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VCE Biology ¾
AOS 1 Revision [1.8]
Workbook

Outline:

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- Recap
- Questions

[1.3] - Gene Expression & The trp Operon — Pg 10-21
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- Questions

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Section A: [1.2] - Nucleic Acids & The Structure of Genes (20 Marks)

Sub-Section: Recap

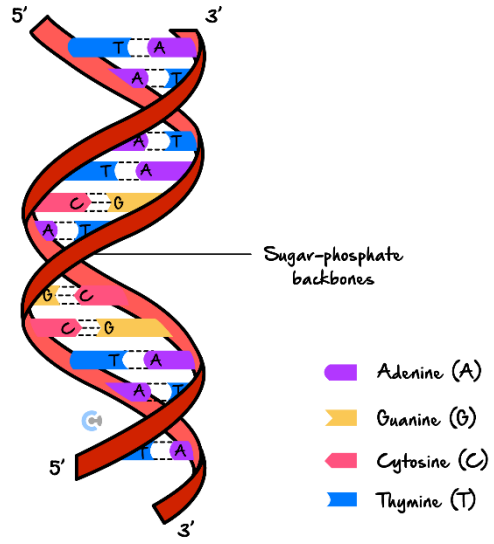
Cheat Sheet

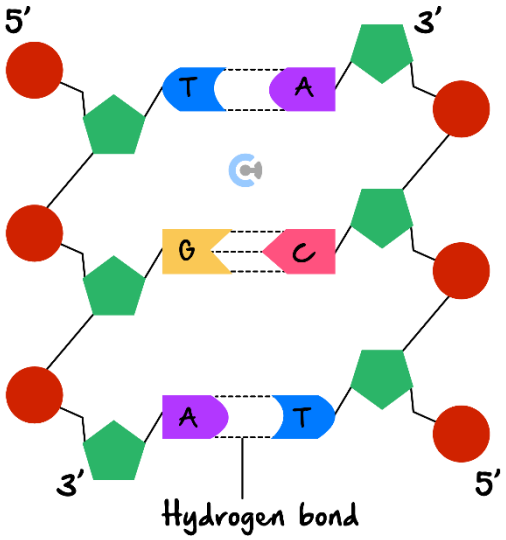
[1.2.1] - Identify and compare the characteristic features of the structures of nucleic acids and their monomers, including DNA, mRNA, tRNA and rRNA, including base pairing

- Nucleic acids (DNA and RNA) encode instructions for protein synthesis.
- DNA is a double-stranded helix composed of nucleotides containing a deoxyribose sugar, phosphate group, and nitrogenous bases (adenine, thymine, cytosine, guanine).
- Complementary base pairing occurs in DNA with adenine pairing with thymine (A-T) and cytosine pairing with guanine (C-G).
- *RNA* exists in three main forms—mRNA (messenger RNA), rRNA (ribosomal RNA), and tRNA (transfer RNA)—each with distinct functions in protein synthesis.
- mRNA carries the genetic code transcribed from DNA to the ribosomes for translation.
- rRNA forms the structural and functional core of ribosomes, where proteins are synthesised.
- tRNA transports specific amino acids to the ribosome during protein synthesis, matching them to the mRNA codons via its anticodon.
- Unlike DNA, RNA is single-stranded, contains the sugar ribose, and uses uracil (U) instead of thymine (T).

[1.2.2] - Identify and describe the structure of a nucleotide in DNA and RNA

- A nucleotide contains a phosphate group, a 5 carbon sugar, and a nitrogenous base - label these on the diagram below!
- DNA nucleotides include deoxyribose, whereas RNA nucleotides include ribose; both share phosphate groups but differ in one nitrogenous base (U in RNA, T in DNA).





[1.2.3] – Define the key components of a gene, including a comparison between the structure of genes in eukaryotes and prokaryotes

Component	Definition
Exons	Regions of DNA that are expressed as a protein.
Introns	Regions of DNA that aren't expressed in the final protein.
Promoter	A sequence of DNA that is responsible for initiating gene expression.
Operator	Binding site for a transcription factor (protein that regulates gene expression).
Terminator	Where transcription of the gene ends.

[1.2.4] – Identify and practically apply the characteristics of the genetic code - universal, unambiguous, degenerate, triplet - to real-life examples

- Characteristics of Genetic Code:

Universal	All living organisms use **DNA** or the language of DNA as the fundamental basis for all their operations.
Triplet	3 bases of DNA (or nucleotides) code for a single amino acid in a protein.
Degenerate	More than 1 triplet/codon codes for a single amino acid.

Sub-Section: Questions

INSTRUCTION: 20 Marks. 20 Minutes Writing.

Question 1 (1 mark)

Some viruses are called retroviruses and contain RNA as their blueprint. Once inside the host cell, an enzyme called reverse transcriptase converts the RNA into DNA. An RNA retrovirus sequence is shown below.

3' GAUCAGUCCA 5'

What would the DNA code be for this sequence?

A. 3′CTAGTCAGGT 5′
5′ GATCAGTCCA 3′

B. 5′CTAGTCAGGT 3′
3′ GATCAGTCCA 5′

C. 3' CATGACTGGA 5'
5' GTACTGACCT 3'

D. 3' CUAGUCAGGU 5'
5' GAUCAGUCCA 3'

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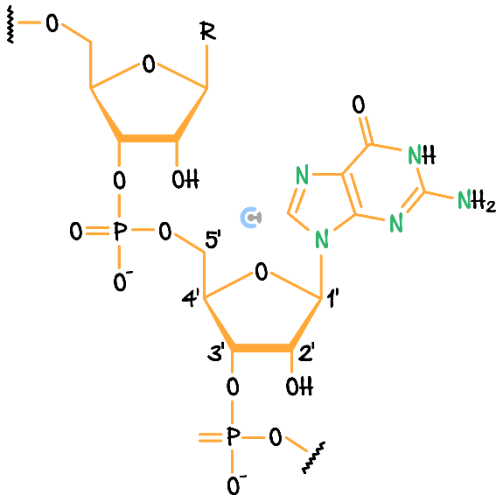
Question 2 (1 mark)

The diagram below shows a small segment of the structure of an organic macromolecule.



Which of the following statements correctly identifies what occurs when this segment is joined to other similar structures?

A. A net output of energy occurs.

B. The reaction is catabolic.

C. A condensation reaction would occur.

D. An input of water is required for this reaction to occur.

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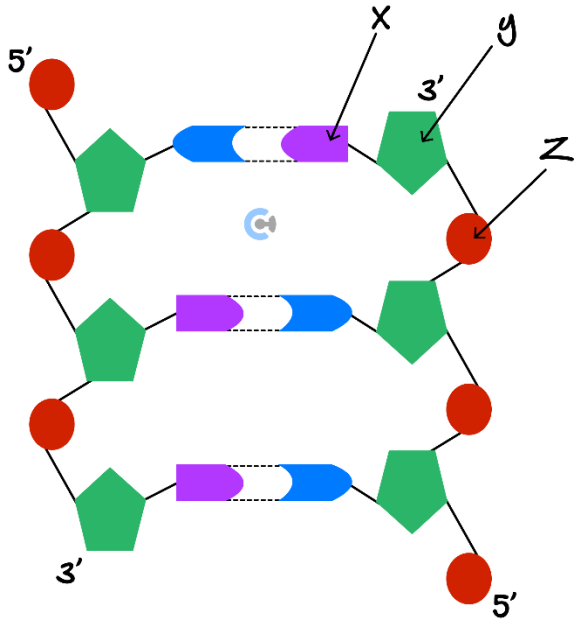
The following information applies to the two questions that follow.

The diagram below represents part of a DNA molecule.



Question 3 (1 mark)

A single DNA nucleotide is shown by sub-unit(s):

A. *X* alone.

B. *X* and *Y* together.

C. *Y* and *Z* together.

D. *X*, *Y* and *Z* together.

Question 4 (1 mark)

A feature of DNA that can be seen in the diagram above is

A. the anti-parallel arrangement of the two strands of nucleotides.

B. the process of semi-conservative replication.

C. its ribose sugar-phosphate backbone.

D. its double-helix structure.

Space for Personal Notes

Question 5 (1 mark)

In eukaryotic cells, this biomacromolecule would be located in the

A. nucleus only.

B. nucleus and mitochondria only.

C. nucleus and chloroplast only.

D. nucleus, chloroplast and mitochondria.

Question 6 (5 marks)

a. In the space below, draw and label the generic structure of a nucleotide. (1 mark)

b. Name two types of nucleic acids and the differences between them. (2 marks)

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c. Briefly explain why it is possible to exchange DNA molecules between different species. (2 marks)

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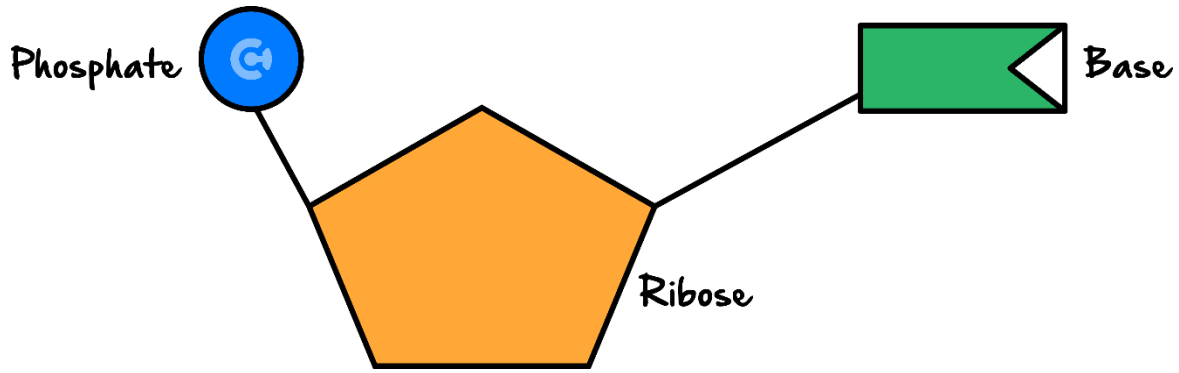
Question 7 (10 marks)

RNA molecules consist of long strands of joined nucleotides. Each nucleotide consists of three sub-units.

a. Label the three sub-units on the diagram of the RNA nucleotide below. (1 mark)



b. Complete the table below by describing the role in a cell of the two types of RNA listed. (2 marks)

Type of RNA	Role in a cell
tRNA	
mRNA	

c. Describe the process in which both of these RNA molecules are involved and state its end product. (4 marks)

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d. What feature of the genetic code allows the same amino acid to be produced despite a change in the DNA? (1 mark)

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e. List two differences between DNA and RNA. (2 marks)

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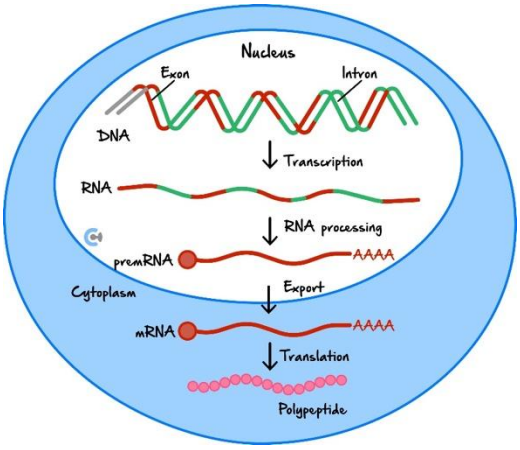
Section B: [1.3] - Gene Expression & The trp Operon (35 Marks)

Sub-Section: Recap

Cheat Sheet

[1.3.1] Identify and recall the process of gene expression in eukaryotes, comparing how it differs in prokaryotes



- Transcription and translation occur simultaneously in prokaryotes, and there is no mRNA processing.

[1.3.2] Describe the processes of transcription, mRNA processing, and translation, recognising the significance of each step to the final product and [1.3.3] Explain how a single gene can give rise to multiple proteins

- RNA polymerase binds to the promoter region and the DNA unwinds and unzips.
- RNA polymerase then catalyses the production of the mRNA strand by joining together complementary RNA nucleotides. mRNA is complementary to the DNA template strand – adenine pairs with uracil in RNA instead.
- Continues until a termination or stop sequence is reached.
- **Addition of Methyl 5′ Cap**:
  - Added to the **5′ end** of the mRNA strand.
  - Prevents degradation.
  - Enables the ribosome to detect the RNA.
- **Addition of Poly-A Tail**:
  - Added to the **3′ end** of the mRNA strand.
  - Composed of adenine bases.
  - Prevents degradation and increases mRNA stability.
- **Splicing: Exons and Introns:**
  - Splicing removes **introns** (non-coding regions) and retains **exons** (coding regions) in the mRNA strand.
  - This process is performed by a complex molecule called a spliceosome.
- **Alternative Splicing:**
  - Allows flexibility in mRNA processing:
    - Exons can be removed, introns can be retained, or the order of exons can be shuffled.
  - Significance:
    - Creates multiple mRNA variants from a single gene.
    - Leads to the production of diverse proteins from the same gene.
- **Translation:**
  - mRNA molecule binds to the ribosome at the 5′ end.
  - tRNA anticodons complementary to the mRNA codons, delivering specific amino acids in their correct order to the ribosome.
  - Adjacent amino acids are joined together by condensation polymerisation by the ribosome.
  - Translation ends when the stop codon is reached.

[1.3.4] Identify and recall the general principles and reasons for gene regulation in both prokaryotes and eukaryotes

- **Gene Regulation**:
  - Ensures specific genes are expressed only when required, conserving energy and resources.
  - **Structural genes**: Code for proteins that perform specific functions (e.g., enzymes).
  - **Regulatory genes**: Code for transcription factors that control the expression of structural genes by interacting with DNA sequences like promoters or operators.
- **Purpose of Gene Regulation:**
  - Avoids unnecessary production of proteins that may not be needed.
  - Responds to environmental changes (e.g., nutrient availability, stress).
  - Maintains cellular efficiency and resource management.
- **What is an Operon?**
  - A cluster of genes with related functions, controlled by a single promoter.
  - Includes a promoter, an operator (binding site for regulatory proteins), and structural genes.
  - Example: The *trp operon* in *E. coli* codes for enzymes that synthesise the amino acid tryptophan.
- **Benefits of Operons**:
  - Simplifies regulation of multiple related genes.
  - Allows a coordinated response to environmental changes (e.g., nutrient levels).
  - Common in prokaryotes but absent in eukaryotes.

[1.3.5] Describe the regulation of the trp operon through the action of the repressor protein

- A regulatory gene upstream of the operon produces the trp repressor protein.
- When tryptophan levels are high:
  - Tryptophan binds to the repressor protein, causing a conformational change that activates it.
  - The active repressor binds to the operator, blocking RNA polymerase from transcribing the structural genes.
  - This halts the production of enzymes needed for tryptophan synthesis, conserving resources.
- When tryptophan levels are low:
  - Tryptophan dissociates from the repressor, rendering it inactive.
  - RNA polymerase can bind to the promoter and transcribe the structural genes.
  - Enzymes for tryptophan synthesis are produced, allowing the cell to make more tryptophan.
- **Importance of Repressor Dissociation**:
  - Prevents unnecessary repression when tryptophan levels drop.
  - Ensures enzymes are available to synthesise tryptophan when needed.

[1.3.6] Describe the regulation of the trp operon through attenuation in high trp environments

- Unique to prokaryotes because transcription and translation occur simultaneously.
- **Mechanism:**
  - The leader sequence between the operator and structural genes contains codons for two tryptophan residues and a region that can form hairpin loops in the mRNA.
  - Depending on tryptophan availability, the mRNA forms one of two types of loops:
    - **Low tryptophan levels:**
      - Ribosome stalls at leader sequence → Anti-terminator loop forms → RNA polymerase continues transcription → Structural genes are expressed.
    - **High tryptophan levels:**
      - Ribosome does not stall → Attenuator loop forms → RNA polymerase is stopped → Structural genes are not expressed.

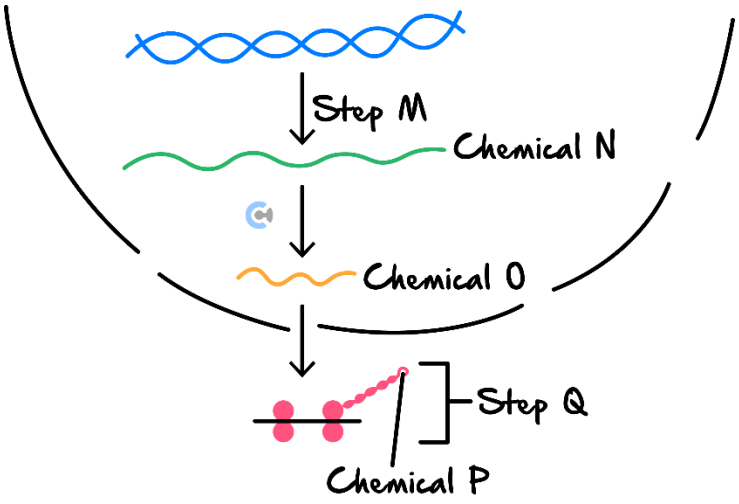
Sub-Section: Questions

INSTRUCTION: 35 Marks. 35 Minutes Writing.

Check off any learning objectives that obtained full marks from the "Contour Check" booklet!

Question 8 (1 mark)

The diagram below illustrates an important cellular process. Labels *M*-*Q* relate to various steps and/or chemicals involved in the process.



Which one of the following correctly names the steps/chemicals represented by *M*-*Q*?

A. *M*: translation; *N*: mRNA; *O*: pre-mRNA; P: protein; *Q*: transcription

B. *M*: transcription; *N*: pre-mRNA; *O*: mRNA; *P*: protein; *Q*: translation

C. *M*: translation; *N*: pre-mRNA; *O*: mRNA; *P*: protein; *Q*: transcription

D. *M*: transcription; N: mRNA; *O*: pre-mRNA; *P*: protein; *Q*: translation

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Question 9 (1 mark)

The graph shows transcription rates of the **trp operon** measured as mRNA synthesis (arbitrary units) under varying tryptophan concentrations (μM).

Tryptophan Concentration (μM)	Transcription Rate (mRNA Units)
0	100
10	80
50	40
100	10
200	0

Based on the data, what pattern is observed in transcription rates of the trp operon as tryptophan levels increase?

A. Transcription rates increase steadily due to the need for tryptophan synthesis.

B. Transcription rates remain constant, regardless of tryptophan levels.

C. Transcription rates decrease sharply as the repressor binds to the operator region.

D. Transcription rates oscillate unpredictably, depending on ribosome activity.

Question 10 (1 mark)

In a prokaryote, what is the primary benefit of regulating gene expression using operons?

A. It prevents unnecessary proteins from being synthesised, conserving energy and resources.

B. It allows eukaryotic-like control of mRNA stability.

C. It eliminates the need for transcription factors in gene expression.

D. It increases the efficiency of post-translational modifications.

Space for Personal Notes

Question 11 (1 mark)

What would occur if a mutation prevented the trp repressor from binding tryptophan?

A. The operon would remain permanently off, regardless of tryptophan levels.

B. Transcription of the trp operon would continue even when tryptophan levels are high.

C. RNA polymerase would fail to bind the promoter region of the operon.

D. Tryptophan biosynthesis would halt, even at low tryptophan concentrations.

Question 12 (1 mark)

How does attenuation regulate the trp operon under high tryptophan levels?

A. The ribosome quickly translates the leader sequence, forming a terminator structure in the mRNA.

B. The repressor protein binds to the operator, blocking RNA polymerase.

C. The ribosome stalls, allowing transcription to continue.

D. Tryptophan molecules bind to RNA polymerase, halting elongation.

Space for Personal Notes

Question 13 (11 marks)

Collagen is a very important protein that is generally found in the extracellular environment of our bodies (the space between our cells).

a. Name the first step of gene expression and describe this process with reference to making collagen. (4 marks)

b. Describe two events that occur during RNA processing and describe their significance. (4 marks)

c. How can a single gene give rise to multiple proteins? (3 marks)

Question 14 (14 marks)

a. Describe the difference between a structural gene and a regulatory gene. (2 marks)

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b. Why is gene regulation so important for organisms to be able to do? (2 marks)

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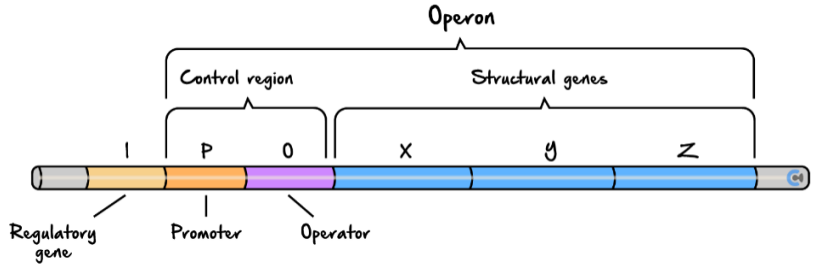
The trp operon is one such process that is useful for bacteria to control the production of tryptophan, an amino acid.



c. What is an operon? Why might this be advantageous? (2 marks)

d. Explain, through the function of the trp repressor, how the level of tryptophan in the cell regulates the expression of the trp operon. (4 marks)

Another method by which the trp operon can be regulated is through attenuation.

e. Explain, using the principle of attenuation, how trp operon can be regulated post-transcriptionally. (4 marks)

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Space for Personal Notes

Question 15 (5 marks)

a. Name the enzyme that is responsible for catalysing transcription. (1 mark)

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b. Name the product of transcription, and describe the processing it undergoes before exiting the nucleus. (3 marks)

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c. What feature of the genetic code allows for this protein to be produced by a bacterial cell? (1 mark)

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Space for Personal Notes

Section C: [1.4] - Proteins, Protein Export & Enzymes (41 Marks)

Sub-Section: Recap

Cheat Sheet

[1.4.1] - Define and compare primary, secondary, tertiary and quaternary structures of proteins

- Proteins are made of amino acids joined by condensation polymerisation.
- Proteins have diverse functions determined by their structure.
- The proteome is the full set of proteins expressed by a cell, tissue, or organism.
- Amino acids have an amine group, a carboxyl group, and an R group.
- The R group determines the chemical properties of the amino acid (non-polar, polar, positive, or negative).
- **Primary structure**: The sequence of amino acids in a polypeptide chain, determining the order of R groups and the foundation for higher levels of structure.
- **Secondary structure**: The folding of the polypeptide chain into alpha-helices or beta-pleated sheets, stabilised by hydrogen bonds between non-adjacent amino acids. These structures increase stability and provide mechanical strength.
- **Tertiary structure**: The overall 3D shape of a polypeptide, formed by interactions between R groups, including hydrophobic interactions, hydrogen bonds, ionic bonds, and disulfide bridges. This structure often determines the protein's specific function.
- **Quaternary structure**: The combination of multiple polypeptide chains into a single functional protein complex. Not all proteins have a quaternary structure.
- Protein diversity comes from their shape and post-transcriptional modifications.
- More proteins exist than genes due to alternative splicing and modifications.

[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

- Ribosome - site of protein synthesis (amino acids joined together to from a polypeptide chain)
- Rough ER - Site of the initial folding of the polypeptide chain, packaged into transport vesicles for delivery to the Golgi apparatus
- Golgi - Its function is to modify and package the protein into a vesicle for transport to various locations within the cell, including to the cell membrane for secretion.
- Transport vesicles take proteins/polypeptides from the rough endoplasmic reticulum to the Golgi Apparatus, and secretory vesicles take the finished product for exocytosis at the membrane.

[1.4.3] - Explain the lock-and-key model, the induced fit model and why enzymes are specific and only catalyse one reaction.

- Enzymes act as organic catalysts, speeding up chemical reactions that would take much longer to occur naturally.
- They are proteins with a 3D active site that is complementary to the shape of their specific substrate.
- Coenzymes assist enzymes by altering the shape of the active site or by providing necessary electrons and protons for the reaction.
- **Process of enzyme function:**
  1. Substrate binds to the enzyme's active site.
  2. The enzyme changes its shape slightly to fit the substrate better (induced fit).
  3. The reaction occurs, transforming the substrate into products.
  4. The products are released, and the enzyme is ready for another reaction.
- **Lock and Key Model:** The enzyme's active site and substrate fit together perfectly, like a key fitting into a lock.
- **Induced Fit Model:** The enzyme's active site moulds itself to fit the substrate upon binding, like a glove fitting a hand.

[1.4.4] - Explain how enzymes change/denature in different pH and at different temperatures

- **Temperature -** As the temperature increases, the rate of enzyme function will INCREASE (as there is an increase in the frequency of successful collisions), until the optimal temperature is reached, after this,
- **pH -** If the pH is within the optimal pH range of an enzyme, the rate of enzyme function will be maximal (as this allows for the greatest frequency of successful collisions). When the pH is outside of this optimal functioning pH range of an enzyme, the rate of enzyme function will decrease (as the active site of the enzyme will be denatured, allowing for less successful collisions).

[1.4.5] - Explain the function of competitive and non-competitive enzyme inhibitors, how they affect the rate of reaction, and how they may/may not be overcome

- **Competitive Inhibitor -** A competitive inhibitor will reduce the activity of an enzyme, as it will compete with the substrate for the active site. This can be overcome by increasing substrate concentration.
- **Non-competitive inhibitor -** binds to the allosteric site of an enzyme, causing a conformational shape change of the active site, preventing the substrate from binding and reducing the activity.

Sub-Section: Questions

INSTRUCTION: 41 Marks. 41 Minutes Writing.

Check off any learning objectives that obtained full marks from the "Contour Check" booklet!

Question 16 (1 mark)

What would be the consequence of a mutation that alters the R-groups of amino acids in a protein's active site?

A. Loss of secondary structure stability due to disrupted hydrogen bonds.

B. Decreased specificity of enzyme-substrate binding.

C. Misfolding of the protein at the primary structure level.

D. Disruption of quaternary structure due to lack of subunit interactions.

Question 17 (1 mark)

Which best explains how protein diversity can exceed the number of genes in an organism?

A. The primary sequence of a protein determines its folding, which increases diversity.

B. Alternative splicing and post-translational modifications create multiple proteins from one gene.

C. The same amino acid sequence can fold into different shapes under environmental conditions.

D. Quaternary structure allows a single polypeptide chain to function as multiple proteins.

Space for Personal Notes

Question 18 (1 mark)

Why does the tertiary structure of a protein determine its specific function?

A. It directly influences how subunits interact in a quaternary structure.

B. It determines the arrangement of disulphide bridges in the protein backbone.

C. It creates a specific three-dimensional active site that interacts with substrates or other molecules.

D. It sets the sequence of amino acids for the protein's primary structure.

Question 19 (1 mark)

What happens to an enzyme that is exposed to a highly acidic pH for an extended period?

A. The enzyme is denatured due to the breaking of peptide bonds in its primary structure.

B. The enzyme's tertiary structure is disrupted, altering the shape of its active site.

C. The enzyme's secondary structure is replaced by random coil regions.

D. The enzyme becomes inactive but regains function when the pH is neutralised.

Question 20 (1 mark)

How do competitive and non-competitive inhibitors differ in their effects on enzyme activity?

A. Competitive inhibitors reduce the enzyme's V_{max}, while non-competitive inhibitors increase K_m.

B. Competitive inhibitors block substrate binding, while non-competitive inhibitors alter enzyme conformation.

C. Non-competitive inhibitors only act on enzymes with quaternary structure, while competitive inhibitors act on all enzymes.

D. Competitive inhibitors bind allosterically, while non-competitive inhibitors compete with the substrate for the active site.

Space for Personal Notes

Question 21 (1 mark)

Which cellular organelle is primarily responsible for the modification and packaging of proteins for extracellular export?

A. Rough endoplasmic reticulum.

B. Golgi apparatus.

C. Lysosome.

D. Ribosome.

Question 22 (1 mark)

Which factor most directly determines whether a protein exhibits quaternary structure?

A. The presence of disulphide bridges within a single polypeptide chain.

B. Interaction between multiple polypeptide chains or subunits.

C. Hydrogen bonding within alpha-helices and beta-sheets.

D. The sequence of amino acids in the primary structure.

Question 23 (1 mark)

What role do enzymes play in chemical reactions, and how is this achieved?

A. Enzymes increase reaction rates by breaking substrate bonds through hydrolysis.

B. Enzymes lower the activation energy required for the reaction to proceed.

C. Enzymes create energy to drive non-spontaneous reactions.

D. Enzymes permanently bind to substrates to stabilise intermediate complexes.

Space for Personal Notes

Question 24 (1 mark)

How does the proteome differ from the genome of an organism?

A. The genome determines cellular function, while the proteome determines gene expression.

B. The genome contains instructions for proteins, while the proteome represents proteins actively expressed at a given time.

C. The proteome contains all RNA transcripts, while the genome contains all DNA sequences.

D. The genome includes only coding regions, while the proteome includes post-translational modifications.

Question 25 (1 mark)

Which of the following best describes why heat permanently denatures an enzyme?

A. It disrupts hydrogen bonds in the active site, rendering it non-functional.

B. It breaks peptide bonds, leading to loss of primary structure.

C. It alters the enzyme's ionic bonds without affecting the hydrophobic interactions.

D. It induces changes in the secondary structure, which the enzyme cannot repair.

Space for Personal Notes

Question 26 (21 marks)

The liver plays an important role in metabolising and breaking down drugs that we consume and enter our body. This ranges from medical drugs, which are important in treating diseases, but also involves other socially acceptable drugs such as alcohol.

The pathway to breaking down these drugs can often be very complex and involves key molecules, called enzymes found within the liver, and if these drugs are left unmetabolised, it may cause issues for the body in general.

a. Using diagrams, describe the two models that describe enzyme function and describe their differences. (4 marks)

When large quantities of these drugs are consumed, this may impact their metabolism in the liver, and after a certain point, there may be excess drug being left unmetabolised, which in the case of alcohol impacts the body's wider function (sensation of being 'drunk').

b. Identifying a factor that impacts the rate of enzyme function, explain why after consuming a lot of alcohol, someone might feel drunk. (2 marks)

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c. Identify and describe 2 other factors that impact enzyme function. (4 marks)

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The enzyme that is involved primarily with the metabolism of many drugs by the liver is the CYP450 enzymes. A recommendation listed on many medications is that you should not drink grapefruit juice prior to or immediately after consuming these drugs.

d. How might grapefruit juice impact the CYP450 enzymes and their ability to metabolise medical drugs? (2 marks)

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Collagen has to be exported outside of the cell to reach the extracellular spaces.

e. Place the structures from which it goes through in order, after the nucleus, and describe their function in relation to protein export. (8 marks)

f. Define the tertiary structure of a protein and its importance. (1 mark)

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Space for Personal Notes

Question 27 (10 marks)

A group of students wanted to investigate the activity of an enzyme that catalyses the breakdown of hydrogen peroxide into water and oxygen.

The students measured oxygen concentration using an oxygen sensor. The oxygen sensor fits into the top of a conical flask, as shown in the photograph below.

The students set up three conical flasks with the contents listed in the table below.

Flask	Contents of flask
1	150 mL of 3% hydrogen peroxide solution. 2 mL of enzyme solution. 50 mL of pH-neutral distilled water.
2	50 mL of 3% hydrogen peroxide solution. 2 mL of enzyme solution. 50 mL of high pH buffer solution.
3	150 mL of 3% hydrogen peroxide solution. 2 mL of enzyme solution. 50 mL of low pH buffer solution.

The buffer solutions and the distilled water did not react with the hydrogen peroxide. All three conical flasks were at room temperature. The students recorded the concentration of the oxygen over a five-minute period.

a. State a hypothesis that the students could be testing with this experiment. (1 mark)

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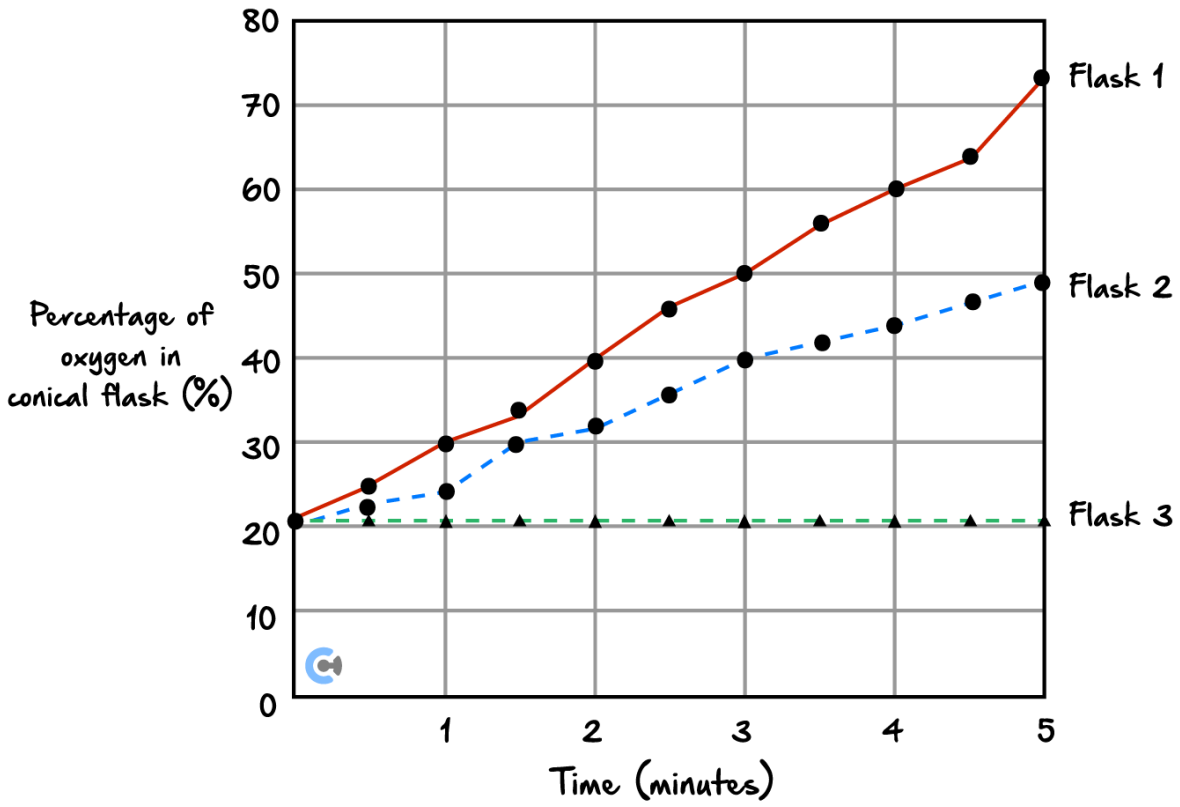
b. The results of the experiment are shown below.



Explain the difference in the results that the students obtained for the three different flasks. (3 marks)

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c. After completing the experiment, the students decided they could improve their experimental design by adding another conical flask.

Flask	Contents of flask
4	150 mL of 3% hydrogen peroxide solution. 2 mL of distilled water.

i. Explain how this additional flask could improve their experimental design. (2 marks)

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ii. Draw on the graph below the results that would be expected for the production of oxygen in Flask 4. (1 mark)

d. Suggest one further improvement that could be made to the students' experimental design. Justify your suggestion. (1 mark)

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e. Consider any experiment. Explain the difference between the accuracy and precision of measurements. (2 marks)

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Section D:[1.5] – Introduction to DNA Manipulation Techniques (28 Marks)

Sub-Section: Recap

Cheat Sheet

[1.5.1] Identify and describe the function of polymerases, endonucleases, and ligases in DNA manipulation

- Polymerases:
  - Copy nucleic acid strands by synthesising DNA using an existing template, requiring a primer and helicase to unzip the DNA.
  - DNA replication is semi-conservative, producing molecules with one original and one new strand.
- Endonucleases:
  - Cut DNA at specific recognition sites, creating either sticky ends (overhangs) or blunt ends (straight cuts).
  - Sticky ends are preferred due to complementary base pairing for easier fragment joining (e.g., EcoRI, AluI).
- Ligases:
  - Join DNA fragments by reforming phosphodiester bonds, sealing both sticky and blunt ends to create continuous DNA strands.

[1.5.2] Identify the ingredients required, describe the process, and recall key applications of PCR

- The materials required for PCR include:
  - **DNA sample**: Provides the template to produce copies.
  - **Primers**:
    - **Forward primer** binds to the start of the DNA segment.
    - **Reverse primer** binds to the complementary strand at the opposite end. These primers designate the sequence to be copied and provide a starting point for DNA synthesis.
  - **Taq polymerase**: A heat-resistant enzyme that makes multiple copies of the DNA strand by adding nucleotides.
  - **Free nucleotides (dNTPs)**: Added by Taq polymerase to produce the new DNA strand.
  - **Mix buffer**: Maintains the appropriate pH and provides the required salts for Taq polymerase activity.
  - **PCR tube**: Serves as the vessel for the reaction, containing all the components.
- The process of PCR involves the following steps:
  1. **Denaturing (90–95°C)**: The two DNA strands are broken apart, performing the role that helicase would in a natural setting. Hydrogen bonds between the complementary bases are broken, separating the strands.
  2. **Annealing (50–55°C):** Lowering the temperature keeps the strands separate while allowing primers to anneal to the DNA strands. The primers bind at the 3' end, allowing polymerase to begin replication.
  3. **Elongation (72°C)**: The raised temperature allows a heat-resistant polymerase (Taq polymerase) to function optimally. It extends the primers, replicating the DNA until the end of the sample or a termination sequence. 72°C is the optimal temperature for Taq polymerase.
- These steps are repeated many times (typically 20–40 cycles) to generate a large sample of DNA.

[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"

- Gel electrophoresis is used to analyse DNA samples by sorting and separating DNA fragments based on size.
- Typically performed after DNA has been cut with a restriction enzyme.
- Allows for size comparison of DNA fragments, visualisation using fluorescent dyes, and separation of DNA based on fragment size.
- **Why DNA moves through the gel**:
  - DNA is negatively charged due to its phosphate backbone.
  - When an electric current is applied, DNA moves toward the positive electrode.
- **Process**:
  1. DNA samples are placed in wells in an **agarose gel**.
     - One well contains a **standard ladder** with fragments of known sizes.
  2. The gel is placed in a **buffer solution** that conducts electricity.
     - A negative electrode is at the well end, and a positive electrode is at the opposite end.
  3. An electric current is applied, and DNA fragments move through the gel:
     - Smaller fragments move faster.
     - Larger fragments move slower.
  4. A dye (commonly **ethidium bromide**) is added to visualise the results under UV light.
     - DNA fragments appear as fluorescent bands.
- **Importance of standard ladders**:
  - Contain DNA fragments of known sizes.
  - Used to compare and estimate the size of unknown DNA fragments.
  - Specific to experimental conditions for accurate results.

[1.5.4] Explain the factors that affect the movement of fragments in gel electrophoresis

- **Voltage**: Higher voltage increases movement but may distort separation.
- **Gel Composition**: Denser gels slow larger fragments.
- **Buffer Concentration**: Affects electrical conductivity and DNA movement.
- **Time**: Longer runs allow DNA to travel further, but excessive time may cause DNA to move out of the gel.

[1.5.5] Define satellite DNA and STRs, and explain their use in identifying people through DNA profiling for crimes and paternity testing

- **Overview**:
  - DNA profiling combines technologies like **PCR** and **gel electrophoresis** to identify individuals using DNA.
  - Widely used in forensic science to solve crimes and in genetic testing to establish relationships.
- **Process**:
  - **DNA Collection**: DNA is collected from a crime scene (e.g., blood, hair, or saliva) and compared to samples from suspects or victims.
  - **PCR Amplification**:
    - The collected DNA is amplified using **PCR** to produce multiple copies of specific DNA regions, particularly **short tandem repeats (STRs)**.
    - STRs are short, repeated DNA sequences found in **non-coding regions (satellite DNA)**, which vary significantly between individuals.
  - **Digestion with restriction enzyme**:
    - DNA is cut with the **same restriction enzyme** to ensure consistent fragment sizes across all samples.
  - **Gel electrophoresis**:
    - The cut DNA fragments are separated based on size using gel electrophoresis.
    - STRs create fragments of varying lengths unique to each individual.
    - Fragments are visualised as fluorescent bands under UV light using dyes like ethidium bromide.
- **Why STRs are used**:
  - STRs are highly variable between individuals, even within non-coding regions.
  - Testing multiple STR loci ensures reliable identification and reduces the chance of matching unrelated individuals.
- **Interpreting results**:
  - Samples with identical banding patterns indicate a match.
  - For forensic cases:
    - Matching the crime scene sample to a suspect's STR profile can link them to the scene.
  - For genetic testing:
    - Banding patterns confirm familial relationships (e.g., paternity tests).
    - Children inherit STR fragments from both parents, which can be observed in shared patterns.

Sub-Section: Questions

INSTRUCTION: 28 Marks. 28 Minutes Writing.

Question 28 (1 mark)

A DNA fragment with a negative charge is placed in a gel for electrophoresis. What causes its migration?

A. The attraction to the negatively charged cathode.

B. The attraction to the positively charged anode.

C. The smaller size of the DNA fragment compared to the gel pores.

D. The application of heat to the gel during the process.

Question 29 (1 mark)

Why are sticky ends often more efficient than blunt ends in creating recombinant DNA?

A. Sticky ends form hydrogen bonds that make ligation more stable.

B. Sticky ends are longer, allowing more opportunities for alignment.

C. Sticky ends prevent DNA degradation during ligation.

D. Sticky ends are recognised by ligase without the need for additional enzymes.

Question 30 (1 mark)

A PCR reaction contains the following components: DNA template, Taq polymerase, dNTPs, and primers. What is the purpose of the primers?

A. To terminate DNA synthesis at specific sequences.

B. To provide a starting point for Taq polymerase to extend the DNA.

C. To unwind the DNA helix during denaturation.

D. To separate DNA strands during the annealing phase.

Question 31 (1 mark)

A gel electrophoresis experiment shows bands for a crime scene sample matching bands from a suspect's DNA. What does this indicate?

A. The suspect's DNA has different sequences than the crime scene DNA.

B. The suspect's DNA matches the crime scene sample, supporting involvement.

C. The suspect's DNA contains fewer restriction sites than the crime scene DNA.

D. The suspect's DNA has undergone more mutations than the crime scene DNA.

Question 32 (1 mark)

What effect would increase the annealing temperature in PCR have on the reaction?

A. Amplification of non-specific products due to less stringent primer binding.

B. Increased specificity as primers bind more accurately to the target DNA.

C. Reduced yield as primers fail to bind effectively to the DNA.

D. Decreased denaturation efficiency, leading to incomplete strand separation.

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Question 33 (12 marks)

With the development of DNA manipulation technologies in the 20th century, forensic science has seen significant improvements in its ability to catch the perpetrators of crimes, with even a single hair or cell left at a crime scene proving enough.

a. What technology can be used to ensure that even trace amounts of DNA can be analysed by technicians? Describe the process. (4 marks)

Once they have enough of a sample, researchers can then apply an endonuclease to a range of DNA samples, and then run the fragments through gel electrophoresis and compare their findings.

b. How does doing this allow the researchers to tell DNA samples apart and match them to a criminal? (3 marks)

c. Which property of DNA explains why it will move through the gel? (1 mark)

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d. Discuss three factors that affect the migration of fragments through the agarose during gel electrophoresis. (3 marks)

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e. What is the function of DNA ligase? (1 mark)

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Question 34 (4 marks)

DNA includes sections that are called short tandem repeats (STR). Mutations in STRs occur, on average, every 500 generations. Different numbers of these repeats have no obvious effect on the individual.

a. What is the likely reason for this? (1 mark)

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A young man, Ben, wants to find out more about his genetic ancestry. He sends a sample of cells, obtained from a swab of his mouth to a laboratory. On receipt of the sample, the laboratory treats the cells to release the DNA to enable the identification of STR markers.

b. Name the process used to produce many copies of the STR markers. (1 mark)

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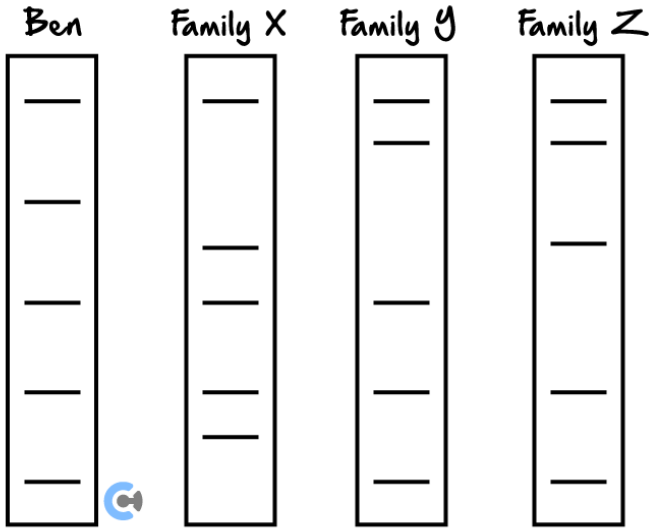
Each of the STR markers produced is labelled with a dye and subjected to gel electrophoresis. Five of Ben's STR markers were compared with three family groups who have the same surname as him. The following gels resulted.



c. Explain which family is Ben's most recent common ancestor. (2 marks)

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Question 35 (7 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)

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b. Outline the three major steps in PCR. (3 marks)

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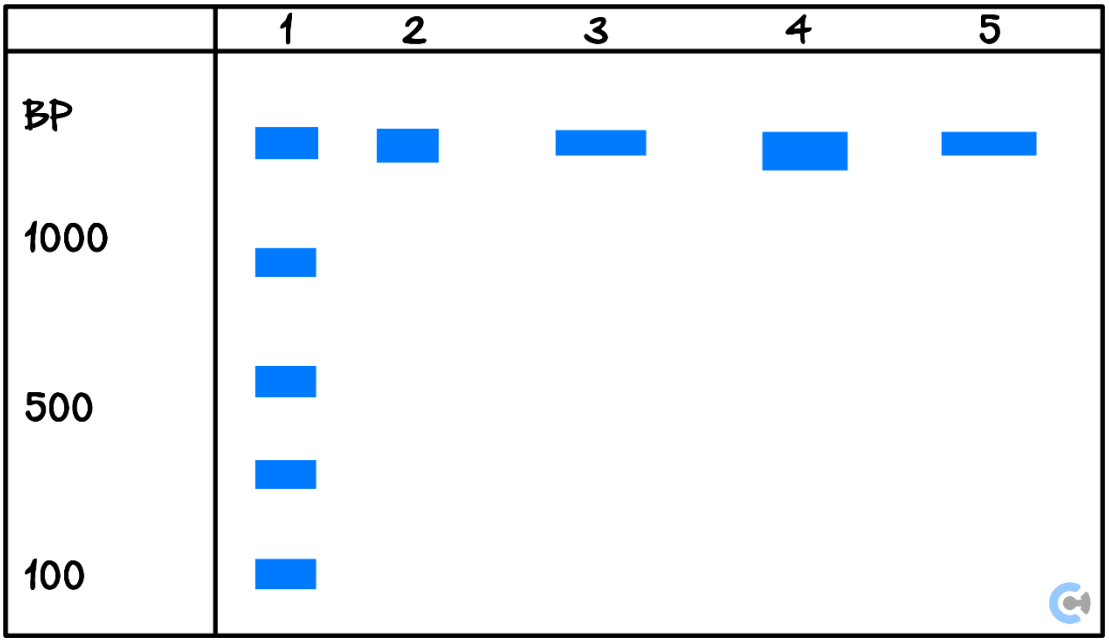
The table below gives the sizes of the various DNA fragments (genes) used in the genetic engineering of the *Arabidopsis* plant.

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 *Arabidopsis* + barley genes	1050	5

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



d. What is placed in well 1? (1 mark)

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Section E: [1.6] – CRISPR-Cas9 & Bioethics (25 Marks)

Sub-Section: Recap

Cheat Sheet

[1.6.1] Describe the function & process of CRISPR-Cas9 as an adaptive defence against viruses in bacteria

- **Overview**:
  - CRISPR-Cas9 is a revolutionary tool derived from bacteria's natural defence mechanism against viruses (bacteriophages).
  - It stands for **Clustered Regularly Interspaced Short Palindromic Repeats**.
  - Function: Allows for precise and efficient gene editing by targeting specific DNA sequences.
- **Analogy**:
  - It works like a surveillance system: bacteria "store" viral DNA to recognise and neutralise future invasions.
- **Key Features**:
  - Cas9: A programmable enzyme (endonuclease) that cuts DNA.
  - Guide RNA (gRNA): Guides Cas9 to the specific target DNA via complementary base pairing.
  - PAM (Protospacer Adjacent Motif): A short DNA sequence that enables Cas9 to bind and cut at the correct site.
- **Process**:
  - **Exposure**: A bacteriophage injects its DNA into a bacterial cell.
    - Endonucleases cut a protospacer (a small viral DNA fragment) adjacent to a PAM sequence.
  - **Incorporation**: The protospacer is integrated into the bacterial genome.
  - **Expression**: The spacer is transcribed into crRNA and combines with tracrRNA to form guide RNA (gRNA).
  - **Extermination**: gRNA directs Cas9 to recognise and cut complementary viral DNA, inactivating the virus.

[1.6.2] Explain the process of using CRISPR-Cas9 as a gene editing tool, including silencing, knock-ins & knock-outs

- CRISPR-Cas9 has been adapted as a powerful and versatile tool for editing genomes in eukaryotic cells.
- The system uses the principles of bacterial immunity but has been optimised to make precise cuts in specific DNA sequences within any organism.
- **How it Works**:
  - **Target Sequence Identification**:
    - A target DNA sequence is selected for editing, such as a gene responsible for a disease.
    - Researchers design a synthetic **single guide RNA (sgRNA)**, which combines the targeting properties of crRNA and tracrRNA into one molecule.
  - **CRISPR-Cas9 Complex Formation**:
    - sgRNA binds to the Cas9 enzyme, forming the CRISPR-Cas9 complex.
  - **DNA Binding**:
    - The complex locates the target DNA by matching the sgRNA sequence to the complementary DNA sequence.
    - A **PAM sequence** (e.g., NGG) adjacent to the target ensures proper binding and initiates the cutting process.

- **DNA Cutting**:
  - Cas9 creates a double-strand break at the target site.
- **Repair Mechanisms**:
  - The cell's natural repair mechanisms are activated:
    - **Non-Homologous End Joining (NHEJ)**: Repairs the break but often introduces errors (e.g., insertions or deletions), which can disable the gene (gene knock-out).
    - **Homology-Directed Repair (HDR)**: Uses a provided DNA template to repair the break precisely, allowing the insertion of new genetic material (gene knock-in).
- **Gene Silencing (Knock-Outs)**:
  - Cas9 introduces a double-strand break at a target DNA sequence.
  - The cell repairs the break via non-homologous end joining (NHEJ), which often introduces mutations, disabling the gene.
  - Example: Disabling genes linked to diseases like cancer or Huntington's disease.
- **Gene Knock-Ins**:
  - Homology-directed repair (HDR) uses an introduced template to insert new DNA sequences.
  - Example: Adding a functional copy of a defective gene in conditions like cystic fibrosis.
- **Targeted Gene Modifications**:
  - Enables precise alterations to DNA to correct errors or enhance traits.

[1.6.3] Describe & compare the function of the PAM sequence in bacteria & gene editing appications of CRISPR-Cas9 technology

- PAM ensures that Cas9 binds to and cuts only the foreign DNA, distinguishing self from non-self DNA. It also ensures that the Cas9 will only bind to DNA and unwind it for detecting a match where it is possible.
- This system is highly specific and efficient, making it adaptable for gene editing.
- In gene editing applications, it allows the system to be more precise and efficient.

[1.6.4] Describe the function & compare the guide RNA (gRNA) & single guide RNA (sgRNA)

- Guide RNA (gRNA) is a combination of crRNA (CRISPR RNA) and tracrRNA (trans-activating crRNA), which directs Cas9 to the target DNA by complementary base pairing.
- Single guide RNA (sgRNA) is a synthetic RNA that combines the functions of crRNA and tracrRNA into one molecule for simplicity and efficiency.
- Both gRNA and sgRNA guide Cas9 to specific DNA sequences for cutting.
- The sgRNA simplifies the CRISPR-Cas9 system by eliminating the need for two separate RNA molecules.

[1.6.5] Apply bioethical principles to the use of CRISPR-Cas9 technology

- **Beneficence**:
  - Obligation to use CRISPR to maximise benefits, such as curing genetic diseases and improving crop productivity.
- **Non-Maleficence**:
  - Avoid causing harm, considering unknown long-term effects or off-target mutations that could negatively impact health or the environment.
- **Consent**:
  - Ethical concerns arise in germline editing where future generations cannot provide consent. Ensuring parents and patients are fully informed is critical.
- **Justice**:
  - CRISPR's potential to widen socio-economic inequalities, with only wealthy individuals having access to enhancements, must be addressed.

[1.6.6] Define & describe the bioethical concepts of integrity, respect, beneficence, non-maleficence & justice as elaborated in the VCAA study design

- **Integrity**:
  - Definition: A commitment to honesty and accurate reporting.
  - Ensures transparency and trust in scientific research.
  - Example: Publishing both positive and negative results from CRISPR experiments.
- **Respect**:
  - Definition: Recognising the intrinsic value and autonomy of living things.
  - Considers welfare, cultural beliefs, and personal rights.
  - Example: Gaining informed consent before experimenting on human subjects.
- **Beneficence**:
  - Definition: Maximising benefits while minimising risks and harms.
  - Encourages actions that contribute positively to individuals or society.
  - Example: Using CRISPR to cure genetic disorders responsibly.
- **Non-Maleficence**:
  - Definition: Avoiding harm, or ensuring harm is proportionate to the benefits.
  - Balances risk and reward in scientific practices.
  - Example: Carefully evaluating potential off-target effects of CRISPR edits.
- **Justice**:
  - Definition: Ensuring fair treatment, distribution of benefits, and avoidance of undue burdens.
  - Focus on equity and accessibility.
  - Example: Avoiding socio-economic inequalities in access to CRISPR-based therapies.

[1.6.7] Define & describe the three ethical approaches as elaborated in the VCAA study design

- **Consequences-Based Approach**:
  - Central focus: Outcomes or consequences of an action.
  - Goal: Maximise positive effects and minimise harm.
  - Example: Supporting CRISPR use for disease eradication if the societal benefits outweigh the risks.
  - Critique: Can justify morally questionable actions if the outcomes are favourable.
- **Duty-/Rule-Based Approach**:
  - Central focus: Adherence to moral rules or duties, regardless of outcomes.
  - Emphasis on ethical responsibilities and strict regulations.
  - Example: Requiring full regulatory approval before using CRISPR on embryos.
  - Critique: May ignore potential benefits if rules are overly restrictive.
- **Virtues-Based Approach**:
  - Central focus: The moral character and intentions of the individual performing the action.
  - Emphasis on qualities such as honesty, fairness, and humility.
  - Example: Scientists exercising restraint and prioritising ethical considerations in CRISPR applications.
  - Critique: Relies on subjective interpretations of virtue.

[1.6.8] Describe briefly how to genetically modify organisms to increase crop productivity & disease resistance, using CRISPR-Cas9

- **Improving Productivity**:
  - Modifications enhance growth, photosynthesis efficiency, or drought resistance.
  - Example: Engineering crops to survive arid climates.
- **Enhancing Disease Resistance**:
  - Targeted edits make crops resistant to diseases or pests.
  - Example: Bananas engineered to resist Panama disease.
- **Increasing Nutritional Value**:
  - Edits improve the nutritional profile of crops.
  - Example: Golden rice enriched with Vitamin A.

[1.6.9] Compare transgenic, cisgenic, & genetically modified organisms

- **Genetically Modified Organisms (GMOs)**:
  - Organisms whose genomes have been altered artificially.
  - Includes any form of genetic modification, whether involving foreign or native genes.
- **Transgenic Organisms**:
  - A subset of GMOs where genes from an unrelated species are introduced.
  - Example: Adding genes from fish to tomatoes for cold resistance.
- **Cisgenic Organisms**:
  - Involves transferring genes from the same or closely related species.
  - Example: Enhancing disease resistance in crops using native plant genes.

Sub-Section: Questions

INSTRUCTION: 25 Marks. 25 Minutes Writing.

Question 36 (1 mark)

A genetically modified crop is resistant to pests but leads to a reduction in local insect biodiversity. Which bioethical principle is most relevant?

A. Beneficence, due to increased crop yields.

B. Non-maleficence, due to harm caused to insect populations.

C. Justice, ensuring equal access to modified crops.

D. Respect, allowing farmers to choose unmodified crops.

Question 37 (1 mark)

A patient is offered CRISPR treatment for a genetic disease but declines due to personal beliefs. Which ethical concept is being upheld?

A. Integrity

B. Non-maleficence

C. Beneficence

D. Respect for autonomy

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Question 38 (1 mark)

CRISPR-Cas9 is used to eliminate genetic diseases in embryos, but this leads to unexpected traits in future generations. Which ethical concern is most relevant?

A. Beneficence, as this prevents genetic diseases.

B. Non-maleficence, as an unintended consequence, harms future generations.

C. Autonomy, as future individuals cannot consent to the modification.

D. Justice, as such treatments, may only be available to the wealthy.

Question 39 (1 mark)

A country bans all human germline editing, citing potential misuse. Which ethical principle is the government prioritising?

A. Justice, to ensure all genetic modifications are regulated equally.

B. Beneficence, to prevent harm from poorly understood technologies.

C. Integrity, to align scientific advances with societal values.

D. Non-maleficence, to avoid long-term unintended effects.

Question 40 (1 mark)

CRISPR-Cas9 is used to enhance crop yields, but local farmers cannot afford the seeds. Which ethical principle is being violated?

A. Beneficence, as the crops could alleviate food shortages.

B. Non-maleficence, as farmers are excluded from economic benefits.

C. Justice, due to inequity in access to technology.

D. Autonomy, as farmers cannot choose non-GMO crops.

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Question 41 (15 marks)

CRISPR-Cas9 is a revolutionary gene editing technique, based upon an immune defence mechanism found in bacteria.

a. Describe how the CRISPR-Cas9 system works as found naturally in bacteria. (3 marks)

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b. What is the significance of the PAM, when looking at the function of CRISPR in bacteria? (2 marks)

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CRISPR can be applied to the medical field especially, with scientists figuring out how to apply this technology for gene editing.

c. Describe how CRISPR-Cas9 could be used to edit an embryo and solve this single mutation. (4 marks)

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d. What are the advantages of CRISPR-Cas9 that separate it from other techniques? (2 marks)

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e. Using a consequences-based approach discuss the consequences of germline edits using CRISPR-Cas9. (4 marks)

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Question 42 (5 marks)

Biotech firm says it can resurrect extinct woolly mammoth.

A technology entrepreneur and a geneticist launched a new biotech firm that they say will bring the extinct woolly mammoth back to life. Calling itself Colossal, the biosciences company claims CRISPR genetic technology can be used to bring back the animal, which went extinct over 11,000 years ago.

"Never before has humanity been able to harness the power of this technology to rebuild ecosystems, heal our Earth and preserve its future through the repopulation of extinct animals," technology entrepreneur and Colossal co-founder Ben Lamm said in a statement.

Scientists have managed to find mammoth tusks, bones and other material to try to sequence the animal's DNA. This DNA would then be inserted into the genome of the Asian elephant to form an "elephant-mammoth hybrid," according to the company.

Advocates of "de-extinction" say the process could help humans gain new knowledge regarding biology, evolution and technology. The resurrection of extinct species could also repair damaged ecosystems. In the case of the woolly mammoth, Colossal believes the animal could revitalise the Arctic grasslands, whose properties can mitigate global warming.

The idea of de-extinction could have its drawbacks, however. A March 2017 study published in the Nature Ecology & Evolution journal found that de-extinction programs would be incredibly expensive. Other downsides include resurrected animals carrying new pathogens that could possibly infect humans, along with how they will impact the environment.

The United Nations said in a 2019 report that 1 million animals, plants, and fungi species face extinction in the coming decades.

Source: <https://learngerman.dw.com/en/biotech-firm-says-it-can-resurrect-extinct-woolly-mammoth/a-59171358#>

a. With reference to evidence from the article, what ethical approach has been used? (2 marks)

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b. Suggest a way in which the company proposing the de-extinction of the woolly mammoth could demonstrate the ethical principle of integrity. (1 mark)

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c. Identify **one** potential political issue and **one** potential economic issue associated with the de-extinction of the woolly mammoth. (2 marks)

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Question 43 (2 marks)

The following table provides information on three commonly grown genetically modified (GM) crops in Australia.

Crop	Genetic Modification	Characteristics Given by Modification
GM cotton	Several bacterial genes inserted	Insect resistance and herbicide tolerance
GM canola	Two genes from two different bacterial species inserted	Tolerance to several herbicides
GM safflower	A selection of genes silenced within the safflower genome	Elevated levels of oleic acid in its seeds

a. Select **one** of the GM crops in the table above and justify whether or not this crop could be described as transgenic. (1 mark)

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b. One issue with GM canola is the accidental release, during transport, of seeds along roadsides. Usually, unwanted plants that grow on the side of the road are killed using the herbicide glyphosate. However, GM canola is resistant to glyphosate.

Suggest **one** practical solution for treating GM canola that is found growing along roadsides. (1 mark)

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Section F: [1.7] - Recombinant Plasmids (21 Marks)

Sub-Section: Recap

Cheat Sheet

[1.7.1] Describe the role of plasmids as a vector to transform bacteria & other cells

- Involves combining DNA from multiple sources to produce desired proteins.
- Transformation is the insertion of recombinant DNA into a bacterium, often using a plasmid or vector.
- A vector carries foreign genetic material into a host organism for replication or expression.
- Plasmids, usually carrying the target gene, transform bacteria to produce the desired protein.
- **Structure of a plasmid**:
  - **Plasmid**: Small circular DNA in bacteria, replicates independently, transferable.
  - **Origin of Replication**: Sequence where replication starts.
  - **Antibiotic Resistance Gene**: Provides resistance to specific antibiotics.
  - **Multiple Cloning Site (MCS)**: Contains recognition sites for inserting genes.
  - **Selectable Marker**: Distinguishes transformant from non-transformant cells.
  - **Screening Marker**: Confirms plasmid is recombinant (e.g., lacZ).
  - **Promoter Region**: Site where RNA polymerase binds to initiate transcription.

[1.7.2] Explain how a gene of interest is isolated & inserted into a plasmid

- **Making a Recombinant Plasmid**:
  - Obtain a gene of interest and plasmid, cut with the same endonuclease to create sticky ends.
  - Complementary tick ends will attract via hydrogen bonding.
  - Use DNA ligase to join the gene and plasmid.
  - Possible outcomes include recombinant and non-recombinant plasmids.

[1.7.3] Explain electroporation & heat shock as methods to transform bacterial cells

- **Transforming Bacteria**:
  - Electroporation and heat shock methods introduce plasmids into bacteria.
  - **Electroporation**: Electric current creates membrane pores for DNA entry, increasing permeability.
  - **Heat Shock**: Uses Ca^{2+} ions, ice, and heat to facilitate DNA uptake, increasing permeability.

[1.7.4] Describe the process by which bacterial cells can be used to produce human proteins, including the application of this to human insulin production

- **Isolation of insulin genes:**
  - mRNA strands coding for the A and B chains of insulin are isolated and used as templates to synthesise complementary DNA (cDNA) via **reverse transcription**. This ensures that introns are not present in the insulin genes.
- **Preparation of plasmids and DNA:**
  - The plasmid (vector) and insulin DNA sequences are cut with the same **restriction endonuclease**, creating complementary **sticky ends**. This ensures the insulin DNA can be inserted into the plasmid.
- **Formation of recombinant plasmids:**
  - The insulin DNA sequences (A and B) are separately inserted into plasmids containing a **beta-galactosidase gene** for detection.
  - **DNA ligase** is used to join the insulin DNA and plasmid, creating **recombinant plasmids**.
- **Introduction to bacteria:**
  - The recombinant plasmids are inserted into ***E. coli*** bacterial cells through **heat shock** or electroporation transforming the bacteria.
  - Each plasmid is introduced into separate bacterial strains to produce the A and B chains independently.
- **Selection of transformed bacteria:**
  - Bacteria are grown on **antibiotic agar plates**, allowing only those with recombinant plasmids (containing antibiotic resistance genes) to survive.
  - Successful incorporation of the insulin genes is confirmed using **beta-galactosidase activity**.
- **Expression and protein extraction:**
  - Transformed bacteria are cultured, producing **fusion proteins** consisting of beta-galactosidase and insulin chains.
  - Fusion proteins are extracted, and enzymes are used to **cleave** the insulin chains (A and B) from the beta-galactosidase portion.
- **Purification and assembly:**
  - The insulin A and B chains are purified and then chemically combined.
- **Disulfide bonds** are formed between the chains, resulting in functional human insulin ready for medical use.

[1.7.5] Explain the significance of beta-galactosidase, other reporter genes, & antibiotic-resistance genes in the selection of transformed, recombinant bacterial cells

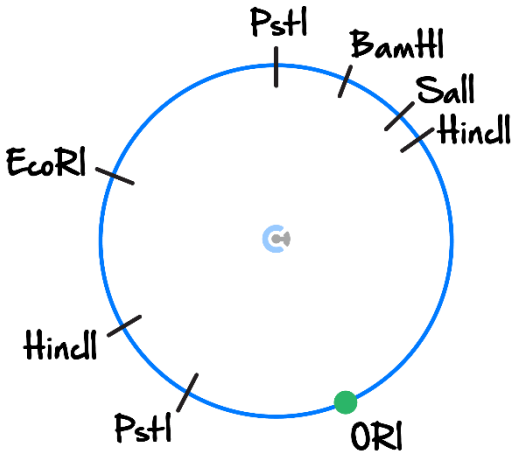
- **Selecting Bacteria**:
  - Identify bacteria with recombinant plasmids using antibiotic selection and reporter genes.
  - Three groups: without plasmid, with non-recombinant plasmid, and with recombinant plasmid.
  - Antibiotic resistance indicates transformed bacteria.
  - Reporter gene function changes indicate recombinant plasmids, often through colour change.
  - Selected bacteria colonies produce the desired protein for purification.
- **Function of b-gal:**
  - The gene for human insulin will be inserted next to a gene coding for the production of beta-galactosidase protein. This will create a fusion protein, which allows for the detection of successful gene insertion and ensures the survival of the insulin peptide when expressed by the bacteria. Detection of gene insertion is done when the fusion protein allows the plated X-gal substrate to turn blue.

Sub-Section: Questions

INSTRUCTION: 21 Marks. 21 Minutes Writing.

Question 44 (1 mark)

The diagram below is a map of a bacterial plasmid showing ORI, the origin of DNA replication, and selected restriction endonuclease sites.



One plasmid was mixed with the restriction enzymes EcoRI, BamHI and HincII.

Which of the following shows the number of restriction sites that have been cut and the resulting number of DNA fragments produced?

	Number of Restriction Sites Cut	Number of DNA Fragments Produced
A.	3	3
B.	3	4
C.	4	4
D.	4	5

Space for Personal Notes

Question 45 (13 marks)

The process of making insulin using bacteria is given below (some parts simplified). This process avoids the previous need to extract it from animals, commonly pigs.

1. A target gene is isolated and cut using a restriction enzyme.
2. A plasmid with ampicillin resistance and a reporter gene is selected and also cut with the same restriction enzyme.
3. Mixed together with DNA ligase.
4. Using a transformation method, delivered to bacteria.
5. Ampicillin applied to the colony.
6. Bacteria that do not express reporter genes are selected and placed in culture.
7. Insulin collected and purified.

a. Describe the action of restriction enzymes and explain which one will you use in this experiment. (3 marks)

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b. Explain two methods that can be used to insert the plasmid into the bacterium containing the insulin gene. (2 marks)

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c. What is the purpose of treating the bacteria with ampicillin? (2 marks)

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d. Describe the function of the reporter gene. (2 marks)

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e. With reference to both ethical implications and social implications, discuss the advantages of this approach to making insulin above the alternatives. (4 marks)

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Question 46 (7 marks)

Gene technology has enabled human insulin to be artificially manufactured within bacteria and then purified for medicinal use by type I diabetics. The steps involved in the procedure from extraction to purification are listed below in the incorrect order.

- Insertion of the modified plasmids into bacteria.
- Amplification of the human insulin gene.
- Isolation of DNA from a human cell.
- Extraction of the insulin protein.
- Selection of the genetically modified bacteria.
- Mixing the restricted plasmid and the restricted human insulin gene together.

a. Complete the table below by placing the correct step of the insulin purification procedure from the list above in the correct order. (2 marks)

Step 1	Isolation of DNA from a human cell.
Step 2	
Step 3	
Step 4	
Step 5	
Step 6	Extraction of the insulin protein.

b. Discuss how the restricted plasmid and restricted human gene would successfully anneal together when they are combined. (2 marks)

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c. Use the following terms to discuss the selection of bacteria that have been genetically modified. (2 marks)

bacterial antibiotic resistance gene restriction enzyme binding site

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d. Is the manufacture of genetically engineered insulin by the bacteria an example of a genetically modified organism or an example of a transgenic organism? (1 mark)

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