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VCE Biology ¾
AOS 1 Revision [1.0]

Contour Check (Part 1) Solutions





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### Section A: [1.2] - Nucleic Acids & the Structure of Genes (Checkpoints) (38 Marks)



# <u>Sub-Section</u>: [1.2.1] - Identify and Compare the Characteristic Features of the Structures of Nucleic Acids and Their Monomers

Qu	estion 1		
De	finitions:		
a.	Nucleic	acids	
		Molecules such as DNA and RNA, which store and transmit genetic information.	
b.	DNA		
		A double-stranded nucleic acid containing genetic instructions for development, functioning, growth, and reproduction.	
c.	mRNA		
		Messenger RNA; a single-stranded RNA that carries genetic information from DNA to ribosomes for protein synthesis.	
d.	tRNA		
		Transfer RNA; a type of RNA that helps decode a messenger RNA sequence into a protein.	
e.	rRNA		
		Ribosomal RNA; a structural component of ribosomes that helps in protein synthesis	s.



### Question 2 (1 mark)



A scientist analyses a nucleic acid and determines that it contains uracil. Which nucleic acid is being analysed?

- A. DNA
- B. mRNA
- C. tRNA
- D. rRNA

### Question 3 (1 mark)



During translation, a strand of RNA with the sequence 5'-GCCAUAAUG-3' is analysed. Which anticodon sequence corresponds to this RNA strand?

- A. 5'-UGGUAUUAC-3'
- B. 5'-CGGAUAAUG-3'
- C. 5'-UCGCUAUAC-3'
- D. 5'-ACCUAUUAC-3'

### Question 4 (1 mark)



A mutation disrupts rRNA synthesis in a cell. What would be the most immediate effect?

- **A.** DNA transcription is halted.
- **B.** Ribosome assembly and protein synthesis are impaired.
- **C.** mRNA is degraded prematurely.
- **D.** Amino acid transport is disrupted.





### Question 5 (1 mark)



Which comparison accurately distinguishes between DNA and RNA?

	DNA	RNA
A.	Contains uracil	Contains thymine
B.	Double-stranded	Single-stranded
C.	Found only in the nucleus	Found throughout the cell
D.	Contains ribose	Contains deoxyribose

### Question 6 (1 mark)



If a DNA strand has 20% adenine, what percentage of guanine does it contain?

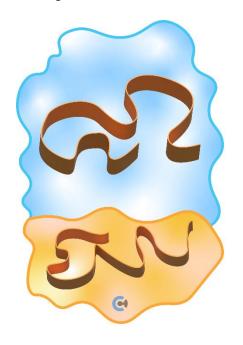
- **A.** 20%
- **B.** 30%
- **C.** 40%
- **D.** 50%



### **Question 7** (6 marks)



The image below shows 2 different molecules found within cells, rDNA and DNA.





**a.** Name the location within a eukaryotic cell where each molecule would be found. (1 mark)

Ribosomal RNA is found in the ribosome, whereas DNA is found in the nucleus.

Only one example is needed for each.

Two correct responses must be given for the differences to attain one mark.

b. Complete the following table comparing molecules of TKNA and DNA. (2 marks)

	rRNA	DNA	
Difference	Ribose sugar Single stranded Uracil	Deoxyribose sugar Double stranded Thymine	
Similarity	Pentose sugar Both composed of nucleotides		

**c.** How would DNA differ in prokaryotic and eukaryotic organisms? (1 mark)

### One of

In prokaryotes, DNA is circular, whereas in eukaryotes, DNA is linear.

In prokaryotes, DNA is found in the cytosol and in eukaryotes, it is found in the nucleus.



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d.	Adenine and guanine are both classified as purines, double-ringed structures of nucleic acids. A cell was foun to contain 10% guanine. What percentage of thymine would be found in the cell? Explain. (2 marks)
	As 10% of the cell is guanine, 10% must also be cytosine due to complementary base pairing (1). The remaining nucleotides would be 40% adenine and 40% thymine (1).
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# <u>Sub-Section</u>: [1.2.2] - Identify and Describe the Structure of a Nucleotide in DNA and RNA

# Ouestion 8

Definition:

Nucleotide.

The basic building block of nucleic acids, consisting of a nitrogenous base, a pentose sugar, and a phosphate group.

### Question 9 (1 mark)



Which of the following correctly describes the components of a nucleotide?

- **A.** A nitrogenous base, a ribose sugar, and a phosphate group
- **B.** A nitrogenous base, a pentose sugar, and a phosphate group
- C. A nitrogenous base, a hexose sugar, and a phosphate group
- **D.** A nitrogenous base, a ribosome, and a phosphate group

### **Question 10** (1 mark)



Two nucleotides are linked together by:

- **A.** Hydrogen bonds between their nitrogenous bases.
- **B.** Peptide bonds between their sugars and phosphates.
- **C.** Covalent bonds between the phosphate of one nucleotide and the sugar of another.
- **D.** Ionic bonds between their sugars.



### Question 11 (1 mark)



A nucleotide with thymine as its nitrogenous base is likely to be part of:

- **A.** DNA only
- **B.** RNA only
- C. Both DNA and RNA
- **D.** Neither DNA nor RNA

### **Question 12** (1 mark)



The difference between ribose and deoxyribose sugars in nucleotides is:

- **A.** Ribose lacks a hydroxyl group on the 2' carbon.
- **B.** Deoxyribose has an additional hydroxyl group on the 3' carbon.
- C. Ribose has a hydroxyl group on the 2' carbon, which deoxyribose lacks.
- **D.** Deoxyribose lacks a phosphate group.

### **Question 13** (5 marks)



Draw the structure of a nucleotide chain, labelling the key features. In your diagram, make sure to label the bonds between the nucleotides and the components of the nucleotide.

Solution Pending



# <u>Sub-Section</u>: [1.2.3] - Define the Key Components of a Gene, Including a Comparison Between the Structure of Genes in Eukaryotes and Prokaryotes

Question 14							
Definitions:							
a.	Gene.						
	A segment of DNA that codes for a specific protein or functional RNA.						
b.	Key Components of a Gene.						
	Include the promoter region, coding sequence (exons), non-coding sequence (introns in eukaryotes), and terminator region.						
c.	Gene Structure in Eukaryotes v/s Prokaryotes.						
	<ul> <li>Eukaryotic genes: Often have introns and exons, require RNA processing, and have complex regulatory elements.</li> <li>Prokaryotic genes: Lack introns, and transcription and translation are coupled.</li> </ul>						

### Question 15 (1 mark)



What is the primary role of the promoter region in a gene?

- **A.** To code for the protein sequence.
- **B.** To initiate the binding of RNA polymerase for transcription.
- **C.** To terminate transcription.
- **D.** To signal the start of translation.





### Question 16 (1 mark)



A researcher is studying a gene with introns. Which organism is the gene most likely from?

- A. Prokaryote
- **B.** Eukaryote
- **C.** Virus
- D. Bacteriophage

### Question 17 (1 mark)



In prokaryotic gene expression, transcription and translation are coupled because:

- A. Prokaryotes lack a nucleus.
- **B.** Prokaryotes have fewer ribosomes.
- C. Prokaryotic DNA is circular.
- **D.** Prokaryotic genes lack promoter regions.

### Question 18 (1 mark)



Which component is not typically part of a prokaryotic gene?

- A. Operator
- **B.** Introns
- C. Promoter
- **D.** Coding sequence





### Question 19 (1 mark)



A gene has the sequence 5'-ATGCCGTAA-3'. After RNA processing, the corresponding mRNA strand is 5'-AUGCGUA-3'. This indicates that:

- **A.** Introns were spliced out during processing.
- **B.** Exons were spliced out during processing.
- **C.** The promoter sequence was removed.
- **D.** Post-transcriptional modifications were unnecessary.

### **Question 20** (4 marks)

A researcher is studying gene expression in a particular organism. They are focusing on the region of the gene that initiates transcription.

**a.** Explain the role of the promoter region in gene expression. (2 marks)

The promoter region is essential in gene expression because it serves as the binding site for RNA polymerase and transcription factors. These elements are required to initiate the transcription process, allowing the gene to be copied into mRNA.

**b.** The researcher is investigating how mutations in the promoter region could affect gene expression. How might a mutation in the promoter region lead to increased or decreased gene expression? (2 marks)

A mutation in the promoter region could either enhance or reduce gene expression. A mutation that strengthens the binding of RNA polymerase or transcription factors could lead to increased transcription, while a mutation that weakens the binding could result in decreased gene expression.





Sub-Section: [1.2.4] - Identify and Practically Apply the Characteristics of the Genetic Code - Universal, Unambiguous, Degenerate, Triplet - to Real-Life Examples

Qu	Question 21						
De	Definitions:						
a.	a. Universal						
	The genetic code is consistent across nearly all organisms; the same codons specify the same amino acids.						
b.	Unambiguous						
	Each codon specifies only one amino acid or a stop signal.						
c.	Degenerate						
	Most amino acids are encoded by multiple codons, providing redundancy in the code.						
d.	Triplet Code						
	Each codon consists of three nucleotides, which encode a single amino acid.						
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### Question 22 (1 mark)



A researcher introduces the human insulin gene into bacterial cells, and the bacteria successfully produce human insulin. What property of the genetic code does this highlight?

- A. Degenerate
- **B.** Universal
- C. Triplet
- **D.** Unambiguous

### Question 23 (1 mark)



A point mutation changes the codon AAA (lysine) to AAG. The resulting protein remains unchanged. This illustrates which characteristic of the genetic code?

- A. Triplet
- B. Degenerate
- C. Universal
- **D.** Unambiguous

### Question 24 (1 mark)



A scientist observes that the codons UAA, UAG, and UGA all signal the termination of translation. Which property of the genetic code does this reflect?

- A. Universal
- **B.** Unambiguous
- C. Degenerate
- **D.** Triplet





### Question 25 (1 mark)



During translation, the codon AUG codes for methionine in human cells, plant cells, and bacterial cells. This demonstrates that the genetic code is:

- A. Specific
- B. Degenerate
- C. Universal
- **D.** Flexible

### Question 26 (1 mark)



A mutation changes the sequence of a gene, but the resulting protein functions identically to the original. Which feature of the genetic code most likely accounts for this?

- A. Triplet
- B. Degenerate
- C. Universal
- **D.** Ambiguous

### **Question 27** (4 marks)



- **a.** A pharmaceutical company develops a drug that blocks specific codons on mRNA during translation. How might the universality of the genetic code impact the effectiveness of this drug across different species? (2 marks)
  - The universality of the genetic code means that the same codons code for the same amino acids across most species. This allows the drug to potentially target similar codons in different organisms, making it effective across a wide range of species, including bacteria and humans.



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•	The drug company notices that a certain mutation in a bacterial gene does not change the resulting protein. How does the degeneracy of the genetic code explain this? (2 marks)		
	The degeneracy of the genetic code means that multiple codons can code for the same amino acid. As a result, a mutation in the gene that changes one codon might still result in the same amino acid being incorporated into the protein, thus leaving the protein function unchanged.		



Section B: [1.3] - Gene Expression & the trp Operon (Checkpoints) (65 Marks)



# <u>Sub-Section</u>: [1.3.1] - Identify and Recall the Process of Gene Expression in Eukaryotes, Comparing How It Differs in Prokaryotes

Qu	estion 28		
De	finitions:		
a.	Gene Expression		
		The process by which the instructions in DNA are transcribed into RNA and then translated into proteins.	
b.	Transcription		
		The synthesis of RNA from a DNA template.	
c.	Translation		
		The process where mRNA is decoded by ribosomes to produce a polypeptide chain.	
d.	RNA Processing		
		A series of modifications to pre-mRNA in eukaryotes, including splicing out introns, adding a 5' cap, and a poly-A tail.	
Sp	ace for Personal No	otes	



### Question 29 (1 mark)



Which of the following correctly describes the steps of gene expression?

- **A.** Translation  $\rightarrow$  RNA Processing  $\rightarrow$  Transcription
- **B.** Transcription  $\rightarrow$  Translation  $\rightarrow$  RNA Processing
- C. Transcription  $\rightarrow$  RNA Processing  $\rightarrow$  Translation
- **D.** RNA Processing  $\rightarrow$  Transcription  $\rightarrow$  Translation

### **Question 30** (1 mark)



In eukaryotes, what is the primary difference between pre-mRNA and mature mRNA?

- **A.** Mature mRNA contains introns, while pre-mRNA does not.
- **B.** Mature mRNA is shorter because introns are removed.
- **C.** Pre-mRNA is double-stranded, while mature mRNA is single-stranded.
- **D.** Pre-mRNA contains a poly-A tail, while mature mRNA does not.

### **Question 31** (1 mark)



Which of the following is unique to prokaryotic gene expression?

- **A.** The presence of introns and exons in genes.
- **B.** Coupling of transcription and translation in the cytoplasm.
- C. RNA splicing before translation occurs.
- **D.** The addition of a 5' cap to mRNA.





### Question 32 (1 mark)



What is the key difference in transcription between prokaryotes and eukaryotes?

- A. Eukaryotic transcription requires fewer enzymes.
- **B.** Transcription occurs in the nucleus of prokaryotic cells.
- C. Prokaryotic RNA does not undergo post-transcriptional modifications.
- **D.** Eukaryotic genes lack promoter regions.

### **Question 33** (1 mark)



A mutation prevents the addition of a poly-A tail to a eukaryotic RNA transcript. What is the likely consequence?

- **A.** The transcript will fail to bind ribosomes.
- **B.** The transcript will degrade quickly and fail to produce a protein.
- C. Transcription will not begin.
- **D.** RNA splicing will not occur.

### Question 34 (1 mark)



Which of the following best describes the function of the 5' cap in eukaryotic gene expression?

- **A.** It protects mRNA from degradation and facilitates ribosome binding.
- **B.** It prevents RNA polymerase from binding to the DNA.
- **C.** It signals the end of translation.
- **D.** It ensures the correct folding of the protein.





### Question 35 (1 mark)



During eukaryotic gene expression, where does RNA polymerase bind to initiate transcription?

- A. Terminator Sequence
- B. Coding Region
- C. Promoter Sequence
- **D.** Poly-A Tail

### Question 36 (1 mark)



In a hypothetical eukaryotic gene, a mutation eliminates the intron-exon boundary. What would be the effect on the resulting protein?

- **A.** The protein would be shorter due to incomplete splicing.
- **B.** The protein would remain unaffected.
- C. The protein would contain additional amino acids due to unspliced introns.
- **D.** Translation would not occur.

### Question 37 (1 mark)



If transcription occurs in the presence of a toxin that inhibits RNA polymerase activity, what will happen?

- **A.** Only RNA splicing is inhibited.
- **B.** No RNA will be synthesised.
- **C.** Translation proceeds as usual.
- **D.** The 5' cap and poly-A tail are still added to mRNA.





### Question 38 (1 mark)



A scientist observes that a prokaryotic mRNA molecule is being translated while it is still being transcribed. This is possible because:

- A. Prokaryotic mRNA lacks introns.
- **B.** Transcription and translation occur in the same cellular compartment in prokaryotes.
- **C.** Prokaryotic genes do not have promoter sequences.
- **D.** Ribosomes are absent in prokaryotes.

# Question 39 (3 marks) Compare the process of gene expression in eukaryotes and prokaryotes. No mRNA processing, and no intron to remove after transcription. Transcription and translation occur simultaneously. Occur in the same location (the cytosol) as there is no nuclear membrane.





Sub-Section: [1.3.2] - Describe the Processes of Transcription, mRNA Processing, and Translation, Recognising the Significance of Each Step to the Final Product

Question 40							
De	Definitions:						
a.	a. Alternative Splicing						
	The process during RNA processing where different combinations of exons are joined, leading to multiple proteins from a single gene.						
b.	Codon						
		A triplet of nucleotides in mRNA that specifies an amino acid.					
c.	Anticodon						
		A triplet of nucleotides in tRNA complementary to an mRNA codon.	-				
d.	Ribosome						
		A cellular structure that facilitates the translation of mRNA into a polypeptide.					
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### Question 41 (1 mark)



During transcription, which strand of DNA serves as the template for RNA synthesis?

- A. Coding Strand
- B. Non-template Strand
- C. Template strand
- **D.** Both strands are transcribed simultaneously.

### **Question 42** (1 mark)



What is the primary purpose of adding a poly-A tail to eukaryotic mRNA?

- **A.** To enhance translation efficiency by ribosomes.
- **B.** To stabilise the mRNA and protect it from degradation.
- C. To signal the start of transcription.
- **D.** To prevent the splicing of introns.

### Question 43 (1 mark)



If a mutation occurs in the spliceosome complex, what would be the likely effect on mRNA processing?

- **A.** The poly-A tail would not be added.
- **B.** Introns would not be removed, resulting in longer mRNA.
- **C.** Exons would be prematurely degraded.
- **D.** Ribosome assembly would be impaired.



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### Question 44 (1 mark)



During translation, which of the following correctly describes the process of elongation?

- **A.** The ribosome binds to the promoter region of mRNA.
- **B.** Aminoacyl-tRNA delivers amino acids to the ribosome, which are joined by peptide bonds.
- **C.** Exons are spliced out, and introns are joined together.
- **D.** Stop codons signal the ribosome to release the polypeptide.

### Question 45 (1 mark)



A mutation alters a stop codon to a codon coding for an amino acid. What is the most likely outcome of this mutation?

- **A.** The protein will be shorter than normal.
- **B.** The protein will continue to elongate until the ribosome encounters another stop codon.
- C. Translation will be completely inhibited.
- **D.** The ribosome will degrade the mRNA.

### **Question 46** (3 marks)



Translation occurs within the cytosol of a cell. Outline the steps that normally occur in translation. Use specific terms and names of the molecules involved. Name the final product of the process.

	Marks	0	1	2	3	Average
Ī	%	39	15	16	30	1.4

- mRNA travels to the ribosomes where its codons are read.
- tRNA carries specific amino acids to the ribosomes **or** complementary base pairing occurs between the codons and anticodons.
- Product; protein/polypeptide.

This question was well answered by many students. However, some students failed to name the final product, which was significantly asked for in the question. Other students used many irrelevant terms, enzymes and structures which detracted from their answers



Question 47 (4 marks)	
Describe two events that occur during the	processing of pre-mRNA in eukaryotes and their significance.
	Solution Pending

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# <u>Sub-Section</u>: [1.3.3] - Explain How a Single Gene Can Give Rise to Multiple Proteins

Question 48				
Definitions:				
a.	a. Alternative Splicing.			
		A process during RNA processing where exons are included or excluded in different combinations, leading to diverse proteins.		
b.	Post-Translational	Modification.		
		Chemical changes to a protein after it has been translated, such as phosphorylation or glycosylation.		
Question 49 (1 mark)				
Wł	nich process allows	a single gene to produce multiple proteins?		
A.	Alternative Splicin	<mark>g</mark>		
В.	Transcription Factor	or Binding		
C.	Ribosome Recyclin	ng		
<b>D.</b> Protein Degradation				
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### Question 50 (1 mark)



A mutation disrupts the recognition sites for the spliceosome in pre-mRNA. What is the likely result?

- **A.** All introns will be removed as usual.
- **B.** The pre-mRNA will fail to undergo splicing, leading to an incomplete protein.
- **C.** The protein will be shorter than normal.
- **D.** Splicing will only occur in the cytoplasm.

### Question 51 (1 mark)



How does post-translational modification contribute to protein diversity?

- **A.** It increases the number of genes in the genome.
- **B.** It allows a single protein to perform multiple functions through chemical changes.
- C. It changes the sequence of mRNA.
- **D.** It replaces alternative splicing in prokaryotes.

### Question 52 (1 mark)



Which of the following is an example of alternative splicing?

- **A.** A ribosome switching between different mRNAs during translation.
- **B.** A pre-mRNA including or excluding different exons to create various mature mRNAs.
- **C.** A DNA mutation altering the coding sequence.
- **D.** A tRNA molecule carrying multiple amino acids.





### Question 53 (1 mark)



A gene codes for a protein with three domains. One cell type produces a shorter version of the protein missing one domain. How is this achieved?

- A. Post-transcriptional Modification
- **B.** Alternative Splicing
- C. Mutation in the Promoter
- D. Poly-A Tail Shortening

# Question 54 (2 marks) Explain how a single gene can give rise to many different proteins. Alternative splicing allows different exons of the pre-mRNA to be joined in various combinations, leading to the production of multiple distinct mRNA transcripts from one gene, and thus different protein variants. After translation, proteins can be chemically modified (e.g., phosphorylation or glycosylation), which can alter their function, activity, or localisation, resulting in different functional forms of the same protein.

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# <u>Sub-Section</u>: [1.3.4] - Identify and Recall the General Principles and Reasons for Gene Regulation in Both Prokaryotes and Eukaryotes

Question 55					
Def	Definitions:				
a.	a. Gene Regulation				
<b>b.</b>	Regulatory Gene	The mechanisms that control the expression of genes to ensure proteins are produced only when needed.			
		A gene that codes for proteins (e.g., repressors or activators) that regulate the expression of other genes.			
c.	Structural Gene				
		A gene that codes for proteins that serve structural or enzymatic functions.			
d.	Repressor Protein				
		A protein that binds to the operator region to inhibit gene expression.			
e.	Activator Protein				
		A protein that binds to the DNA to enhance the binding of RNA polymerase and promote transcription.			



### Question 56 (1 mark)



What is the primary purpose of gene regulation in prokaryotes?

- **A.** To conserve energy by producing proteins only when required.
- **B.** To increase genetic variation.
- **C.** To ensure every gene is expressed at all times.
- **D.** To modify the DNA sequence in response to environmental changes.

### Question 57 (1 mark)



In eukaryotes, which of the following is an example of gene regulation at the transcriptional level?

- **A.** Splicing out introns from pre-mRNA.
- **B.** Methylation of DNA to silence genes.
- C. Modification of the protein's active site.
- **D.** Folding of the protein after translation.

### Question 58 (1 mark)



A mutation in the operator region of a prokaryotic operon prevents the binding of a repressor protein. What is the likely consequence?

- **A.** RNA polymerase will fail to bind to the promoter.
- **B.** The structural genes will be transcribed continuously.
- **C.** The operon will not respond to environmental signals.
- **D.** The structural genes will never be expressed.





Question 59 (1 mark)



How does an activator protein increase transcription of a gene?

- **A.** By removing introns from the mRNA.
- **B.** By assisting RNA polymerase in binding to the promoter.
- **C.** By altering the genetic code to improve efficiency.
- **D.** By repressing competing genes in the same operon.

### Question 60 (1 mark)



In a eukaryotic cell, a signal molecule triggers a cascade that leads to histone acetylation in the promoter region of a gene. What is the most likely result?

- **A.** The gene becomes transcriptionally active.
- **B.** The gene's mRNA is rapidly degraded.
- **C.** The gene becomes permanently silenced.
- **D.** The DNA sequence of the gene is modified.



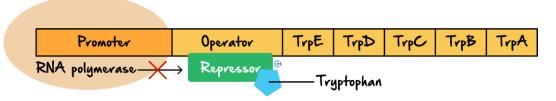
### Question 61 (5 marks)



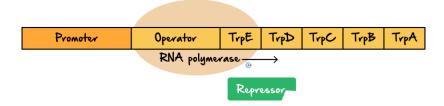
The trp operon is a method of gene regulation in some species of bacteria. It contains a series of three genes that code for the production of enzymes that facilitate the ability of the bacteria to metabolise tryptophan. These genes are switched on or off depending on whether tryptophan is present or absent.

**a.** Draw a labelled diagram to illustrate how the trp operon regulates the expression of the structural genes in the presence and absence of tryptophan. (2 marks)

When tryptophan is present, the trp repressor binds the operator, and RNA synthesis is blocked.



In the absence of the tryptophan, the repressor dissociates from the operator, and RNA synthesis proceeds.



### Mark allocation:

- ▶ 1 mark is awarded for providing a diagram that shows the repressor protein bound to the operator region in the absence of tryptophan.
- I mark is awarded for providing a diagram that shows the repressor protein changing shape and releasing the operator region in the presence of tryptophan.

**Note:** Annotations must be provided to be awarded each mark.

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**b.** The trp operon is an example of gene regulation.

Explain how the bacterium benefits by being able to regulate the expression of the three genes that code for the production of the enzymes that facilitate the metabolism of tryptophan. (1 mark)

Protein synthesis is an endergonic process. Prevention of the expression of the structural genes in the absence of tryptophan enables the bacterium to conserve energy that can be used for other life-sustaining processes.

### Mark allocation:

- 1 mark is awarded for providing an answer that demonstrates that protein synthesis is a process that uses energy and for explaining an advantage, such as enabling energy conservation.
- c. Outline the role played by structural and regulatory genes in the trp operon system.

Include a reference to the functional distinction between structural and regulatory genes in your answer. (2 marks)

Regulatory genes code for the production of proteins that regulate the expression of other genes. Structural genes code for the production of any protein that plays a structural or functional role in an organism. In the trp operon, regulatory genes code for the production of the repressor protein, which regulates transcription. The three genes (lacZ, lacY and lacA) are structural genes because they code for the production of the enzymes that facilitate the metabolism of tryptophan.

### Mark allocation:

- ▶ 1 mark is awarded for explaining the distinction between structural and regulatory genes.
- ▶ 1 mark is awarded for identifying examples of structural and regulatory genes in the trp operon.

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# <u>Sub-Section</u>: [1.3.5] - Describe the Structure and Function of the trp Operon, Including the Role of the Repressor Protein

Question 62				
De	Definitions:			
a.	trp Operon			
		A group of genes in prokaryotes responsible for synthesising the amino acid tryptophan, regulated based on tryptophan availability.		
b.	Repressor Protein			
		A protein coded by the regulatory gene that binds to the operator to prevent transcription of the operon.		
c.	Corepressor			
		A molecule (e.g., tryptophan) that binds to the repressor protein, enabling it to bind to the operator.		
d.	Operator			
		A DNA sequence where the repressor protein binds to regulate transcription.		
e.	Promoter			
		The site where RNA polymerase binds to initiate transcription of the operon.		



#### Question 63 (1 mark)



In the absence of tryptophan, the trp operon:

- **A.** Is repressed by the repressor protein.
- **B.** Is transcribed because the repressor protein is inactive.
- **C.** Is permanently silenced.
- **D.** Activates the synthesis of the repressor protein.

#### Question 64 (1 mark)



How does tryptophan regulate the trp operon?

- **A.** It acts as a corepressor, enabling the repressor protein to bind the operator and block transcription.
- **B.** It binds to RNA polymerase, preventing it from initiating transcription.
- **C.** It serves as an activator, increasing the transcription of the operon.
- **D.** It enhances the removal of introns from pre-mRNA.

#### Question 65 (1 mark)



What happens to the trp operon when tryptophan levels are high?

- **A.** The operon is silenced because the repressor binds to the operator.
- **B.** The operon is transcribed at a faster rate to compensate.
- **C.** RNA polymerase cannot bind to the promoter.
- **D.** Tryptophan degrades the repressor protein.





#### Question 66 (1 mark)



A mutation prevents the binding of tryptophan to the repressor protein in the trp operon. What would be the consequence?

- **A.** The operon would always be repressed.
- **B.** The operon would be transcribed continuously, even in the presence of tryptophan.
- **C.** RNA polymerase would no longer bind the promoter.
- **D.** Tryptophan synthesis would cease permanently.

#### **Question 67** (1 mark)



Which of the following best explains why the trp operon is an example of negative feedback?

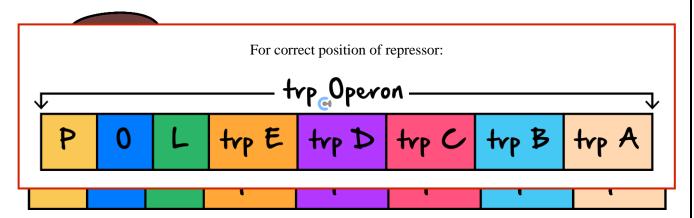
- **A.** Transcription of the operon stops when its product, tryptophan, is abundant.
- **B.** The operon requires a repressor protein to initiate transcription.
- **C.** Transcription occurs only when tryptophan is present.
- **D.** The operon contains genes coding for activator proteins.

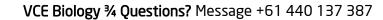
#### **Question 68** (8 marks)



Repression is used by E. coli in order to regulate the levels of tryptophan that are present in the cell.

**a.** In the figure below, draw where a repressor protein would bind when tryptophan levels are high in the bacterial cell. (2 marks)







<b>b.</b> Explain why bacteria regulate the ge (1 mark)	ne expression of enzymes involved in the synthesis of tryptophan.	
synthesis enal	che expression of enzymes involved in tryptophan bles the bacteria to conserve energy/ATP when phan levels in the environment are high.	
c. Explain the role of the trpR gene in t	he regulation of the trp operon. (3 marks)	
As it says solution pending, the answer can be:  The trpR gene codes for the trp repressor protein, which plays a key role in regulating the trp operon.  1. Production of the trp repressor: The trpR gene is transcribed into mRNA and then translated to produce the trp repressor protein, which is inactive on its own.  2. Activation by tryptophan: When tryptophan levels are high, tryptophan molecules bind to the trp repressor. This binding causes a conformational change in the repressor protein, activating it.  3. Repression of the operon: The active repressor, now bound to tryptophan, attaches to the operator region of the trp operon. This prevents RNA polymerase from binding to the promoter and transcribing the genes needed for the synthesis of tryptophan. This ensures that the genes involved in tryptophan production are not expressed when tryptophan is abundant in the cell.  Thus, the trpR gene helps regulate the trp operon by controlling the activity of the repressor protein in response to tryptophan levels.		
<b>d.</b> Andy thinks that once the trp produc	tion has stopped, it cannot be restarted. Is this true? (2 marks)	
•	will dissociate after a while to "check" if trp levels and then a new repressor will respond accordingly.	
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# <u>Sub-Section</u>: [1.3.6] - Describe the Regulation of the trp Operon Through Attenuation in High trp Environments

Question 69		
Definitions:		
a.	Attenuation:	
	A regulatory mechanism where transcription of an operon is prematurely terminated, depending on environmental conditions, such as the availability of tryptophan.	
b.	Leader Sequence:	
	A region in the mRNA that can form different secondary structures (attenuator or anti-terminator loops) based on ribosome activity and tryptophan levels.	
c.	Anti-Terminator Loop:	
	A structure in the leader sequence that prevents transcription termination, allowing the expression of structural genes.	
d.	Attenuator Loop:	
	A structure in the leader sequence that halts transcription when tryptophan levels are high.	
e.	Ribosome Stalling:	
	A process where the ribosome slows or stops at specific codons due to insufficient charged tRNA molecules.	



#### Question 70 (1 mark)



In the trp operon, what determines whether the attenuator loop or anti-terminator loop forms in the leader sequence?

- **A.** The binding of RNA polymerase to the promoter.
- **B.** The availability of tryptophan influencing ribosome stalling.
- **C.** The strength of the repressor protein binding to the operator.
- **D.** The speed at which RNA polymerase transcribes the structural genes.

#### **Question 71** (1 mark)



What happens during attenuation when tryptophan levels are high?

- **A.** The ribosome stalls at the leader sequence, allowing the anti-terminator loop to form.
- **B.** The ribosome moves quickly through the leader sequence, causing the attenuator loop to form.
- **C.** The repressor protein directly blocks RNA polymerase at the promoter.
- **D.** Transcription proceeds without any termination.

#### **Question 72** (1 mark)



Which part of the trp operon is critical for the formation of the attenuator loop?

- **A.** The promoter.
- **B.** The operator.
- C. The leader sequence.
- **D.** The structural genes.





#### Question 73 (1 mark)



A mutation prevents the ribosome from stalling at the leader sequence. How does this affect the regulation of the trp operon?

- **A.** The operon will be transcribed continuously, even at high tryptophan levels.
- **B.** The anti-terminator loop will form, halting transcription prematurely.
- **C.** The attenuator loop will always form, reducing unnecessary gene expression.
- **D.** Tryptophan synthesis will stop permanently.

#### Question 74 (1 mark)



How does attenuation in the trp operon balance resource usage in bacterial cells?

- **A.** By preventing the synthesis of unnecessary enzymes when tryptophan is abundant.
- **B.** By accelerating the production of tryptophan even when it is not needed.
- **C.** By regulating translation instead of transcription.
- **D.** By degrading tryptophan to inhibit feedback loops.





uestion 75 (5 marks)		
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## Section C: [1.4] - Proteins, Protein Export & Enzymes (Checkpoints) (45 Marks)



# <u>Sub-Section</u>: [1.4.1] - Define and Compare Primary, Secondary, Tertiary and Quaternary Structures of Proteins

Question 76			
Definitions:			
a. Primary Structure:			
The unique sequence of amino acids in a polypeptide chain, linked by peptide bonds.			
<b>b.</b> Secondary Structure:			
Local folding of the polypeptide chain into structures such as alpha-helices or beta-pleated sheets, stabilised by hydrogen bonds.			
c. Tertiary Structure:			
The overall 3D shape of a protein, formed by interactions between R-groups, such as hydrogen bonds, ionic bonds, and disulfide bridges.			
d. Quaternary Structure:			
The structure formed when two or more polypeptide chains join to form a functional protein.			
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#### Question 77 (1 mark)



Which level of protein structure is characterised by hydrogen bonding between the backbone atoms?

- A. Primary
- **B.** Secondary
- C. Tertiary
- D. Quaternary

#### Question 78 (1 mark)



A protein consists of two different polypeptide chains. What is the highest level of structure observed?

- A. Primary
- B. Secondary
- C. Tertiary
- **D.** Quaternary

#### Question 79 (1 mark)



Which of the following contributes to the tertiary structure of a protein?

- A. Peptide bonds.
- **B.** Hydrogen bonding between amino acids.
- C. Interactions between R-groups.
- **D.** The sequence of amino acids.





#### Question 80 (1 mark)



A mutation changes a single amino acid in a protein. Which level of structure is directly affected?

- A. Primary
- **B.** Secondary
- C. Tertiary
- D. Quaternary

#### Question 81 (1 mark)



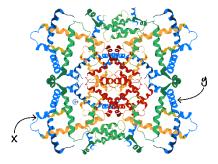
What is the primary difference between the tertiary and quaternary structures of a protein?

- **A.** Tertiary structure involves the arrangement of multiple chains, while quaternary structure involves a single chain.
- **B.** Quaternary structure is the 3D arrangement of a single chain, while tertiary structure involves interactions between multiple chains.
- C. Tertiary structure refers to the 3D arrangement of a single polypeptide, while quaternary structure refers to the arrangement of multiple polypeptides.
- **D.** Both refer to the sequence of amino acids.

#### **Question 82** (5 marks)



The diagram below shows a transmembrane protein, found in the cell membranes of mice. There are two secondary structures labelled as X and Y.



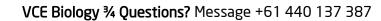
**a.** Give one function of a transmembrane protein. (1 mark)

Worked solution

 $Any\ of\ cell\ recognition,\ signalling,\ transport,\ structural\ support\ or\ enzyme.$ 

Mark allocation: 1 mark

1 mark for any of the functions given above.





	Worked solution  X: Alpha helix Y: Beta pleated sheet	
	<ul> <li>Mark allocation: 2 marks</li> <li>1 mark for identifying the alpha helix.</li> <li>1 mark for identifying the beta pleated sheet.</li> </ul>	
The protein also	o displays a primary structure. Explain how the primary s	tructure would be related to the
The primar	y structure is the linear sequence number and type of ampreptide chain. This determines the way the protein will f	no acids joined together to
The primar form a poly	y structure is the linear sequence number and type of ampreptide chain. This determines the way the protein will f	no acids joined together to





Sub-Section: [1.4.2] - Identify and Describe the Roles of Ribosomes,

Rough Endoplasmic Reticulum and Golgi Apparatus in the Transport and Export of

Proteins from a Cell

Question 83			
Definitions:			
a.	Ribosome:		
	- The site of protein synthesis where mRNA is translated into a polypeptide chain.		
b.	Rough Endoplasmic Reticulum (RER):		
	A network of membrane-bound structures studded with ribosomes, responsible for folding and modifying proteins.		
c.	Golgi Apparatus:		
	An organelle that modifies, sorts, and packages proteins for transport to their destination.		
d.	Vesicles:		
	Small, membrane-bound structures that transport proteins between organelles.		
e.	Exocytosis:		
	The process by which vesicles fuse with the plasma membrane to release proteins outside the cell.		



#### Question 84 (1 mark)



What is the primary function of ribosomes during protein synthesis?

- **A.** To synthesise lipids for cellular membranes.
- **B.** To translate mRNA into a polypeptide chain.
- C. To modify and package proteins.
- **D.** To transport proteins out of the cell.

#### **Question 85** (1 mark)



After a protein is synthesised by a ribosome on the rough endoplasmic reticulum, where does it go next for further modification?

- A. Cytoplasm
- **B.** Nucleus
- C. Golgi apparatus
- **D.** Plasma membrane

#### Question 86 (1 mark)



What role does the Golgi apparatus play in the transport of proteins?

- **A.** Synthesises proteins from mRNA.
- **B.** Packages proteins into vesicles for transport.
- C. Degrades damaged proteins.
- **D.** Facilitates DNA replication.





#### Question 87 (1 mark)



Which process is responsible for releasing proteins from the cell into the extracellular environment?

- A. Endocytosis
- B. Phagocytosis
- C. Exocytosis
- **D.** Pinocytosis

#### Question 88 (1 mark)



A mutation prevents vesicles from fusing with the plasma membrane. How does this affect protein export?

- **A.** Proteins will accumulate in the cytoplasm.
- **B.** Proteins will be degraded by lysosomes.
- **C.** Proteins will be trapped inside the vesicles and not released.
- **D.** Proteins will be exported directly without vesicles.

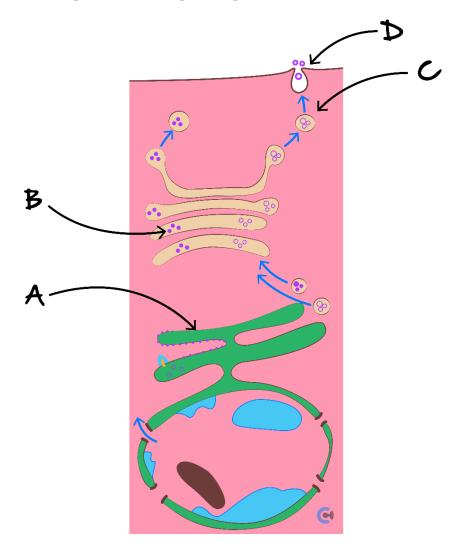




Question 89 (6 marks)



The diagram below shows the production and export of a protein from a cell.



**a.** Name the final **cellular process** shown at point D in the diagram. (1 mark)

Worked solution
 Exocytosis
Mark allocation: 1 mark
 1 mark for exocytosis.
· ·

**b.** Identify the organelles labelled A, B, and C in the diagram. (2 marks)

Worked solution  A: Rough endoplasmic reticulum  B: Golgi apparatus	
C: Vesicle  Mark allocation: 2 marks	
 <ul> <li>2 marks for all three correct terms.</li> <li>1 mark for two correct terms.</li> <li>0 marks for one or no correct terms.</li> </ul>	



c. Describe the process shown in the diagram for exporting a protein from a cell. (3 marks)

#### **Worked solution**

A protein is synthesised at the rough endoplasmic reticulum by translation. After synthesis at the attached ribosome, the protein enters the membrane of the rough endoplasmic reticulum and folds into its secondary and tertiary structure. Vesicles transport the protein to the Golgi apparatus for further packaging and modification. Another vesicle then transports the protein to the cell membrane and fuses with it to release the protein from the cell.

#### Mark allocation: 3 marks

- ▶ 1 mark for referencing rough endoplasmic reticulum, protein synthesis or translation.
- ▶ 1 mark for explaining that the protein is packaged and modified at the Golgi apparatus.
- 1 mark for explaining that the vesicle transports the protein to the cell membrane.

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# <u>Sub-Section</u>: [1.4.3] - Explain the Lock-And-Key Model, the Induced Fit Model and Why Enzymes are Specific and Only Catalyse One Reaction

Qu	Question 90			
Definitions:				
a.	a. Lock-And-Key Model:			
	A theory suggesting that the active site of an enzyme is precisely shaped to fit a specific substrate, like a key fits into a specific lock.			
b.	Induced Fit Model:			
	A theory where the enzyme's active site undergoes a conformational change to better accommodate the substrate.			
c.	Catalyst:			
	A substance that speeds up a chemical reaction without being consumed.			
d.	Substrate:			
	The reactant molecule upon which an enzyme acts.			
e.	Active Site:			
	The region on an enzyme where the substrate binds and the reaction occurs.			



#### Question 91 (1 mark)



Which statement best describes the lock-and-key model of enzyme action?

- **A.** The active site of an enzyme adapts its shape to fit the substrate.
- **B.** The substrate changes shape to fit into the enzyme's active site.
- **C.** The enzyme's active site is perfectly complementary to the substrate.
- **D.** Both the enzyme and substrate undergo conformational changes during binding.

#### Question 92 (1 mark)



How does the induced fit model differ from the lock-and-key model?

- **A.** The active site is rigid in the induced fit model.
- B. The enzyme changes shape to better accommodate the substrate in the induced fit model.
- **C.** The induced fit model does not involve substrate binding.
- **D.** Substrate molecules are altered to fit into the enzyme in the induced fit model.

#### Question 93 (1 mark)



Why are enzymes specific to particular reactions?

- **A.** They are consumed during the reaction, limiting their reuse.
- **B.** Their active site is shaped to only bind specific substrates.
- **C.** They can only function at specific temperatures.
- **D.** They bind to all molecules equally but catalyse reactions selectively.



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#### Question 94 (1 mark)



A scientist observes that an enzyme binds to a substrate, causing a conformational change in the enzyme. Which model of enzyme action does this describe?

- A. Lock-and-key model
- B. Induced fit model
- C. Competitive inhibition
- D. Allosteric regulation

#### Question 95 (1 mark)



How does the specificity of an enzyme ensure the efficiency of a biochemical reaction?

- **A.** By reducing the need for cofactors.
- **B.** By lowering the activation energy specifically for one type of substrate.
- **C.** By denaturing at high temperatures.
- **D.** By altering the equilibrium of the reaction.

#### Question 96 (3 marks)



The equation below describes a reaction that occurs in the cells of plants. The enzyme amylase breaks down starch into maltose.

$$\begin{array}{ccc} (C_6H_{10}O_5)n \ \rightarrow \ nC_{12}H_{22}O_{11} \\ starch \rightarrow maltose \end{array}$$

**a.** What is the substrate in the reaction? (1 mark)

#### Worked solution

Starch (or  $(C_6H_{10}O_5)n$ )

Mark allocation: 1 mark

- 1 mark for correctly recognising the substrate, starch.
- **b.** Can the enzyme amylase catalyse other reactions in plant cells? Explain your answer. (2 marks)

No. Enzymes only catalyse one type of reaction because their specifically shaped active sites can only bond to specific substrates.

Mark allocation: 2 marks

- ▶ 1 mark for referencing the specific shape of the active site.
- 1 mark for explaining that only specific substrates are complementary to active sites.





# <u>Sub-Section</u>: [1.4.4] - Explain How Enzymes Change/Denature in Different pH and at Different Temperatures

Qı	Question 97		
Definitions:			
a.	• Denaturation:		
	The process by which an enzyme loses its functional shape due to the disruption of bonds, often caused by extreme pH or high temperatures.		
b.	Optimal pH:		
	The specific pH range where an enzyme exhibits maximum activity.		
c.	Optimal Temperature:		
	The temperature range where an enzyme is most active and catalyses reactions efficiently.		
d.	Thermal Denaturation:		
	The loss of enzymatic function due to high heat breaking bonds in the protein structure.		
e.	pH Sensitivity:		
	The effect of acidic or basic conditions on the charge and structure of an enzyme's active site.		



#### **Question 98** (1 mark)



The enzyme amylase breaks down starch into maltose. Two test tubes were prepared with identical amounts of starch and amylase. Test tube one was incubated at 37°C, while test tube two was incubated at 5°C. After 15 minutes, the amount of maltose in test tube two compared to test tube one would be:

- **A.** Higher as the enzyme is more active at lower temperatures.
- **B.** Equal since the amount of starch and enzyme was identical.
- **C.** Lower as fewer collisions occur between the enzyme and substrate at lower temperatures.
- **D.** Lower because the enzyme denatures at 5°C.

#### Question 99 (1 mark)



Pepsin functions optimally at pH 2. Two samples containing the same concentration of pepsin and protein substrate were prepared. Sample one was adjusted to pH 7, and sample two to pH 2. After 10 minutes, the rate of reaction in sample one compared to sample two would be:

- **A.** Higher because the enzyme functions more effectively at neutral pH.
- **B.** Lower because the enzyme's active site would be altered at pH 7.
- C. Equal as pepsin can function across a wide pH range.
- **D.** Lower because the protein substrate is degraded at neutral pH.

#### Question 100 (1 mark)



The enzyme catalase breaks down hydrogen peroxide into water and oxygen. A catalase solution is heated to 70°C before the reaction is started. Compared to a reaction at 30°C, the amount of oxygen produced would be:

- **A.** Higher because the reaction rate increases with temperature.
- **B.** Equal since catalase is unaffected by high temperatures.
- **C.** Lower because the enzyme denatures at high temperatures.
- **D.** Lower because hydrogen peroxide degrades at higher temperatures.





#### Question 101 (1 mark)



A student investigates the effect of pH on enzyme activity by measuring the rate of a reaction at pH 4, pH 7, and pH 10. The enzyme shows peak activity at pH 7. What would happen to the reaction rate at pH 10?

- **A.** It would increase as the substrate concentration increases.
- **B.** It would decrease because the enzyme's structure is altered at pH 10.
- C. It would remain constant since the enzyme can adapt to pH changes.
- **D.** It would stop completely because pH 10 causes the substrate to degrade.

#### Question 102 (1 mark)



The enzyme lipase is used to break down fats. Two test tubes were prepared with equal amounts of lipase and a fat substrate. Test tube one was kept at 37°C, and test tube two was kept at 50°C. After 20 minutes, the reaction in test tube two compared to test tube one would be:

- **A.** Faster because higher temperatures speed up enzymatic reactions.
- **B.** Slower because the enzyme would denature at 50°C.
- **C.** Equal because the same amount of substrate and enzyme was used.
- **D.** Faster because the substrate binds more efficiently at higher temperatures.



#### Mark allocation: 2 marks

1 mark for a gradual slope increasing up to optimum temperature.

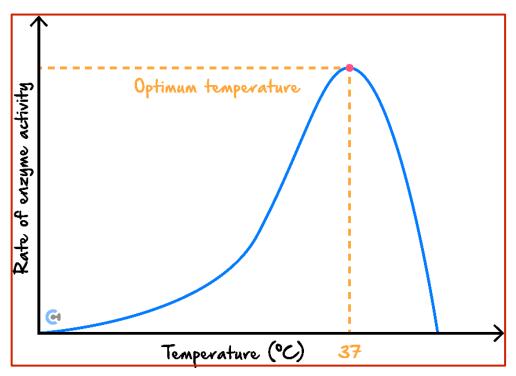
1 mark for steep decline above 40°C.



**Question 103** (6 marks)

a. The average human body temperature is 37°C, which is the optimum temperature for catalase.

Complete the graph below to show the rate of catalase-controlled reactions at temperatures above and below 37°C. (2 marks)



**b.** Explain what happens to the enzyme if the body temperature is over 40°C for an extended period. (2 marks)

#### Worked solution

The enzyme becomes denatured because the heat energy causes bonds in the tertiary level of the protein structure to break. This causes the active sites of the enzymes to lose their shape and they then cannot bind to the substrate.

#### Mark allocation: 2 marks

- ▶ 1 mark for recognising that the enzyme denatures due to heat energy.
- ▶ 1 mark for stating that the active site shape changes and is unable to bind to the substrate.
- c. State the likely optimal pH for this enzyme, and describe how pH can impact enzyme function. (2 marks)

The likely optimal pH for catalase is around pH 7, as it functions best in neutral conditions. The pH impacts enzyme function by affecting the ionic bonds in the enzyme's structure, which can alter the shape of the active site and lead to reduced activity or denaturation at extreme pH levels.



# Sub-Section: [1.4.5] - Explain the Function of Competitive and Non-Competitive Enzyme Inhibitors and How They Affect the Rate of Reaction, and How They May/May Not Be Overcome

Qu	estion 104		
De	finitions:		
a.	Competitive Inhibitor:		
	A molecule that binds to the active site of an enzyme, preventing the substrate from binding. Its effect can often be overcome by increasing substrate concentration.		
b.	Non-Competitive Inhibitor:		
	A molecule that binds to an enzyme at a site other than the active site, altering the enzyme's structure and function. This inhibition cannot be overcome by increasing substrate concentration.		
c.	Allosteric Site:		
	The specific site on an enzyme where a non-competitive inhibitor binds, causing conformational changes.		
d.	Inhibition:		
	The process by which an enzyme's activity is reduced or halted.		
e.	Rate of Reaction:		
	The speed at which a reaction proceeds, influenced by factors such as enzyme concentration, inhibitors, and substrate availability.		



#### Question 105 (1 mark)



A molecule binds to the active site of the enzyme carbonic anhydrase, competing with its substrate carbon dioxide. What type of inhibition is this?

- A. Non-competitive inhibition
- **B.** Allosteric inhibition
- C. Competitive inhibition
- **D.** Irreversible inhibition

#### Question 106 (1 mark)



A non-competitive inhibitor binds to an enzyme at an allosteric site. How does this affect the enzyme's activity?

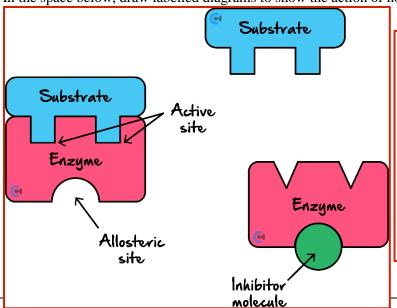
- **A.** It permanently blocks the active site.
- **B.** It alters the enzyme's shape, reducing its ability to bind to the substrate.
- **C.** It increases substrate binding to speed up the reaction.
- **D.** It prevents the substrate from binding to the allosteric site.

#### **Question 107** (3 marks)



Catalase activity in humans can be affected by toxins that act as non-competitive inhibitors.

In the space below, draw labelled diagrams to show the action of non-competitive inhibitors on catalase.



#### Mark allocation: 3 marks

- 1 mark for correct labels of enzyme, substrate, and inhibitor.
- 1 mark for enzyme and substrate binding or showing enzyme and substrate having complementary shapes.
- I mark for showing the changing shape of the active site and the substrate being unable to bind.



## <u>Section D:</u> [1.5] - Introduction to DNA Manipulation Techniques (Checkpoints) (46 Marks)



## <u>Sub-Section</u>: [1.5.1] - Identify and Describe the Function of Polymerases, Endonucleases, and Ligases in DNA Manipulation

Question 108					
Definitions:					
a.	Endonuclease:				
	An enzyme that cuts DNA at specific recognition sites, producing fragments with sticky or blunt ends.				
b.	Recognition Site:				
	A specific sequence of DNA where an endonuclease binds and cuts.				
c.	Sticky End:				
	DNA fragments with overhanging ends that can form base pairs with complementary sequences.				
d.	Blunt End:				
	DNA fragments with no overhangs, where both strands are of equal length.				
e.	Polymerase:				
	An enzyme that synthesises a new DNA strand complementary to the template strand by adding nucleotides.				
_					



f. Ligase:

An enzyme that joins DNA fragments by forming phosphodiester bonds between the sugar-phosphate backbones.

#### Question 109 (1 mark)



A scientist aims to insert a gene of interest into a plasmid. Which combination of enzymes would they use?

- **A.** Helicase and polymerase
- B. Endonuclease and ligase
- C. Polymerase and ligase
- D. Endonuclease and helicase

#### Question 110 (1 mark)



During DNA repair, which enzyme seals the gap between adjacent nucleotides?

- A. Polymerase
- **B.** Ligase
- C. Endonuclease
- **D.** Restriction enzyme

#### Question 111 (1 mark)



A student observes that after cutting a DNA sample, the fragments have sticky ends. Which enzyme likely produced these fragments?

- A. DNA ligase
- **B.** DNA polymerase
- C. Endonuclease
- D. Helicase



#### Question 112 (1 mark)



During PCR, which enzyme is responsible for synthesising a new DNA strand?

- A. Restriction enzyme
- **B.** TAQ polymerase
- C. DNA ligase
- **D.** Topoisomerase

#### Question 113 (1 mark)



If an endonuclease cuts DNA into fragments but a ligase enzyme fails to function, what is the result?

- **A.** DNA fragments remain unattached.
- **B.** The DNA forms sticky ends.
- C. DNA fragments rejoin naturally.
- **D.** DNA replicates incorrectly.

#### **Question 114** (2 marks)



Explain why sticky ends are preferred over blunt ends when cutting DNA from manipulation using an endonuclease.

They form complementary overhangs, which allow for specificity when joining the fragments together, via hydrogen bonding.







# <u>Sub-Section</u>: [1.5.2] - Identify the Ingredients Required, Describe the Process, and Recall Key Applications of PCR

Question 115						
Definitions:						
a.	. Primer:					
<b>b.</b>	A short sequence of nucleotides that provides a starting point for DNA synthesis during PCR.  PCR (Polymerase Chain Reaction):					
	A method used to amplify specific sections of DNA.					
c.	Denaturation:					
	The process of heating DNA to separate its two strands.					
d.	Annealing:					
	The binding of primers to complementary DNA sequences at a lower temperature.					
e.	Elongation:					
	The synthesis of a new DNA strand by TAQ polymerase at an optimal temperature, usually 72°C.					



#### Question 116 (1 mark)



A scientist observes that a temperature of 95°C is used during the PCR process. What is the purpose of this step?

- **A.** To denature the primers.
- **B.** To separate the double-stranded DNA into single strands.
- **C.** To enable primers to bind to the DNA template.
- **D.** To activate DNA ligase.

#### Question 117 (1 mark)



During PCR, the purpose of primers is to:

- **A.** Move along the DNA strand and add complementary nucleotides.
- **B.** Provide a starting point for TAQ polymerase to synthesise DNA.
- C. Act as 'glue' to join complementary nucleotides together.
- **D.** Separate the DNA strands for synthesis.



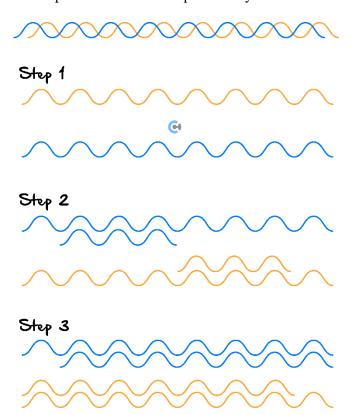


Question 118 (1 mark)



Polymerase chain reaction (PCR) has been used to primarily 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 the step: Question 32

- **A.** 1 only.
- **B.** 2 only.
- **C.** 3 only.
- **D.** 2 and 3 only.

A PCR cycle is repeated many times to generate copies of target sections of DNA. A temperature of about 90°C is involved in step 1 which breaks the hydrogen bonds holding the original DNA strand together. The sample is then cooled to about 50°C where primers are then able to anneal (stick) onto complementary sections of each DNA strand, as shown in step 2. Finally, in step 3, the sample is warmed to about 70°C and the taq polymerase enzyme works to replicate each strand using the primer as an anchor for the enzyme. This completes one cycle and it then begins again.



#### Question 119 (1 mark)



During a PCR experiment, a researcher finds that no DNA amplification occurs even though the correct primers were used. What is the most likely cause of the failure?

- **A.** The annealing temperature was set within the primer's melting temperature range.
- **B.** Taq polymerase was missing or inactive during the reaction.
- C. The elongation temperature was set to 72°C.
- **D.** The reaction included too much template DNA, preventing amplification.

#### Question 120 (1 mark)



A researcher is using PCR to amplify a segment of DNA. After completing the reaction, the product is found to include unintended DNA fragments. What is the most likely reason for this?

- **A.** The primers were non-specific and annealed to multiple regions of the DNA template.
- **B.** The annealing temperature was set too high, preventing proper primer binding.
- **C.** Taq polymerase degraded during the elongation step.
- **D.** The denaturation temperature was too low, leading to incomplete strand separation.

#### Question 121 (6 marks)



Scientists investigating a transgenic strain of the *Arabidopsis* plant called Kojak carried out a gel electrophoresis to find the root hair gene that had been transferred into the Kojak strain from a species of barley.

The root hair gene was removed along with some other DNA using restriction enzymes and underwent PCR prior to the gel electrophoresis being run.

a. Why was PCR performed on the DNA sample prior to the gel electrophoresis being carried out? (1 mark)

 PCR is performed to amplify the DNA segment so that there is enough to be visible in the gel electrophoresis.	
Mark allocation: 1 mark	
<ul> <li>1 mark for recognising that PCR is performed to increase the amount of DNA in the sample</li> </ul>	

Worked solution



	Worked solution	
	1. Heat the DNA to 90 °C	
	2. Cool to attach primers	
	Taq polymerase copies DNA strands	
	Mark allocation: 3 marks	
	1 mark for identifying that the DNA must be heated to 90 °C	
	<ul> <li>1 mark for stating 'cool to attach primers' or equivalent</li> <li>1 mark for stating 'Taq polymerase copies DNA strands' or equivalent</li> </ul>	
Describe	the role of primers in PCR. (2 marks)	
	➤ Primers will bind to the 3'end of each DNA strand allowing TAQ polymerase to	
	hand and rankageta the LMA atrenda	
	<ul> <li>bind and replicate the DNA strands.</li> <li>There are two different primers which will bind to each strand, allowing for a specific sequence to be transcribed.</li> </ul>	



Sub-Section: [1.5.3] - Describe the Process of Gel Electrophoresis, and Describe How it May be Used to Differentiate DNA Samples or to Obtain a "DNA Profile"

Question 122					
De	efinitions:				
a.	Gel Electrophoresis:				
	A method used to separate DNA fragments based on their size by applying an electric current to a gel matrix.				
b.	Well:				
	A small indentation in the gel where DNA samples are loaded.				
c.	Standard Ladder:  A set of DNA fragments of known sizes used as a reference to estimate the size of unknown fragments.				
d.	Buffer:				
	A solution that maintains pH and provides ions to carry the current during electrophoresis.				
e.	Electrode:				
	A conductor that transfers electric current into the gel to drive DNA fragment movement.				



f. Band:

A visible line of DNA fragments in the gel, representing fragments of a specific size.

#### Question 123 (1 mark)



In a gel electrophoresis experiment, DNA samples are loaded into wells at one end of the gel. Why do DNA fragments move towards the positive electrode?

- **A.** DNA fragments are positively charged and are attracted to the positive electrode.
- **B.** DNA fragments are negatively charged due to their phosphate backbone and are attracted to the positive electrode.
- C. DNA fragments have no charge and are moved by diffusion.
- **D.** DNA fragments move randomly in the gel due to electrical currents.

#### Question 124 (1 mark)



During a gel electrophoresis experiment, a researcher observes that larger DNA fragments remain closer to the wells, while smaller fragments migrate further. What is the reason for this difference?

- A. Larger fragments have a higher charge-to-mass ratio, making them move slower.
- **B.** Smaller fragments can move through the gel matrix more easily, encountering less resistance.
- **C.** Larger fragments are repelled by the negative electrode.
- **D.** Smaller fragments degrade faster, making them travel further.





#### Question 125 (1 mark)



A DNA sample contains fragments of 300 bp, 600 bp, and 1000 bp. After running gel electrophoresis, which fragment would appear closest to the positive terminal?

- **A.** 1000 bp
- **B.** 600 bp
- C. 300 bp
- **D.** All fragments would travel the same distance.

#### Question 126 (1 mark)



A researcher uses a standard ladder during gel electrophoresis. What is the purpose of the standard ladder?

- **A.** To identify the exact sequence of DNA fragments.
- **B.** To serve as a positive control for the experiment.
- C. To estimate the sizes of DNA fragments in the sample by comparing their positions to the ladder.
- **D.** To prevent the DNA fragments from degrading during electrophoresis.

#### Question 127 (1 mark)



During gel electrophoresis, a band appears faint or is absent in the results. What is the most likely explanation?

- **A.** The DNA fragments degraded completely during the process.
- **B.** An insufficient amount of DNA was loaded into the well.
- **C.** The buffer solution did not contain enough ions to carry the current.
- **D.** The DNA fragments were too large to enter the gel.

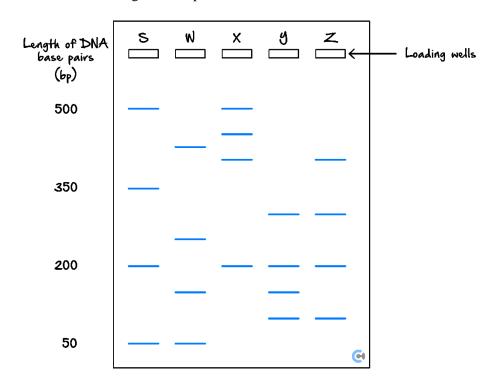




Question 128 (1 mark)



Four samples of DNA were loaded into four different wells in lanes W, X, Y and Z. A standard ladder was loaded into the well in lane S. The results of gel electrophoresis are shown below.



Which lane represents a sample that was loaded with DNA fragments of four different lengths: 100 bp, 150 bp, 200 bp and 300 bp?

**A.** *W* 

**B.** *X* 

**C.** *Y* 

**D.** *Z* 





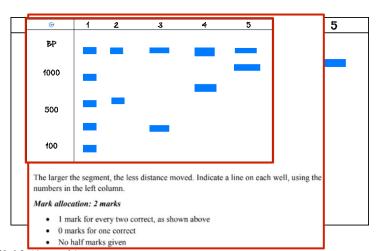
#### Question 129 (4 marks)



The table below gives the sizes of the various DNA fragments (genes) used in the genetic engineering of the *Arabidopsis* plant.

Gene	Size (base pairs)	Well (gel electrophoresis)
Normal Arabidopsis root hair gene	520	2
Mutant Kojak Arabidopsis gene	450	3
Barley root hair gene	600	4
Recombined mutant <i>Arabidopsis</i> + barley genes	1050	5

**a.** On the diagram below, indicate where each of the DNA fragments would be positioned after the gel electrophoresis has been run. (2 marks)



**b.** What is placed in well 1? (1 mark)

Worked solution

Standard DNA fragments of known lengths

Mark allocation: 1 mark

- 1 mark for identifying a standard DNA fragments of known lengths
- **c.** What is the purpose of the standard ladders? (1 mark)

To provide a point of reference by which unknown fragments can be compared with known ones.





# <u>Sub-Section</u>: [1.5.4] - Explain the Factors That Affect the Movement of Fragments in Gel Electrophoresis

Qu	estion 130		
De	Definitions:		
a.	Electric Currer	ıt:	
	Drive	es the movement of DNA fragments through the gel towards the positive electrode.	
b.	Gel Concentrat	ion:	
	Determines the	ne resolution of fragment separation; higher concentrations are used for smaller fragments.	
c.	Fragment Size		
	·	Smaller fragments move faster and travel further through the gel matrix.	
d.	Buffer:		
		Maintains pH and ionic conditions necessary for DNA movement.	
e.	Loading Dye:		
		Added to DNA samples for visualisation and tracking during electrophoresis.	



#### Question 131 (1 mark)



A DNA sample is loaded into a gel with a high agarose concentration. How would this affect the movement of DNA fragments compared to a gel with a lower agarose concentration?

- **A.** All fragments would move faster.
- **B.** Larger fragments would move further, while smaller fragments would remain closer to the wells.
- C. Smaller fragments would move more slowly, while larger fragments would not enter the gel.
- **D.** Smaller fragments would separate more distinctly from larger fragments.

#### Question 132 (1 mark)



A researcher runs gel electrophoresis using a buffer with insufficient ions. What would likely happen?

- **A.** DNA fragments would move faster due to reduced resistance.
- **B.** The DNA fragments would degrade due to ion imbalance.
- C. The electric current would not pass effectively through the gel, preventing DNA movement.
- **D.** The gel would overheat, melting the DNA fragments.

#### **Question 133** (1 mark)



In a gel electrophoresis experiment, why do smaller DNA fragments travel further through the gel?

- **A.** They are less dense and lighter than larger fragments.
- **B.** They experience less resistance within the gel matrix.
- **C.** They have a stronger negative charge than larger fragments.
- **D.** They are degraded into smaller pieces during electrophoresis.





#### Question 134 (1 mark)



A student loads a DNA sample into the gel but forgets to add the loading dye. What would likely happen?

- **A.** DNA fragments would degrade before reaching the positive terminal.
- **B.** DNA movement through the gel would be slower.
- **C.** DNA fragments would still separate, but their positions would be harder to track.
- **D.** DNA fragments would not separate due to the absence of the dye.

#### Question 135 (1 mark)



What happens if gel electrophoresis is run for too long?

- **A.** DNA fragments continue to separate more clearly.
- **B.** All DNA fragments migrate out of the gel into the buffer solution.
- C. DNA fragments denature due to prolonged exposure to current.
- **D.** Smaller fragments move closer to the wells as larger fragments exit the gel.

#### **Question 136** (4 marks)



List and explain the factors that affect the movement of fragments in a gel electrophoresis experiment.

Suitable responses for factors affecting the migration of DNA fragments through the agarose gel during gel electrophoresis included three of the following:

- · the size of the molecules, as the larger molecule will move more slowly
- the charge of the molecule, as the negative charge means that DNA moves towards the positive electrode
- the length of time the voltage is applied, as there may not be enough time for the DNA to migrate through the gel
- the concentration of the agarose, as denser agarose results in the molecules moving more slowly.





<u>Sub-Section</u>: [1.5.5] - Define Satellite DNA and STRs, and Explain Their Use in Identifying People Through DNA Profiling for Crimes and Paternity Testing

Question 137	1
Definitions:	
a. Satellite DNA:	
Repeated sequences of non-coding DNA that vary greatly between individuals and are used in DNA profiling	
<b>b.</b> Short Tandem Repeats (STRs):	
Short sequences of DNA, typically 2-6 base pairs long, repeated multiple times in a row at specific locations in the genome.	
c. DNA Profiling:	
A technique used to distinguish between individuals based on their unique DNA sequences, especially STR patterns.	
d. Genetic Fingerprinting:	
Another term for DNA profiling, often used in forensic science to link biological samples to individuals.	
e. Allele:	
A variant of a specific gene or DNA marker, including STR repeats at a given locus.	



#### Question 138 (1 mark)



Which of the following best describes the function of STRs in DNA profiling?

- **A.** STRs encode proteins essential for cell function.
- **B.** STRs are variable regions of DNA that are compared between individuals to create a genetic fingerprint.
- **C.** STRs are sections of DNA removed during transcription.
- **D.** STRs are unique sequences only found in certain populations.

#### Question 139 (1 mark)



A forensic scientist analyses DNA from a crime scene and matches it to a suspect using STR profiles. Which factor makes STRs ideal for this purpose?

- **A.** STRs are located in the coding regions of genes.
- **B.** STR patterns are identical among all family members.
- C. STRs are highly variable and unique to individuals.
- **D.** STRs degrade faster than other DNA regions, making them easy to sequence.

#### Question 140 (1 mark)



Why is satellite DNA, such as STRs, preferred over coding regions of DNA for forensic identification?

- **A.** Coding regions are too small for reliable comparison.
- **B.** Satellite DNA is non-coding and shows greater variation between individuals.
- **C.** Satellite DNA is present only in blood samples.
- **D.** Coding regions are unstable during PCR amplification.

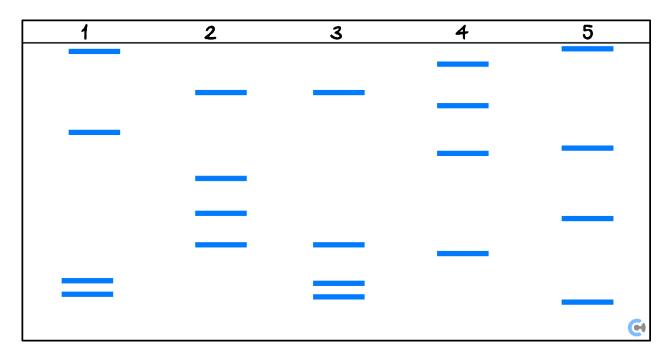




#### Question 141 (1 mark)



A woman with a child marries a man. The couple then have a child of their own, after which they adopt a third child. Genetic fingerprinting was carried out and the results are shown below. Lane 1 contains the woman's DNA; lane 2 contains the man's DNA; and lanes 3, 4 and 5 contain the children's DNA.



Use the information provided to identify each of the children as being the woman's child, the couple's child or the adopted child.

	Lane 3	Lane 4	Lane 5
A.	Woman's child	Couple's child	Adopted child
В.	Adopted child	Couple's child	Woman's child
C.	Couple's child	Woman's child	Adopted child
D.	Couple's child	Adopted child	Woman's child

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uestion 142 (3 marks)	))
escribe how STRs can be used to differentiate between criminals and their DNA samples.	
Everyone has different STRs in their satellite DNA (non-coding regions)	
Cleaving these with the same restriction enzymes will mean that fragments of different sizes are created	i
Running that through gel electrophoresis will therefore create different band patterns that can be used to	)
distinguish between suspects.	



### Section E: [1.6] - CRISPR-Cas9 & Bioethics (Checkpoints) (55 Marks)



### <u>Sub-Section</u>: [1.6.1] - Describe the Function and the Process of CRISPR-Cas9 as an Adaptive Defense Against Viruses in Bacteria

Qu	Question 143		
De	Definitions.		
a.	a. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats):		
	DNA sequences in bacterial genomes that serve as a memory bank of past viral infections.		
b.	Cas Proteins:		
	Enzymes that cut and degrade viral DNA, guided by CRISPR-derived RNA.		
c.	Spacer DNA:		
	Viral DNA fragments incorporated into CRISPR loci, used to recognise and combat future infections.		
d.	Guide RNA (gRNA):		
	RNA that directs Cas9 to complementary viral DNA for precise cutting.		
e.	Adaptive Immunity:		
	A bacterial defense mechanism where CRISPR-Cas9 identifies and destroys viral DNA based on prior encounters.		



#### Question 144 (1 mark)



What is the primary role of CRISPR-Cas9 in bacteria?

- **A.** To synthesise proteins for cell division.
- **B.** To defend against viral infections by targeting their DNA.
- C. To facilitate bacterial conjugation for genetic diversity.
- **D.** To promote mutations for adaptation.

#### Question 145 (1 mark)



How does CRISPR-Cas9 identify specific viral DNA to cut?

- **A.** It binds randomly to any DNA in the cell.
- B. It uses guide RNA transcribed from CRISPR arrays to locate complementary viral DNA.
- **C.** It relies on physical interaction with viral proteins.
- **D.** It produces enzymes that degrade all DNA in the cell.

#### Question 146 (1 mark)



What happens when Cas9 encounters viral DNA that matches its guide RNA?

- **A.** Cas9 deactivates, allowing the viral DNA to replicate.
- **B.** Cas9 cleaves the viral DNA, rendering it non-functional.
- C. Cas9 incorporates the viral DNA into the host genome for immunity.
- **D.** Cas9 triggers the production of additional guide RNAs.





#### Question 147 (1 mark)



Why is spacer DNA important in the CRISPR-Cas9 system?

- A. It prevents Cas9 from cutting bacterial DNA.
- **B.** It encodes the proteins required for bacterial immunity.
- C. It serves as a molecular memory of past viral infections.
- **D.** It stabilises guide RNA for Cas9 activity.

#### **Question 148** (1 mark)



How does the CRISPR-Cas9 system enhance bacterial survival against viruses?

- **A.** By synthesising proteins that inactivate viruses.
- **B.** By degrading viral DNA through targeted cleavage.
- **C.** By using RNA to bind and neutralise viral proteins.
- **D.** By generating random mutations in viral DNA.

#### Question 149 (8 marks)



A research team has been investigating a strain of bacteria, *E. coli*, which has been exposed to a virus. They have observed that the bacteria are able to resist future infections by the same virus. The research team believes that CRISPR-Cas9 is involved in this process.

**a.** A student states that Cas9 is involved in incorporating a proto-spacer into the CRISPR array in bacteria. Explain where the student has gone wrong, and correctly describe the function of the Cas9 enzyme in this process. (2 marks)

The student has gone wrong by suggesting that Cas9 is involved in incorporating the proto-spacer into the CRISPR array. The correct process involves Cas1 and Cas2 enzymes, which are responsible for cutting and incorporating the proto-spacer into the CRISPR array. Cas9 functions to cleave viral DNA during re-exposure.



b.	Based on the scenario above, describe how CRISPR-Cas9 functions as a defense system in <i>E. coli</i> against viral infections. (3 marks)
	Upon infection, viral DNA is injected into the <i>E. coli</i> by a bacteriophage, and Cas1 and Cas2 enzymes cleave a section of the viral DNA, incorporating it into the bacterium's CRISPR array.  The CRISPR array is transcribed into gRNA, which combines with Cas9 enzyme to form the CRISPR-Cas9 complex. Upon re-infection, the gRNA guides Cas9 to cleave the viral DNA, eventually inducing a mutation that inactivates the virus.
2.	A new experiment shows that when the bacteria were re-infected with the virus, they were unable to resist the infection. The research team suspects a mutation in the <i>E. coli</i> CRISPR array. How could the loss of a functional CRISPR-Cas9 system lead to the bacteria's inability to defend itself? (2 marks)
	If the CRISPR-Cas9 system is mutated or lost, the bacteria would be unable to incorporate new spacers into the CRISPR array. Without the ability to recognise and target viral DNA during re-exposure, the bacteria would be unable to defend itself against subsequent infections by the same virus.
l.	Given that the CRISPR array contains several spacers from previous viral infections, how might the diversity of spacers within the array affect the bacteria's? (1 mark)
	The diversity of spacers within the CRISPR array would allow the bacteria to target a broader range of viruses by recognising different viral DNA sequences. For example, if the <i>E. coli</i> had spacers from multiple viruses, it could potentially resist infections from a variety of bacteriophages.
Spa	ace for Personal Notes





# <u>Sub-Section</u>: [1.6.2] - Explain the Process of Using CRISPR-Cas9 as a Gene Editing Tool, Including Silencing, Knock-Ins & Knock-Outs

Qu	Question 150		
De	Definitions.		
a.	Gene Editing	;:	
		A method to precisely alter DNA sequences to add, delete, or modify genes.	
b.	Knock-In M	utation:	
		A technique where specific DNA sequences are inserted into the genome.	
c.	Knock-Out M	Autation:	
	A gene	e modification where the target gene is disrupted or deleted, rendering it non-functional.	
d.	Guide RNA (	gRNA):	
		A synthetic RNA molecule that directs Cas9 to the target DNA sequence.	
e.	Cas9 Protein	:	
		An enzyme that introduces double-stranded breaks at specific DNA sequences.	





#### Question 151 (1 mark)



In a CRISPR-Cas9 experiment, a researcher aims to disrupt the function of a target gene. Which component is necessary to achieve this?

- A. A donor DNA template for homology-directed repair.
- **B.** A guide RNA to direct Cas9 to the target gene.
- C. A reverse transcriptase enzyme to copy RNA into DNA.
- **D.** An RNA polymerase enzyme to synthesise guide RNA.

#### Question 152 (1 mark)



Researchers use CRISPR-Cas9 to modify a gene associated with disease. After the experiment, they observe that no protein is produced from the gene. Which CRISPR technique was most likely used?

- A. Gene silencing
- B. Gene knock-in
- C. Gene knock-out
- D. Gene duplication

#### Question 153 (1 mark)



A scientist designs a gRNA and provides a donor DNA template for an experiment using CRISPR-Cas9. What is the expected outcome?

- A. Random mutations in the genome.
- **B.** Silencing of the target gene.
- C. Precise insertion of the donor DNA into the target site.
- **D.** Deletion of the target gene.





Question 154 (1 mark)



How does CRISPR-Cas9 facilitate gene knock-ins?

- A. By silencing genes through blocking transcription.
- **B.** By cutting DNA and inserting new genetic material during repair.
- **C.** By using guide RNA to degrade messenger RNA before translation.
- **D.** By introducing random mutations to alter gene expression.

#### Question 155 (2 marks)



Researchers used CRISPR-Cas9 to modify the PIGM1 gene, which regulates pigmentation in mice. They designed a guide RNA targeting the gene's coding region but did not provide a donor DNA template for repair. After the experiment, the mice showed no detectable PIGM1 protein and their fur colour was significantly lighter compared to controls.

Which CRISPR-Cas9 technique was likely used in this experiment? Justify.



Knock out.

The PIGM1 protein is no longer detected, and it has not been replaced with a different allele.





## Sub-Section: [1.6.3] - Describe & Compare the Function of the PAM Sequence in Bacteria & Gene Editing Applications of CRISPR-Cas9 Technology

Question 156		
De	finitions	
a.	PAM Sequence (Protospacer Adjacent Motif):	
	A short DNA sequence required for Cas9 to bind and cut target DNA.	
b.	Self-DNA Protection:	
	In bacteria, the absence of a PAM sequence prevents Cas9 from cutting the host DNA.	
c.	Gene Editing Application:	
	The PAM sequence determines which DNA regions Cas9 can target for editing.	
d.	Sequence Specificity:	
	PAM ensures high precision in CRISPR-Cas9 targeting during both bacterial immunity and gene editing.	
e.	Cas9 Binding:	
	Cas9 must identify a PAM sequence near the target DNA for successful activity.	
	<u> </u>	



#### Question 157 (1 mark)



What is the role of the PAM sequence in the natural bacterial CRISPR-Cas system?

- **A.** It prevents Cas9 from cutting host DNA by recognising self-DNA.
- **B.** It enhances the transcription of CRISPR arrays.
- C. It triggers the synthesis of guide RNA (gRNA) for Cas9.
- **D.** It recruits repair enzymes to fix DNA cuts.

#### Question 158 (1 mark)



Why is the PAM sequence critical in CRISPR-Cas9 gene editing?

- **A.** It eliminates the need for guide RNA (gRNA).
- **B.** It ensures that Cas9 targets specific *DNA* sequences by requiring a PAM near the target site.
- C. It allows Cas9 to cut RNA sequences.
- **D.** It increases the versatility of Cas9 by binding any DNA sequence.

#### Question 159 (1 mark)



In bacteria, what is the natural role of the PAM sequence?

- **A.** To stabilise the bacterial genome.
- **B.** To differentiate viral DNA from bacterial DNA.
- **C.** To inhibit the expression of viral genes.
- **D.** To allow viral DNA to integrate into the bacterial genome.





Question 160 (1 mark)



How does the presence of a PAM sequence influence CRISPR - Cas9 targeting in gene editing?

- **A.** It enables Cas9 to bind and cut any *DNA* sequence.
- **B.** It restricts Cas9 activity to sequences adjacent to a specific *PAM* motif.
- C. It prevents off-target cuts by enhancing gRNA specificity.
- **D.** It deactivates Cas9 after the cut is made.

#### Question 161 (3 marks)



a. Compare and contrast the role of the PAM sequence in both bacteria and gene editing. (2 marks)

The PAM (Protospacer Adjacent Motif) sequence is essential in the bacterial CRISPR-Cas system to distinguish foreign DNA from self-DNA. Cas9 requires the PAM sequence adjacent to the target DNA for binding and cleavage, ensuring that bacterial DNA, which lacks a PAM near stored spacer sequences are not targeted. In gene-editing applications, the PAM sequence determines where Cas9 can bind and cut DNA, limiting target sites to those with a PAM nearby. This ensures specificity but also constrains the flexibility of targetable DNA sequences.

**b.** Other than preventing self-harm to the bacteria, what role does the PAM sequence play naturally? (1 mark)

Efficiency-Cas9 searches for PAM sequences.







# Sub-Section: [1.6.4] - Describe the Function and Compare Guide RNA (gRNA) and Single Guide RNA (sgRNA)

Question 162		
Definitions		
a. Guide RNA (gRNA):		
A molecule consisting of two parts, crRNA (CRISPR RNA) and tracrRNA (trans-activating CRISPR RNA), that directs Cas9 to the target DNA sequence.  b. Single Guide RNA (sgRNA):		
A synthetic version of gRNA, combining crRNA and tracrRNA into a single strand for simplicity and efficiency in gene editing.		
c. crRNA (CRISPR RNA):		
The part of gRNA that is complementary to the target DNA sequence.		
d. tracrRNA (Trans-activating CRISPR RNA):		
The part of gRNA that stabilises the crRNA and assists Cas9 binding.		
e. Off-Target Effects:		
Unintended DNA modifications caused by imperfect gRNA or sgRNA targeting.		





#### Question 163 (1 mark)



What is the primary difference between guide RNA (gRNA) and single guide RNA (sgRNA)?

- **A.** gRNA consists of separate crRNA and tracrRNA, while sgRNA combines them into a single molecule.
- **B.** gRNA is more specific than sgRNA.
- C. sgRNA is naturally occurring in bacteria, while gRNA is artificially engineered.
- **D.** sgRNA is used only for gene silencing, while gRNA is used for knock-outs.

#### Question 164 (2 marks)



Provide one structural and one functional difference between gRNA and sgRNA.

**>** 

crRNA and tracrRNA are fused in sgRNA whilst they exist as separate molecules in gRNA. gRNA is utilised in bacteria's natural defense whereas sgRNA is utilised in gene editing.





### <u>Sub-Section</u>: [1.6.5] - Apply Bioethical Principles to the Use of CRISPR-Cas9 Technology and,

<u>Sub-Section</u>: [1.6.6] - Define & Describe the Bioethical Concepts of Integrity, Respect, Beneficence, Non-Maleficence & Justice as Elaborated in the VCAA Study Design

Qu	estion 165					
Definitions.						
a.	Bioethics:					
				<del>-</del>		
	The study of ethical issues arising from advances in biology and medicine.					
b.	Beneficence:					
			Acting in ways that promote the well-being of others.			
c.	Non-Maleficer	nce:				
			Avoiding harm to individuals or populations.			
d.	Justice:					
	Ensuring fairness and equity in the distribution of benefits and risks.					



e. Integrity:

Upholding honesty and strong moral principles in research and application.

#### Question 166 (1 mark)



A company develops a CRISPR-Cas9-based therapy that could cure a rare genetic disease. However, the therapy is priced so high that only wealthy patients can afford it. Which bioethical principle is most relevant in addressing this issue?

- **A.** Beneficence ensuring the therapy maximises benefits for all patients.
- **B.** Justice ensuring equitable access to the therapy.
- **C.** Non-Maleficence ensuring the therapy does not harm patients.
- **D.** Integrity ensuring honest communication about the therapy's benefits.

#### Question 167 (1 mark)



A CRISPR-Cas9 trial aims to modify human embryos to prevent genetic diseases, but long-term effects on future generations are unknown. Critics argue that this raises significant ethical concerns. Which bioethical principle is primarily being questioned?

- **A.** Beneficence ensuring maximum benefit to future individuals.
- **B.** Non-Maleficence avoiding potential harm to future generations.
- **C.** Justice ensuring equitable access to genetic technologies.
- **D.** Integrity ensuring transparency in the trial's aims.





#### Question 168 (1 mark)



A scientist uses CRISPR-Cas9 to edit the genomes of laboratory animals for medical research, ensuring humane treatment and minimising suffering. However, activists argue the research is unethical. Which bioethical principle best supports the scientist's position?

- **A.** Justice ensuring fair treatment of research animals.
- **B.** Respect acknowledging the autonomy of the animals involved.
- C. Beneficence ensuring the research benefits society.
- **D.** Integrity conducting the research with honesty.

#### Question 169 (1 mark)



A research team proposes using CRISPR-Cas9 to modify crops for higher yields but faces concerns about long-term ecological impacts. What bioethical principle is most relevant in evaluating the project?

- **A.** Non-Maleficence assessing potential harm to the environment.
- **B.** Justice ensuring equitable access to the modified crops.
- **C.** Beneficence maximising benefits for global food security.
- **D.** Integrity ensuring accurate reporting of risks and benefits.

#### Question 170 (1 mark)



Which ethical principle is most relevant when ensuring all populations, regardless of socioeconomic status, have access to CRISPR-Cas9 medical therapies?

- A. Beneficence
- B. Integrity
- C. Non-Maleficence
- **D.** Justice





#### Question 171 (1 mark)



A research team proposes a CRISPR-Cas9 trial that may lead to significant advances in medicine but excludes participants from underprivileged backgrounds due to high costs. Which bioethical principle is being violated?

- A. Beneficence
- B. Non-Maleficence
- C. Justice
- D. Integrity

#### Question 172 (1 mark)



A company uses CRISPR-Cas9 to modify crops to improve yield. However, they fail to inform farmers about potential long-term environmental risks. Which bioethical principle is being compromised?

- A. Respect
- B. Integrity
- C. Non-Maleficence
- **D.** Justice

#### Question 173 (1 mark)



A scientist refuses to conduct CRISPR-Cas9 experiments on human embryos due to the potential risks of unintended mutations. Which bioethical principle does this decision demonstrate?

- A. Justice
- B. Beneficence
- C. Non-Maleficence
- **D.** Respect





#### Question 174 (1 mark)



In designing CRISPR-based therapies, researchers prioritise ensuring patient autonomy by offering comprehensive information and obtaining informed consent. Which principle is this?

- A. Justice
- B. Respect
- C. Integrity
- D. Beneficence

#### Question 175 (1 mark)



Which ethical principle requires researchers to be transparent and truthful in reporting CRISPR-Cas9 experimental results, even if the outcomes are unfavourable?

- A. Beneficence
- **B.** Non-Maleficence
- C. Justice
- **D.** Integrity





#### **Question 176**



Scientists are exploring the use of CRISPR-Cas9 technology to modify the genes of crops to enhance yield and resistance to pests. For example, CRISPR could be used to make wheat resistant to a specific fungal disease. However, concerns have been raised about the long-term environmental impact and the potential consequences of genetically modified organisms interacting with wild species.

Discuss an ethical concern that could be raised about the use of CRISPR-Cas9 technology in crops. Propose a feasible solution to this ethical concern. State the ethical concept or approach that has been addressed in your discussion.

- ▶ Unintended gene flow from genetically modified crops to wild species could result in ecological disruptions.
- This is related to the principle of non-maleficence.
- Non-maleficence involves minimising harm and preventing adverse consequences, such as ecological damage caused by unintended gene transfer to wild populations.
- A feasible solution is to perform thorough environmental impact assessments and conduct trials in isolated environments. In addition, containment measures like buffer zones around genetically modified crops can be implemented to prevent gene flow to wild species.

Space for Personal Notes		

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