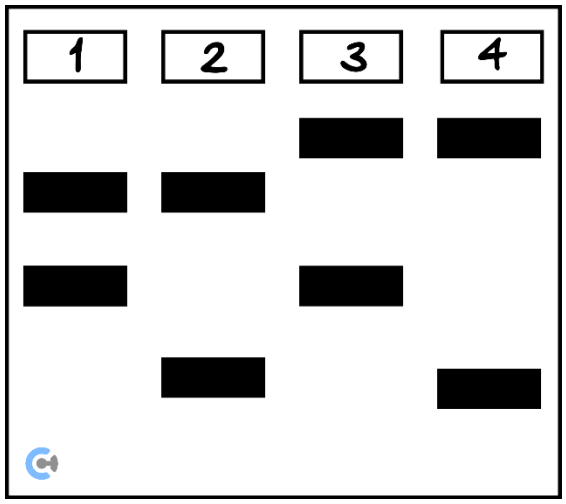
_____

_____

_____

_____

c. The results of the gel electrophoresis are illustrated below. Lanes 1 and 2 are the potential fathers, lane 3 is the mother and lane 4 is the child.



Which lane contains the DNA of the child's father? Justify your response. (1 mark)

_____

_____

d. State **two** conditions that need to be applied to the electrophoretic gel so that the DNA fragments in each sample separate successfully. (2 marks)

Question 24 (6 marks)

Sickle cell anaemia is a common genetically inherited autosomal disease that affects about 5% of the world's population. Genetic testing for the presence of the disease is an important diagnostic procedure in many countries where the disease is more predominant (America and Africa). PCR, restriction enzymes and then gel electrophoresis are used to diagnose the genetic status of individuals.

a. What is the purpose of PCR? (1 mark)

AMPLIFY DNA

b. There are two alleles for the sickle cell gene: one codes for normal haemoglobin and the other for sickle cell haemoglobin (where the haemoglobin is unable to absorb oxygen very well). The difference between the two alleles is a single point mutation changing one amino acid in the haemoglobin gene.

Describe how a single point mutation could change a single amino acid in a protein. (1 mark)

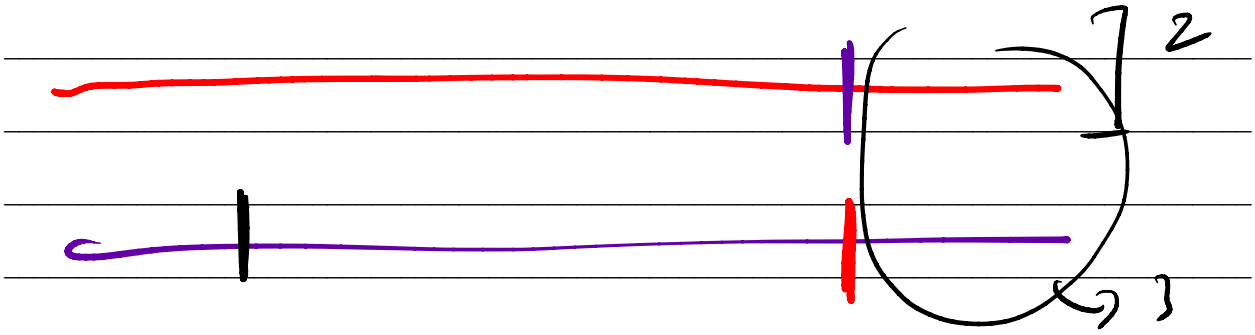
codon changes

AAA → AAT — same

AAA AUA change

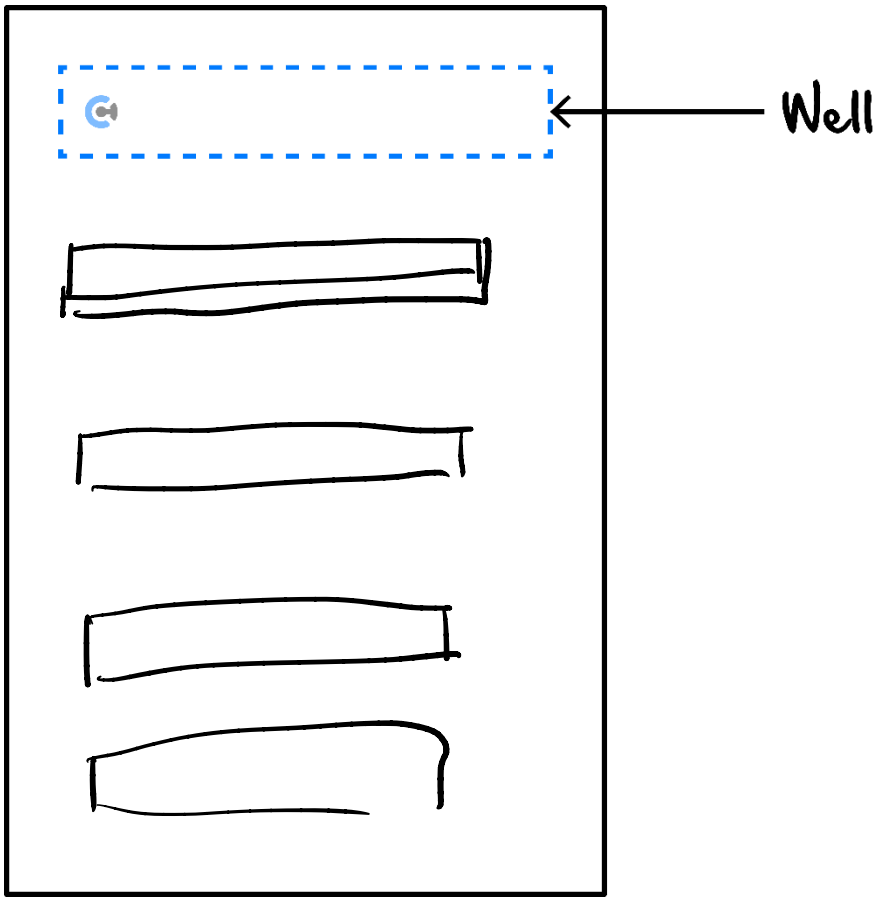
c. After an individual's DNA undergoes PCR it is mixed with a restriction enzyme, DdeI. There is one restriction enzyme binding site that is the same in both alleles. The only difference is one extra restriction enzyme binding site along the normal allele. Each allele, once cut with DdeI, produces fragments of unequal size.

Complete the diagrams below by placing *X*s along the alleles, showing the DdeI binding sites for both the sickle cell allele and the normal allele. (2 marks)



d. Gel electrophoresis is used to provide a profile so that an appropriate diagnosis can be made. A couple who are both carriers of the sickle cell trait (heterozygous) conceive a child.

Draw a band pattern within the box below that would lead to a positive diagnosis for a carrier of the sickle cell trait. Show on the diagram where the smallest fragment is located within the gel. (2 marks)



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