2016 'A' Level
H2 Biology
Mark Scheme

PAPER 1 (MCQ)

Question Number	*Key*	*Question Number*	*Key*
1	**A**	21	**B**
2	**D**	22	**D**
3	**B**	23	**B**
4	**C**	24	**A**
5	**B**	25	**B**
6	**D**	26	**B**
7	**A**	27	**D**
8	**C**	28	**C**
9	**B**	29	**B**
10	**C**	30	**A**
11	**D**	31	**D**
12	**B**	32	**B**
13	**D**	33	**D**
14	**B**	34	**D**
15	**A**	35	**B**
16	**B**	36	**B**
17	**C**	37	**A**
18	**A**	38	**A**
19	**D**	39	**A**
20	**B**	40	**C**

PAPER 2 (CORE)

QUESTION 1

Fig.1.1 shows the effect of increasing substrate concentration on the rate of an enzyme-catalysed reaction in the presence and absence of a non-competitive inhibitor.

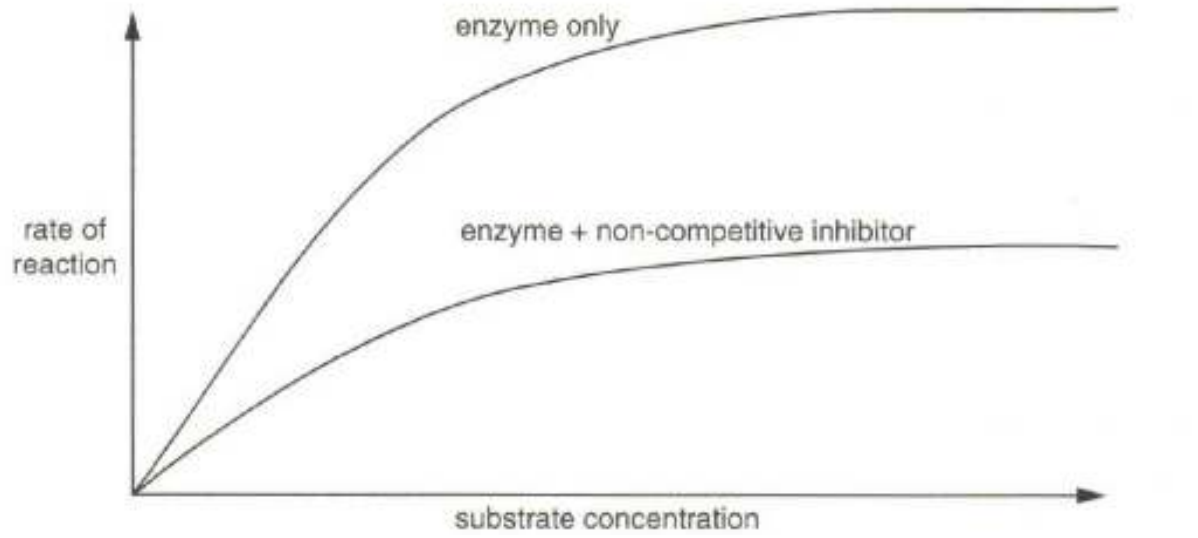


Fig. 1.1

(a) Explain why, in the reaction with the enzyme only, as substrate concentration increases :

(i) the rate of reaction increases at first **[2]**

1 Active sites of available enzyme molecules are not fully occupied by substrate, increase in <u>frequency of effective collisions</u> between enzyme and substrate molecules (as substrate concentration increases) ;
2 Increase in concentration of <u>enzyme-substrate complexes</u> formed <u>per unit time</u>, increase in concentration of <u>products</u> formed <u>per unit time</u> ;
3 Substrate concentration is limiting factor at low substrate concentration,

(ii) the rate of reaction becomes constant **[2]**

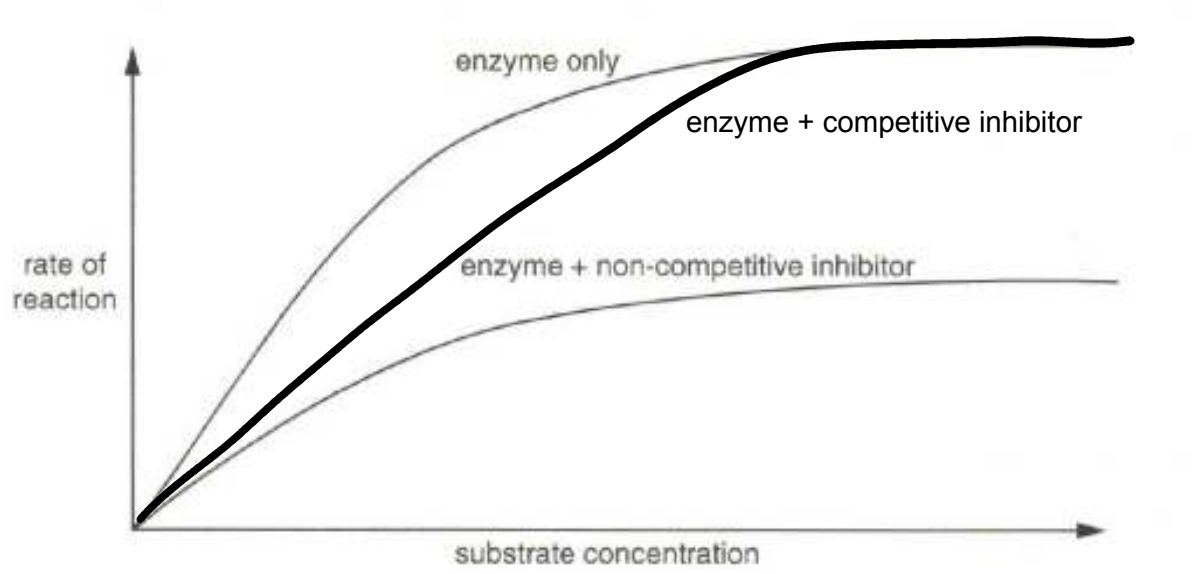
1 Active sites of available enzyme molecules are saturated with substrate molecules ;
2 Any extra substrate molecule has to wait until the E-S complex in the active site of the enzyme is released as products before the substrate can enter the active site ;
3 Enzyme concentration is limiting factor at high substrate concentration ;

(b) Explain why, in Fig. 1.1, the addition of a non-competitive inhibitor causes the reaction to become constant at a lower rate. **[2]**

1 Non-competitive inhibitor binds to the enzyme at its allosteric site,
results in change in 3D conformation of the enzyme,
3D conformation at active site altered ;

2 Substrate cannot bind to the active site,
decrease in frequency of effective collisions between enzymes and substrate molecules
/ decrease in concentration of enzyme-substrate complexes formed per unit time,
decrease in concentration of products formed per unit time ;

(c) Draw, on Fig. 1.1, the approximate shape of the curve if a competitive inhibitor were added to the enzyme instead of a non-competitive inhibitor. **[2]**



1 (Labelled) Curve beginning at 0 ;

2 V_{max} reached at higher substrate concentration ;

(d) The antibiotic penicillin irreversibly inhibits the activity of transpeptidase. Transpeptidase is a bacterial enzyme that cross-links cell wall peptides during the formation of bacterial cell walls.

Fig.1.2 shows part of each of the molecular structure of a cell wall peptide and penicillin.

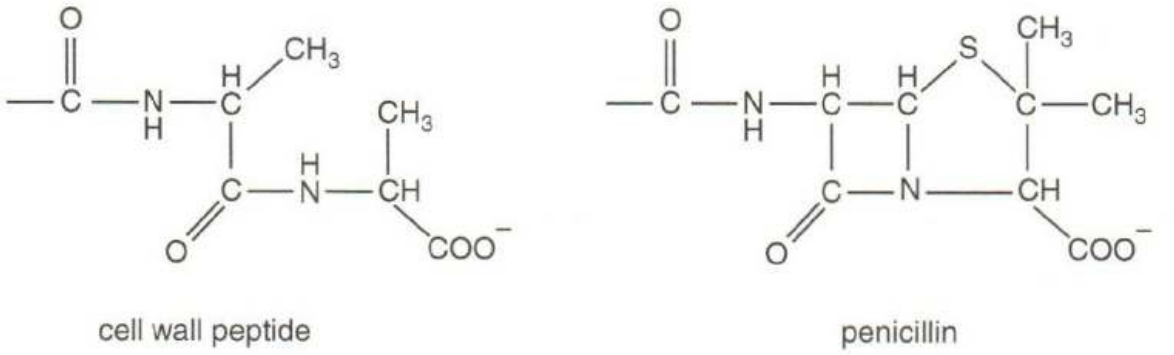


Fig. 1.2

Suggest why the penicillin molecule is an effective inhibitor of transpeptidase.**[2]**

1 Penicillin has similar 3D conformation to the cell wall peptide (substrate for transpeptidase) and competes for binding to the same active site on transpeptidase as the cell wall peptide ;

2 Prevents cell wall peptide from binding to the active site,
decreasing the concentration of the enzyme-substrate complexes formed per unit time,
preventing the cross-linking of cell wall peptides at the active site ;

Examiner's comment:
Candidates were able to suggest an explanation for the inhibition of the transpeptidase enzyme by penicillin. Not all considered binding of the inhibitor or the role of the active site.

[Total: 10]

QUESTION 2

Fig. 2.1 shows a eukaryotic transcription complex.

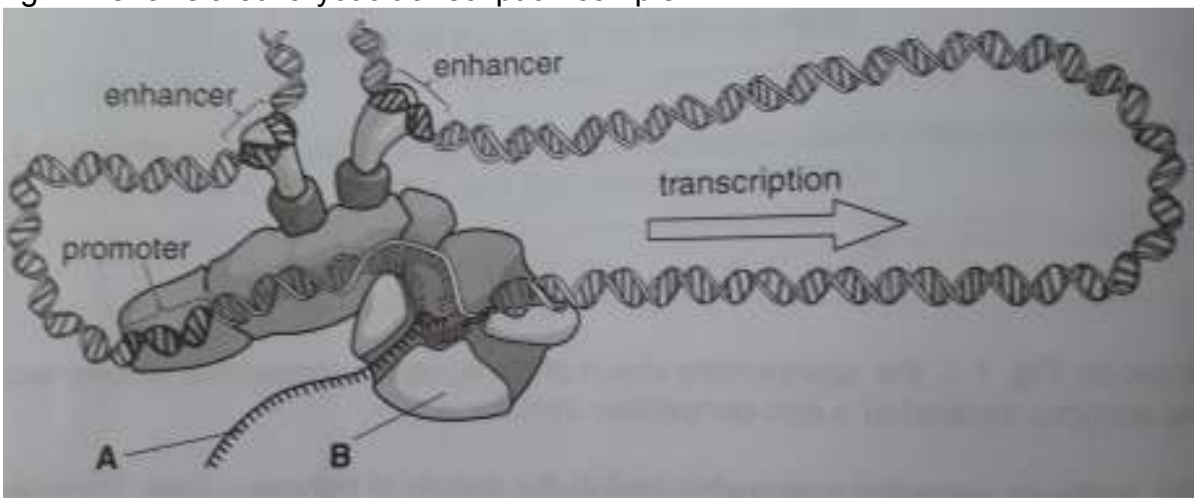


Fig. 2.1

(a) Identify the molecules labelled **A** and **B** on Fig. 2.1. **[2]**

A – Ribonucleic acid (RNA)
B – RNA polymerase

Examiner's comment: Most candidates recognised the labelled molecules.

(b) Explain how the control elements shown in Fig. 2.1 influence transcription. **[4]**

Promoter:

1. DNA sequence upstream of a gene that contains the **TATA box** to which **one transcription factor** recognises and **binds**, initiating a series of interactions between **multiple transcription factors**;
2. **RNA polymerase II binds** to the promoter of the DNA template with the **aid of transcription factors**, forming the **transcription initiation complex**, allowing the **transcription process to start**;

Enhancer:

1. Control element that **activator** protein (a type of specific transcription factor) **binds** to via the proteins' **DNA-binding domain**;
2. Activator proteins then binds to **co-activators / mediator proteins** via its **activation domain** which in turn bind to **general transcription factors and RNA polymerase II** via **protein-protein interactions**;
3. facilitate the **correct positioning of transcription initiation complex** on the promoter to **increase the rate** of transcription;

Examiner's comment: Many candidates gave full and detailed answers to this question and clearly understood the different control elements shown.

(c) Describe the role of a silencer control element in transcription. **[2]**

1. Silencer is a control element that **repressor** protein (a type of specific transcription factor) binds to. This **turns off** transcription even in the presence of activator proteins;
2. by blocking the binding of activator proteins to the control elements or to components of the transcription machinery; **OR**
3. repressor binds to its control element which is within an enhancer and act to turn off transcription even in the presence of activator proteins;

Examiner's comment: Most candidates fully appreciated the role of silencer control elements in transcription and were able to provide detailed responses.

(d) There are a number of differences between prokaryotic transcription and the eukaryotic transcription shown in Fig. 2.1.

Describe a feature of the control of prokaryotic transcription that is not shown in Fig. 2.1. **[2]**

1. The control of prokaryotic transcription involves an **operator** (a region of **DNA**) that lies **close to the promoter**;
2. An **active repressor binds** to the **operator**, preventing the **RNA polymerase** from **accessing** and **transcribing** the structural genes;

Examiner's comment: Most candidates were familiar with the control of transcription in prokaryotes and were able to describe a feature not shown in the diagram of eukaryotic transcription.

[Total: 10]

QUESTION 3

(a) Describe the changes shown in Fig. 3.1.

……..[3]

1. Number of helper T cells decreases from weeks 1-3 and increases between weeks 3-9
2. Correspondingly, number of HIV viruses increases from weeks 1-3 and decreases between weeks 3-9
3. Number of helper T cells continue to decrease from year 1 to 10 while number of HIV viruses increase gradually between year 1-6, and then sharply from year 6-10

Examiner's comments:
Most candidates provided detailed descriptions of the changes. Some candidates went on to
develop explanations for the changes, which was not a requirement of this question.

(b) Suggest how the changes in the number of T helper cells shown in Fig. 3.1 would affect the health of an untreated HIV-infected individual over the course of the infection.

……..[3]

1. As number of helper T cells decreases from weeks 1-3 and subsequently from years 1-10, the number of HIV viruses increase correspondingly;
2. Number of HIV decreases when number of T cells increase from weeks 3-9
3. [explain] When number of helper T cells decreases, there are less helper T cells to secrete cytokines to activate B cells into plasma cells which secretes antibodies / There are also less helper T cells to secrete cytokines to activate CD8 T cells into T cytotoxic cells;
4. immune system is weakened and cannot eliminate the HIV viruses effectively

Examiner's comments:
Some candidates did not make full use of the data shown in the graph to answer the question.

(c) Why is HIV described as a retrovirus?

……..[2]

1. HIV is able to perform reverse transcription using RNA genome as a template to form double stranded cDNA before integration into host gehome
2. Presence of reverse transcriptase enzyme

Examiner's comments:
The majority of candidates were familiar with the specific characteristics that make HIV a retrovirus.

(d) Explain why viruses are described as obligate parasites

……..[2]

1. They contains either DNA or RNA, but never both,
   AND
   They lack certain enzymes such as DNA polymerase and DNA-dependent RNA polymerase,
2. hence relies on host cell's enzymes for replication or transcription

3. Unable to synthesise ATP on its own.
4. ATP is used as a source of energy to allow metabolic activities to occur.

5. They lack transcription and translation machineries
   such as enzymes to transcribe DNA to mRNA and ribosomes to translate mRNA to polypeptides / proteins,
6. and hence relies on host cell's enzymes and ribosomes to make viral proteins

7. They lack raw materials for reproduction,
8. and hence relies on the host cell's nucleotides, amino acids and ATP for the synthesis of nucleic acids and (viral) proteins.

[Any 2 sets]

Examiner's comments:
Many candidates were able to address this question with a full explanation.

[Total: 10]

QUESTION 4

Fig. 4.1 shows the synthesis of polypeptides on the rough endoplasmic reticulum.

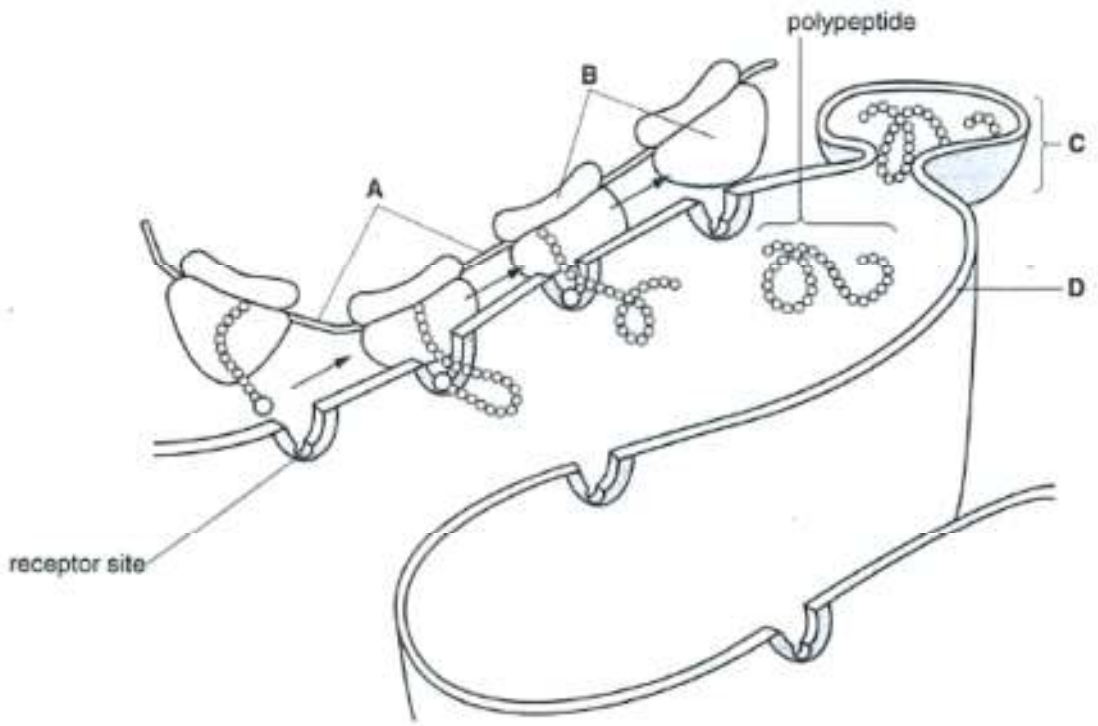


Fig. 4.1

(a) Identify structures **A**, **B**, **C** and **D**, as shown on Fig. 4.1. [4]

A messenger RNA
B ribosome / ribosomal subunits
C transport vesicle
D cisternae

Examiner's comment: Candidates were able to correctly identify structures A and B. The identification of C and D proved to be more challenging.

(b) Use Fig. 4.1 to suggest how the structures labelled **B** attach to the endoplasmic reticulum through the growing polypeptide. [2]

1. **Signal recognition particle (SRP) recognises and binds to the signal sequence of the growing polypeptide chain.**
2. **SRP recognises and binds to the SRP receptor on the RER membrane.**
3. **The SRP-signal sequences-mRNA-ribosome complex docks to the SRP receptor on the membrane.**

Examiner's comment: Careful reference to the sequence of events in translation shown in Fig. 4.1 provided all the necessary information for candidates to address this questions effectively.

(c) Suggest why the newly synthesised polypeptides shown in Fig. 4.1 cannot pass directly into the cytosol. [1]

1. **Protein is too large to pass through the membrane**

/ Protein may have hydrophilic or charged domains which are not able to bypass the hydrophobic core of the membrane

Examiner's comment: Many candidates found this question to be challenging. Those who considered the question in terms of moving polypeptides across a membrane were able to make relevant suggestions.

(d) Describe what happens to the newly synthesised polypeptides released from the rough endoplasmic reticulum. [3]

4. **Polypeptides are packaged into transport vesicles which move and fuse with the cis face of the Golgi apparatus**
5. **The polypeptides are then released into the lumen of the Golgi apparatus.**
6. **Polypeptides may undergo biochemical modifications (i.e. proteolysis, glycosylation)**
7. **Packaged into Golgi/secretory vesicles which bud off from the trans face of the Golgi apparatus and transported to various cellular locations**

Examiner's comment: Most candidates were very familiar with the processes required and were able to provide detailed responses.

QUESTION 5

Rapid-cycling brassicas ("Fast Plants") were developed to help breed disease-resistant crop plants such as cabbage and broccoli.

They are quick growing and flower two weeks after seed germination.

Many genetic traits that demonstrate Mendelian inheritance have been identified in Fast Plants.

Pure-breeding Fast Plants with purple stems and yellow leaves were crossed with pure-breeding Fast Plants with white stems and green leaves.

All the offspring (F1) had purple stems and green leaves.

These plants were allowed to self-pollinate and the phenotypes of the resultant offspring (F2) were recorded.

315	purple stems and green leaves
108	purple stems and yellow leaves
101	white stems and green leaves
32	white stems and yellow leaves

(a) Draw a genetic diagram to explain the results of the first cross in which all offspring had purple stems and green leaves.

Use the symbols **N** for the allele for purple stems, **n** for the allele for white stems, **G** for the allele for green leaves and **g** for the allele for yellow leaves. **[3]**

Parental phenotype: Purple stem, yellow leaves X White stem, green leaves
Parental genotype: NNgg X nnGG ;
Parental gametes: (Ng) (nG)

F1 genotypes ; NnGg
F2 genotypes correspond to phenotype: Purple stem, green leaves

Examiner's comment:
Nearly all candidates used the symbols given to draw a correct genetic diagram to explain the results of the first cross (F1). Some candidates did not note the requirements of the question sufficiently and used different symbols or explained the results of the second cross (F2).

1 Parental genotype ;
2 Parental gametes ;
3 F1 genotypes ;

A scientist suggested the hypothesis that the phenotypic ratio of the offspring from the second cross was 9:3:3:1. A chi-squared test was carried out to test this hypothesis and the value calculated was 0.47. There were three degrees of freedom. Table 5.1 shows part of the chi-squared distribution table for three degrees of freedom.

Table 5.1

	probability				
number of degrees of freedom	0.90	0.50	0.10	0.05	0.01
3	0.58	2.37	6.25	7.81	11.34

(b) Explain the significance of the chi-squared value for these results. **[4]**

1 Calculated χ^2 value of 0.47 corresponds to p-value of >0.90 or >90%, More than 90% probability that any difference between observed and expected results is due to chance ;
2 p value is larger than 0.05 ;
3 No significant difference between observed and expected results, predicted ratio of 9:3:3:1 is correct ;
4 Two alleles are randomly segregated into different gametes during meiosis, there is independent assortment of genes ;

(c) Suggest the advantages of using Fast Plants, instead of conventional crop varieties, as sources of useful mutations that can be introduced into broccoli and cabbage through cross-breeding. **[3]**

1 Short generation time from seedling to flowers allows quicker selection of useful traits ;
2 They grow quickly, possibly having higher rates of DNA replication which may lead to higher probability of mutations

Examiner's comment:
Most candidates found this to be a challenging question. Some did not address the focus of the question and considered the desirability of introducing mutations from Fast Plants into broccoli and cabbage rather than why Fast Plants were a better source of mutations than conventional crops.

[Total: 10]

QUESTION 6 (OUT OF SYLLABUS)

[Total: 11]

QUESTION 7

Fig. 7.1 shows the distribution of the clouded leopard, *Neofelis nebulosa*, and the Sundaland clouded leopard, *N. diardi*, in part of South-East Asia. *N. nebulosa* only occurs in mainland Asia while *N. diardi* is found only on the islands of Borneo and Sumatra.

Until 2006, all clouded leopards were thought to be members of the same species.

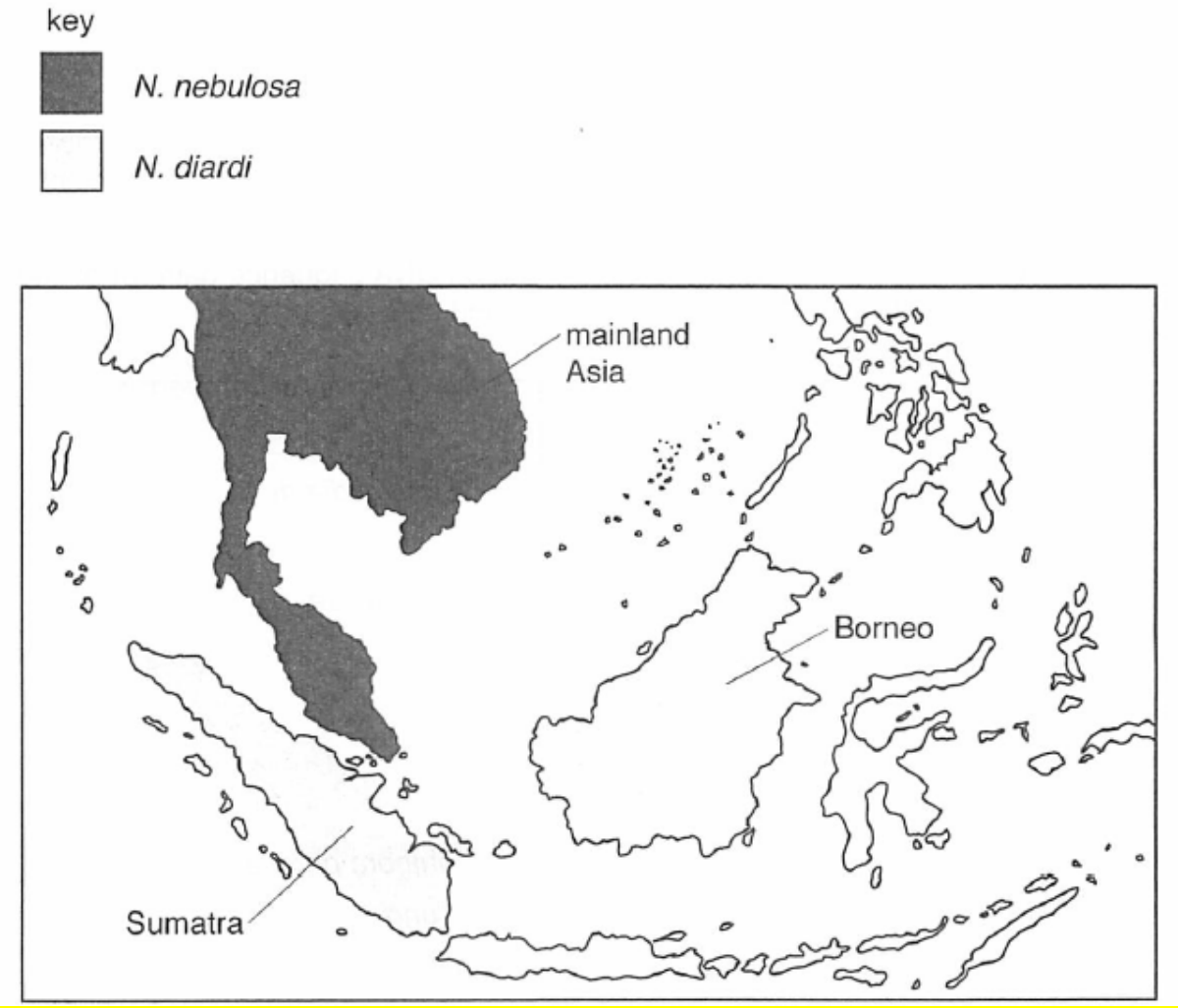


Fig. 7.1

(a) Suggest how *N. diardi*, of Borneo and Sumatra, evolved as a separate species from *N. nebulosa*, found in the rest of Asia.

……..…….. [3]

1 There is <u>geographical isolation</u> due to ocean body separating mainland Asia from Borneo and Sumatra.

There is <u>disruption of gene flow</u> between cloudy leopard populations.

2 <u>Genetic variations</u> exist among the cloudy leopard populations due to <u>mutations</u>.

3 Processes of <u>natural selection</u> and <u>genetic drift</u> occur.

4 **Different selection pressures** in different habitats causes different individuals with a selective advantage in that particular environment to survive better, reproduce more offspring and pass on their alleles to their offspring

5 Over time, changes in allele frequency occurred resulting in allopatric speciation.

Examiner's comments:
Speciation was well known by most candidates and this was reflected in detailed and clear responses.

(b) There are a number of differences between populations of *N. diardi* on Borneo and Sumatra. These are regarded as different sub-species.

Suggest why they are not regarded as separate species.

……..…….. [2]

1 Individuals in the two sub-species are still able to interbreed with each other to produce viable and fertile offspring.

2 Individuals in the two sub-species still share the same ecological niche.

3 Individuals in the two sub-species still share a lot of morphological similarities.

4 Individuals in the two sub-species still share a high level of molecular homology / similarities in their DNA/amino acid/RNA sequences.

[Any two]

Examiner's comments:
A majority of candidates limited their responses to a single aspect of what defines a species. Candidates should note the mark allocation and number of answer lines provided when considering the degree of detail that is likely to be expected in a response.

Fig. 7.2 shows the phylogeny and timescale, based on DNA sequence data, of *N. nebulosa*, two sub-species of *N. diardi* and some other closely related cats.

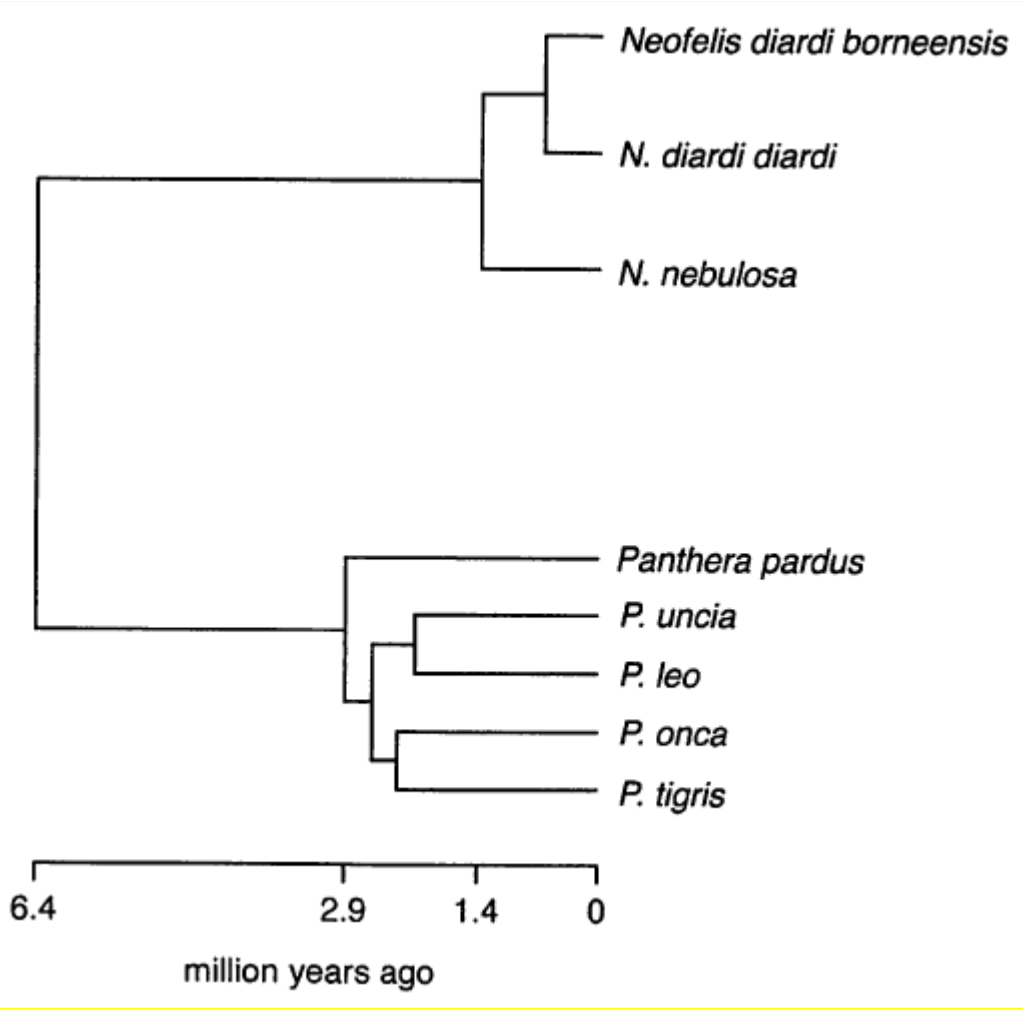


Fig. 7.2

(c) Explain whether the data in Fig. 7.2 provide sufficient evidence on their own for the existence of two separate species of *Neofelis*.

…….. [2]

1 No. The phylogenetic relationship between the two species of *Neofelis* constructed in Fig. 7.2 is based on molecular homology only.

2 Other sources of evidences are required to determine the existence of two species, such as **reproductive isolation, biogeography data and fossil records** (name at least two).

OR

1 Yes. Fig. 7.2 shows that the two species of *Neofelis* diverged from each other 1.4 million years ago.

2 Sufficient time was available for the two species to **accumulate significant differences** between them (morphological and genetic) to result in reproductive isolation.

Examiner's comments:
Candidates found this to be a challenging question. Some candidates considered the two sub-species of *N. dardi*, rather than the two species of *Neofelis*.

(d) Describe the advantages of using DNA sequence data in constructing phylogenies such as that shown in Fig. 7.2.

…….. [3]

1 Molecular data such as nucleotide and amino acid sequences are quantifiable, in abundance and open to statistical analysis, while little morphological data is available.

2 Molecular data can be easily described in an unambiguous manner for the objective assessment of evolutionary relationships.
Morphological data may be subjective, depending on the way in which it was classified.

3 Molecular data provides a clear model of evolution by comparing the nucleotide and amino acid sequence as the rate of molecular change in genes and proteins is **regular like a molecular clock**. Morphological evidence could be due to convergent evolution as similar morphology may not have been inherited from common ancestor.

4 Molecular data is based strictly on heritable material.
Morphological data is based on anatomical characters which may be influenced by environmental factors as well as variation due to genotype of the organism.

Examiner's comments:
Most candidates considered quantitative and objective aspects of data interpretation.

[Total: 10]

Question 8

Fig. 8.1 shows the main phases in the Calvin cycle.

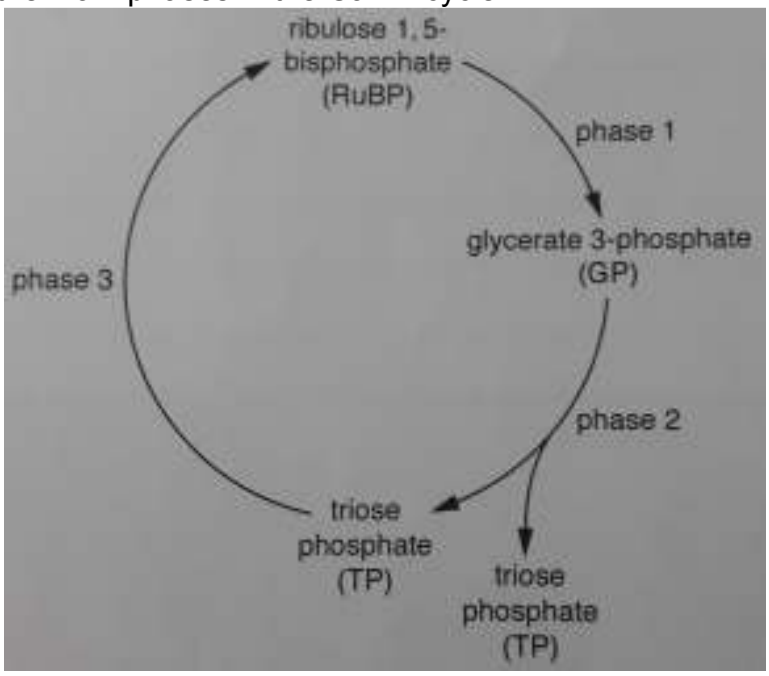


Fig. 8.1

(a) Describe what is happening in:

(i) phase 1: **[2]**

1. **Carbon dioxide fixation** where **CO_2** (1-carbon molecule) **enters** the Calvin cycle, and is **fixed by combining** with a **5C compound** called **ribulose 1,5-bisphosphate (RuBP)**, catalysed by the enzyme **rubisco (RuBP carboxylase)**, to form an **unstable intermediate 6C compound**;
2. The unstable 6C compound immediately splits into half to form **2 molecules** of a **3C compound** called **glycerate-3-phosphaste (GP)**;

Examiner's comment: Some candidates referred only to carbon fixation but did not specifically mention carbon dioxide.

(ii) phase 2: **[2]**

1. **glycerate-3-phosphaste (GP) reduction** where **GP** is **reduced** to **triose phosphate (TP)**;
2. The **electrons (hydrogen)** for this **reduction** come from **NADPH** produced from the **light-dependent reactions** and the **energy** for this step comes from **ATP** produced from the **light-dependent reactions**;
3. For every ***3*** molecules of **CO_2**, there are ***6*** molecules of **TP**, but only **1** molecule of **TP** (**3C**) **exits** the cycle, and will be used by the plant cell to synthesise carbohydrate like glucose (sugar). The other **5** molecules of **TP** must be **recycled** to **regenerate 3** molecules of **RuBP**;

Examiner's comment: Some references to reduction were unclear in candidates' responses.

(iii)phase 3: **[2]**

1. In a complex series of reactions, the carbon skeletons of **5** molecules of **TP** (**3C**) are **rearranged** by the last steps of the Calvin cycle into **3** molecules of **RuBP** (**5C**);
2. **3** more molecules of **ATP** from **light dependent reaction** used. The RuBP is now prepared to receive **CO_2** again, and the cycle **continues**;

Examiner's comment: Responses needed to be appropriate for a question with an allocation of two marks

(b) Light is not a factor that directly limits the rate of the Calvin cycle but it is needed for the Calvin cycle to continue.

Outline why light is needed for the Calvin cycle to continue. **[2]**

1. Calvin cycle uses **ATP** and consumes **NADPH** generated from **light-dependent reactions** where **phase 2 requires both ATP and NADPH** and **phase 3 requires ATP**;
2. **Without light**, **no ATP and NADPH** would be **produced** in the light-dependent reactions, thus, **phases 2 and 3 and hence Calvin cycle cannot continue**;
3. **ATP** functions as **chemical energy**. **NADPH provides a source of electrons**, and thus, provides the **reducing power** for the **reduction of GP to TP** in phase 2 of the Calvin cycle;

Examiner's comment: Most candidates correctly made the link to the products of the light dependent reaction.

(c) State two environmental factors that can directly limit the rate of the Calvin cycle and explain how they act. **[2]**

[Factor 1] Carbon dioxide concentration
[explanation] **Carbon dioxide** is a **substrate** for the enzyme **rubisco (RuBP carboxylase)** in **carbon dioxide fixation** in **phase 1**. Thus, a low concentration of CO_2 leads to a low frequency of effective collision between enzyme and substrate, a low concentration of enzyme-substrate complexes formed per unit time and hence a low concentration of products (GP) formed per unit time, limiting phase 1 of Calvin cycle;

[Factor 2] Temperature
[explanation] At low temperatures (below optimum temperature), there would be a low **kinetic energy** of both **enzyme and substrate molecules**, thus, low frequency of effective collision between enzyme and substrate, low concentration of enzyme-substrate complexes formed per unit time and hence

a low concentration of products formed per unit time, limiting the rate of enzymatic reactions (eg phase 1) in Calvin cycle;

Examiner's comment: Some candidates considered environmental factors that do not directly limit the rate of the Calvin cycle.

[Total: 10]

Question 9

(a) Describe the different functions of proteins in cell surface membranes. **[7]**

1. Membrane proteins allow **transport of polar and charged substances** across the membrane via **active transport** or **facilitated diffusion**;
2. **Channel** or **carrier proteins** are involved in **facilitated diffusion** where the net movement of molecules / substances from a region of higher concentration to a region of lower concentration, down a concentration gradient, across the cell surface membrane **does not need** the **expenditure of energy (ATP)**;
3. **Carrier proteins** known specifically as **protein pumps** are involved in **active transport** where the net movement of molecules from a region of lower concentration to a region of higher concentration requires the **expenditure of ATP** as an energy source to move substances across the membrane **against a concentration gradient**;
4. Acts as a **recognition site** that is recognised by other cells during the process of **cell-cell recognition**;
5. Acts as a **receptor site** in the process of **cell signalling**;
6. Eg. the receptor protein has a **binding site** with a specific **3D conformation** that is **complementary** to the 3D conformation of an **external** chemical messenger (eg. insulin hormone) where binding of external messenger (signal) may cause a **conformational change** in the protein receptor that **relays the message** to the **inside** of the cell;
7. Membrane proteins may function as **enzymes**. E.g. ATP synthase, which is located on the inner mitochondrial membrane, is an important enzyme catalyzing the formation of ATP;
8. Microfilaments or other elements of the **cytoskeleton** may be attached to membrane proteins, maintaining the **cell shape** and stabilizing the **location** of certain membrane proteins.

Examiner's comment: Many candidates provided detailed and wide-ranging responses.

(b) Describe the process of endocytosis. **[6]**

1. Endocytosis involves the **movement** of molecules/substances **across the membrane into the cell** via the formation of **vesicles** where **large quantities** of molecules/substances can be moved via **bulk transport**;
2. Involves the **expenditure of ATP** (from cellular respiration) as movement of vesicles along the microtubules of the cell cytoskeleton requires the **hydrolysis of ATP** as an energy source;
3. The three types of endocytosis are **phagocytosis**, **pinocytosis**, and **receptor-mediated endocytosis**;

4. Formation of the vesicle involves the **surrounding** of the **molecules/substance** by the **cell surface membrane**;
5. In **phagocytosis** (cell eating), **pseudopodia** (projections of the cell surface membrane) are formed for the **engulfing** of large particles such as micro-organisms and cell debris, forming large vesicles (or vacuoles);
6. In **pinocytosis** and **receptor-mediated endocytosis**, molecules/substances enter cells via vesicles **pinched inward from the cell surface membrane**;
7. **Receptor-mediated endocytosis** involve **specific macromolecules** **binding** to **specific recognition sites** on cell surface protein receptors. The cell surface membrane region containing the receptor-ligand complex then undergoes endocytosis, becoming a transport vesicle;
8. Receptor-ligand complexes are selectively incorporated into the intracellular transport vesicles. Examples of molecules entering the cell via receptor-mediated endocytosis include cholesterol-containing particles called low-density lipoprotein (LDL); transferrin, an iron-binding protein; insulin and most other protein hormones;

Examiner's comment: Candidates were clearly familiar with this process and many good descriptions were seen.

(c) Outline the functions of membranes within cells. **[7]**

1. Provides a **boundary** between the cytoplasm and specific contents within the membrane-bound organelles so that **separate compartments** can be formed within a cell – **compartmentalization**;
2. This allows the **maintenance of a constant internal environment** within each organelle, maintaining high concentrations of **reactants** at specific site and preventing intermediates of one **pathway** from mixing with those of another;
3. It also provides many different **local environments** that facilitate biochemical pathways. E.g. pH within the **lysosome** or **mitochondrion** can be maintained at a value which would otherwise be detrimental to the processes occurring in other parts of the cell;
4. It also allows for the establishment of a **concentration gradient / selective passage of materials** to facilitate sequential reactions to occur.
5. E.g. proton gradient generated across thylakoid membrane in chloroplast or inner mitochondrial membrane;
6. It also **increases surface area** for reaction to occur;
7. E.g. Folding of inner mitochondrial membrane results in a greater surface area for the attachment of more proteins and enzymes of the electron transport chain and ATP synthase for higher rates of ATP synthesis;

Examiner's comment: Some candidates referred to the cell surface membrane, rather than membranes within cells. Effective responses considered the full range of membranes within cells.

QUESTION 10

(a) Explain how different types of bonding hold protein molecules in shape. [7]

[Primary level]

1 Amino acids in a linear polypeptide chain are held together by peptide bonds ;

2 Sequence, number and types of amino acids determine the structure of polypeptide at its secondary, tertiary and quaternary level by determining the types of bonds that can form at these levels ;

[Secondary level]

3 Polypeptide chain folds into regular structures such as alpha helices or beta-pleated sheets, which are held together by hydrogen bonds ;

4 formed between peptide bonds found within the same polypeptide chain / the –CO group of peptide bond on one amino acid and the –NH group on peptide bond of another amino acid ;

[Tertiary level]

5 Polypeptide chain folds into its unique 3-dimensional globular conformation held by R-group interactions between amino acids (within the same polypeptide chain) ;

6 hydrogen bonds between polar R groups (of amino acid residues),
ionic bonds between charged (acidic and basic) R groups (of amino acid residues),
disulfide bonds between sulfhydryl (R) groups of cysteine residues and
hydrophobic interactions between non-polar R groups (of amino acid residues)
maintain 3D structure of polypeptide chain ;

[Quaternary level]

7 Polypeptide chain associates with other polypeptide subunits within a protein through R-group interactions between amino acids (from different polypeptide subunits) ;

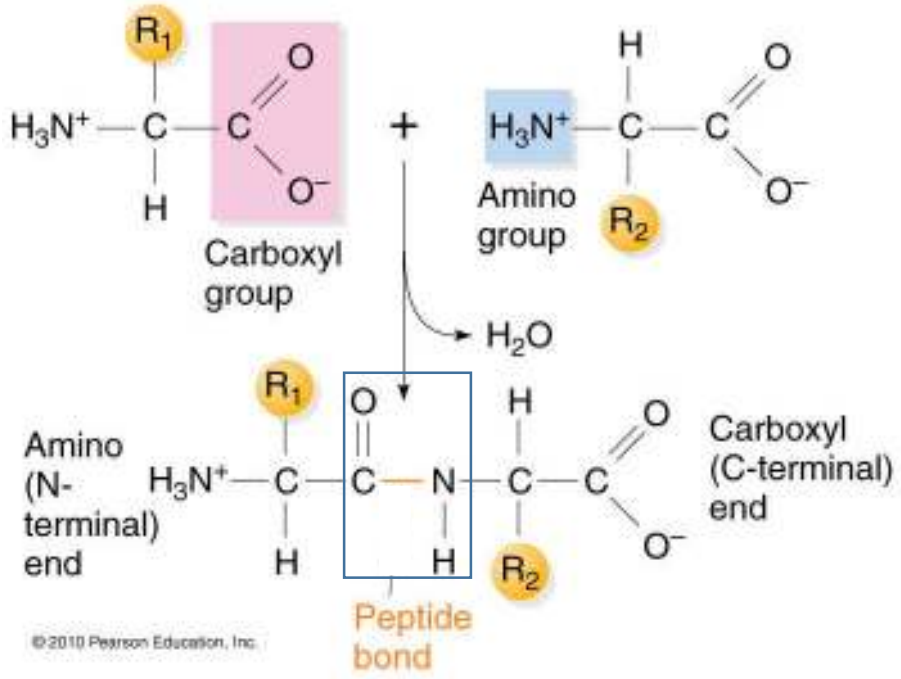
8 Held together by hydrogen bonds, ionic bonds, disulfide bonds and hydrophobic interactions ;

(b) Describe how amino acids are joined together. [6]

1 Condensation reaction between two adjacent amino acids ;
2 Reaction involving loss of a water molecule per peptide bond formed ;

3 Peptide bond formed between carboxyl group of first amino acid and amino group of following amino acid ;
4 Occurs between amino acid attached to tRNA at the P site and the amino acid of tRNA at the A site **;**
5 rRNA on large ribosomal subunit catalyses peptide bond formation, functioning as a ribozyme with peptidyl transferase activity ;

6 Annotated diagram / equation ;



Examiner's comment:
Some candidates went into great detail in an effort to cover translation of a complete polypeptide, rather than restricting their answers to joining amino acids together. Candidates often supported their answer appropriately with an equation.

(c) Outline the structure of haemoglobin and relate this to its function. [7]

Structure of haemoglobin	Property & Function
• Each polypeptide (both α and β chains) folds into primarily α-helical structure, maintained by intrachain hydrogen bonds between the peptide linkages of amino acids within the same chain	
• Each polypeptide (both α and β chains) folds into a globular shape, maintained by non-covalent **intrachain** interactions such as ionic bonds and hydrogen bonds between the R groups of amino acids within the same polypeptide. Hydrophobic interactions between hydrophobic R groups of non-polar amino acids ensure that they are shielded from the aqueous medium while the hydrophilic R groups of polar or charged amino acids are on the surface of the globular structure.	• Property : soluble in aqueous medium • Function : aids in its transport of oxygen in blood • Property : compact in shape • Function : allows maximum packing of haemoglobin into RBC for transport of oxygen in blood
• Association of prosthetic haem group per subunit to form a conjugated polypeptide.	• Property : Fe^{2+} within haem group combines reversibly with oxygen • Function : enhances release of oxygen in metabolically active tissues
• The 4 subunits associate together to form the functional haemoglobin protein which is a tetramer. The protein is globular in shape.	• Property : each functional haemoglobin molecule consists of four subunits. Each subunit is capable of binding one oxygen molecule • Function : maximises the transport of oxygen within each haemoglobin molecule
• Non-covalent interchain interactions such as ionic bonds, hydrogen bonds and hydrophobic interactions between the R groups of amino acids from different subunits are involved in the association of the tetrameric subunits. These subunits can move because of the weak interactions.	• Property : cooperative binding of oxygen / allostery • Function : slight changes in oxygen concentration causes a conformational change in one subunit which is relayed to the other subunits, resulting in greater ease of loading / unloading oxygen.

Award 1 mark for each level of protein structure (max3) ;
Award 3 marks for each correct association of structure to function (max4) ;

PAPER 3

QUESTION 1

(b) P1 nuclease is an enzyme that can completely break down both single-stranded DNA and RNA. It was originally obtained from the fungus *Penicillium citrinum*.

The enzyme can be covalently bonded to the surface of a matrix (immobilised) for use in various processes. The matrix is an inert solid material with a very high surface area to volume ratio. A substrate in solution can come into contact with the enzyme held in place at the surface of the matrix, allowing reaction to occur.

Table 1.1 and Table 1.2 show the effects of pH and temperature on immobilised and free enzyme.

Table 1.1

pH	Immobilised P1 nuclease activity /arbitrary units	Free P1 nuclease activity /arbitrary units
4.5	34	34
5.0	49	56
5.5	54	65
6.0	68	48
6.5	47	42
7.0	36	31

Table 1.2

Temperature /°C	Immobilised P1 nuclease activity /arbitrary units	Free P1 nuclease activity /arbitrary units
30	20	20
40	41	35
50	56	54
60	67	65
70	78	52
80	58	47

(i) Use the information in Table 1.1 to compare the effect of pH on immobilised P1 nuclease and free P1 nuclease.

…… [4]

1 The optimum pH for immobilized P1 nuclease is 6.0 while the optimum pH for free P1 nuclease is 5.5.

2 As pH increased from 4.5 to 5.5, the activity of both immobilized and free P1 nuclease increased: from 34 to 54 arbitrary units for immobilized P1 nuclease and 34 to 65 arbitrary units for free P1 nuclease.

3 As pH increased from 5.5 to 6.0, the activity of immobilized P1 nuclease increased from 54 to 68 arbitrary units but the activity of free P1 nuclease decreased from 65 to 48 arbitrary units.

4 As pH increased further from 6.0 to 7.0, the activity of immobilised P1 nuclease decreased sharply from 68 to 36 arbitrary units while the activity of free P1 nuclease decreased **less** sharply, from 48 to 31 arbitrary units.

Examiner's comments:
Most candidates provided sufficiently detailed comparisons of immobilised and free P1 nuclease. Some candidates described each type of separately and did not develop this further into a comparison.

(ii) With reference to your knowledge of enzyme structure and the information in **(b)**, explain the higher activity of immobilized P1 nuclease, compared to free P1 nuclease, at temperatures above 30°C, as shown in Table 1.2.

…… [3]

1 The activity of both immobilized and free P1 nuclease increased as temperature increased to an optimum temperature. Further increase in temperature decreased the enzyme activity.
2 Higher temperature increases kinetic energy of the enzyme molecules, disrupting the hydrogen bonds, ionic interactions and hydrophobic interactions between the R groups.
3 Hence, the enzyme loses its specific 3D conformation of its active site and is denatured.
4 But the optimum temperature for free P1 nuclease, 60◦C is lower than that for immobilized P1 nuclease, which is 70◦C.
5 This is because binding of enzyme to matrix can stabilize the enzyme's structure at higher temperature.

Examiner's comments:
Most candidates recognised relevant aspects of the explanation. Fewer recognised how binding of the enzyme to matrix can stabilise the enzyme's structure at higher temperature.

QUESTION 2 (OUT OF SYLLABUS)

QUESTION 3a, c(i)

(a)(i) The bone marrow contains blood stem cells which are described as being multipotent

State what is meant by multipotent.
…….[1]
1. Able to differentiate into a limited range of cell types of a specific lineage

Examiner's comments:
Most candidates correctly answered this question.

(ii) Describe the characteristics that identify all stem cells.
…….[2]
1. Self-renewal ability / divide continuously by mitosis to produce new stem cells
2. Unspecialized / do not have cell-specific structures to carry out specific functions
3. Undifferentiated

Examiner's comments:
Most candidates correctly described the characteristics of stem cells.

(b) During human reproduction, only the egg supplies the cell organelles, including the mitochondria, to the zygote. Chromosomes are supplied by both the egg and sperm.

There are a number of genetic diseases caused by mutations in the mitochondrial genome. The mitochondrial genome is very similar in structure to that of the prokaryotic genome.

The human mitochondrial genome contains about 16 600 base pairs, coding for about 40 genes. In comparison, the X-linked SCID gene contains 4227 base pairs.

(i) Suggest how the mitochondrial genome can code for about 40 genes when it is only four times the size of the SCID gene
…….[2]
1. There are very little non-coding DNA e.g. intergenic spaces in mitochondrial genome compared to nuclear genome; most sequences are coding.
2. There are no introns in mitochondrial genome as compared to genes of nuclear genome; thus, gene sequences are short in mitochondrial genome
3. There are less control elements in mitochondrial genome compared to nuclear genome

Examiner's comments:
Candidates were able to derive valid reasons based on their understanding of the nature of gene
organisation in the eukaryotic nucleus.

QUESTION 4 (SPA PLANNING)

4 Planning question

Penicillin is an antibiotic that is used to treat infections by killing bacteria.

In an investigation, a student was provided with Petri dishes containing nutrient agar. Each Petri dish had already been inoculated with *Staphylococcus* sp. and incubated to produce an evenly distributed growth of the bacteria (a bacterial lawn).

In a trial investigation, the student cut four wells into the agar and added distilled water to one of them and three different concentrations of penicillin solution to the others.

Fig. 4.1 shows the result after incubating the Petri dish for 24 hours.

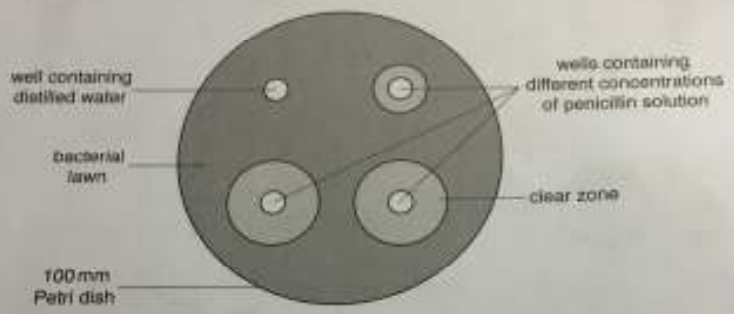


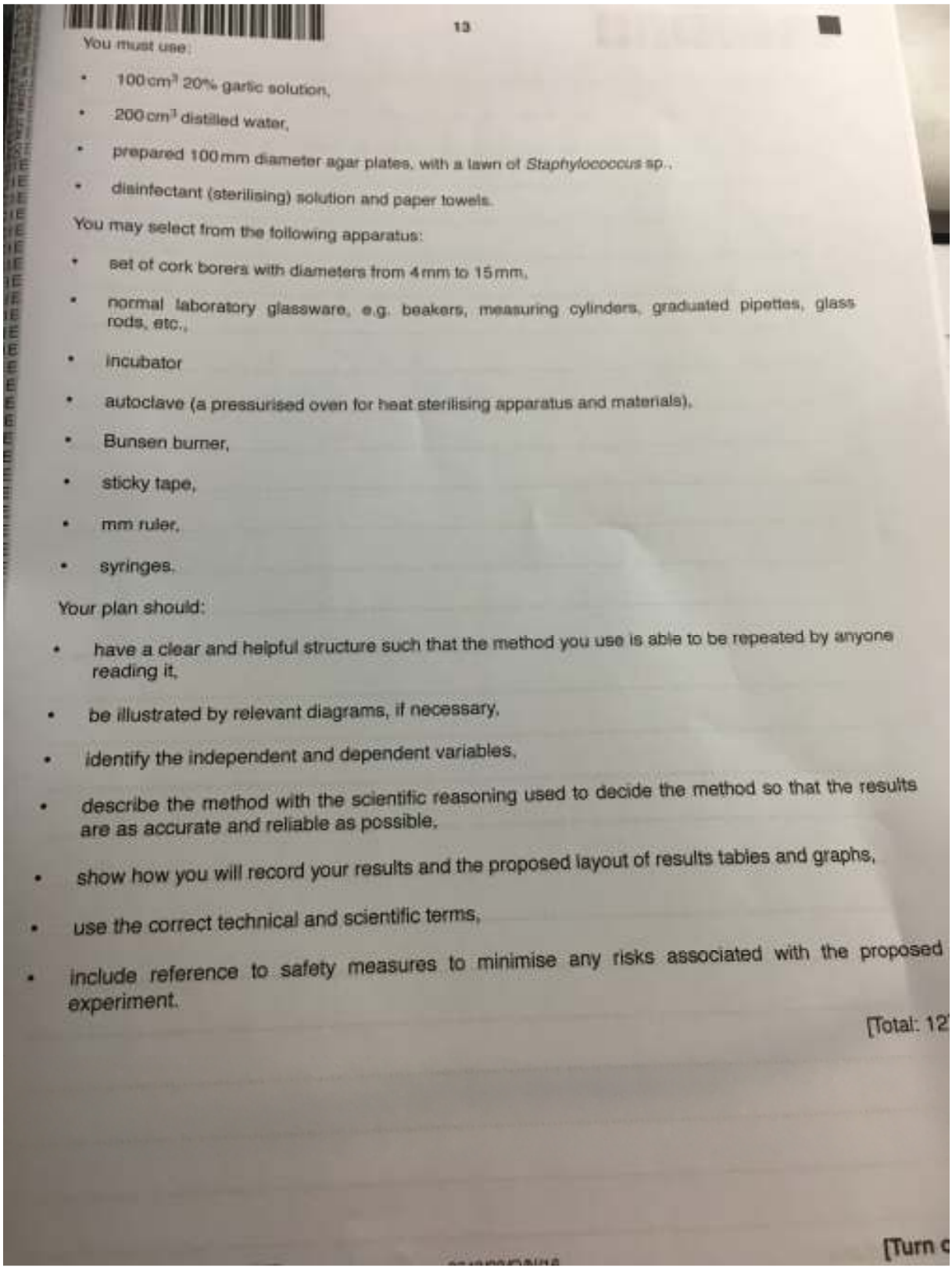
Fig. 4.1

The clear zones around the wells containing penicillin solution showed that the *Staphylococcus* sp. had been killed, whilst none had been killed by the distilled water. The student noticed that the two highest concentrations of penicillin tested had clear zones that were the same size.

The student read that garlic has long been used to treat wound infections. During the Second World War, the Russian army ran out of penicillin and used garlic as an antibiotic, leading to garlic being called the Russian penicillin.

The student wanted to find the most effective concentration of garlic solution. This is the **lowest** concentration of garlic solution that gives the largest clear zone possible.

Using this information and your own knowledge, design an experiment to find the **lowest** concentration of garlic solution that gives the largest clear zone possible.



13

You must use:

- 100 cm^3 20% garlic solution,
- 200 cm^3 distilled water,
- prepared 100 mm diameter agar plates, with a lawn of *Staphylococcus* sp.,
- disinfectant (sterilising) solution and paper towels.

You may select from the following apparatus:

- set of cork borers with diameters from 4 mm to 15 mm,
- normal laboratory glassware, e.g. beakers, measuring cylinders, graduated pipettes, glass rods, etc.,
- incubator
- autoclave (a pressurised oven for heat sterilising apparatus and materials),
- Bunsen burner,
- sticky tape,
- mm ruler,
- syringes.

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it,
- be illustrated by relevant diagrams, if necessary,
- identify the independent and dependent variables,
- describe the method with the scientific reasoning used to decide the method so that the results are as accurate and reliable as possible,
- show how you will record your results and the proposed layout of results tables and graphs,
- use the correct technical and scientific terms,
- include reference to safety measures to minimise any risks associated with the proposed experiment.

[Total: 12

[Turn o

Mark scheme

Theoretical considerations:
[Theory]
- Bacteria walls made of peptidoglycans;
- bacterial transpeptidases form cross-links between peptidoglycans;
- penicillin acts as irreversible inhibitor of bacterial transpeptidases ;
- Ref. to competitive inhibition;
- cross-links between peptidoglycans do not form ;
- cell wall weakened ;
- bacteria take in water by osmosis ; increased turgor pressure causes cell to burst ;

[Rationale]
- Garlic solution with same antibiotics properties as penicillin is placed in the form of dug-out wells on the agar gel plated with Staphylococcus bacteria.
- Measurement of the size of the clear zone formed after incubation is a measure of the antibiotic effectiveness of the garlic solution.
- The lowest concentration of garlic solution which gives the largest clear zone can be obtained based on the observations.

[Hypothesis]
- Increasing concentration of garlic solution will increase the diameter of the clear zone until certain high concentrations which produce the same diameter of the clear zone.

Variables:
Independent variable
- 5 different concentrations of garlic solution (4, 8, 12, 16, 20 %) prepared by simple dilution using distilled water.

Dependent variable:
- Diameter of clear zone / mm , measured using a mm ruler.

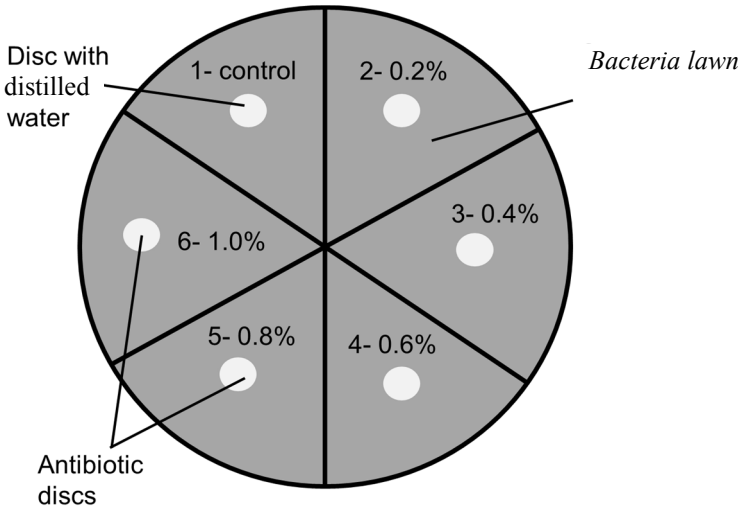
Variables to be kept constant [Minimum 2]
- Concentration and volume of bacterial culture added onto each plate, fixed at 100 mm using measuring cylinder / syringe
- Concentration and volume of agar used
- Size of wells fixed at 10mm by the 10mm cork borer
- Temperature of incubation, fixed at 30°C using the incubator
- Volume of garlic solution added into wells, fixed at 1ml using a 1ml syringe

Control:
- Replace garlic solution in one well with **equal** volume (1ml) of distilled water. This is to prove that any clear zones is due to antibiotic properties of the garlic solution.

Procedure:
1. Clean the work bench using aseptic techniques.
2. Prepare 5 different concentrations of garlic solution (4, 8, 12, 16, 20 %) from the 20% garlic stock solution, using **simple** dilution and distilled water according to the table below. Label the beakers accordingly.

Concentration of garlic solution / %	Volume of 20% garlic stock solution / cm^3	Volume of distilled water / cm^3	Total volume / cm^3
20	10.0	0.0	10.0
16	8.0	2.0	10.0
12	6.0	4.0	10.0
8	4.0	6.0	10.0
4	2.0	8.0	10.0

3. Draw lines on the base of the petri dish plastic so that the base is divided into 6 equal parts. Label the sections 1 to 6.
4. Using the 10mm cork borer, make 6 wells in each section of the agar gel.
5. Using a 1ml syringe, add in 1ml of garlic solution of varying concentration, each concentration into each well.
6. For control, prepare a well containing same volume of distilled water at 1ml.
7. The setup of the petri dish in the experiment is as shown below:



8. Conduct 3 replicates from steps 1-7 for each prepared concentrations of garlic solution. Conduct 2 repeats using fresh chemicals and apparatus.

9. Seal the petri dish with sticky tape and incubate the petri dishes at 30°C overnight.

10. Without opening the lid measure the diameter of the clear zone around each disc using mm ruler.

11. Tabulate the results to show the effect of different garlic solution concentrations on bacterial growth.

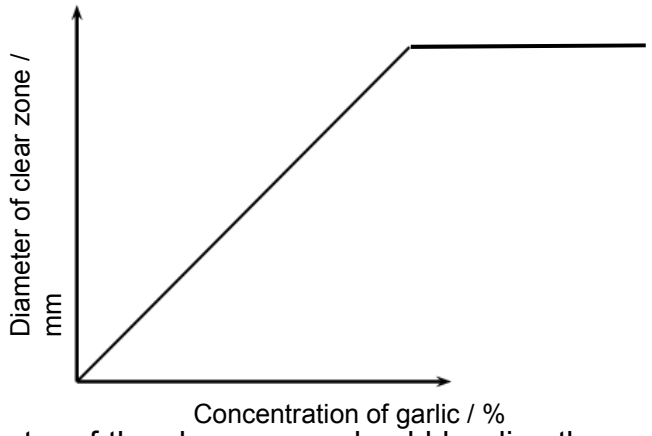
Table showing the effect of different concentration of garlic solution/% on the diameter of clear zone /mm

Conc. of garlic solution	Diameter of clear zone /mm			
	Reading 1	Reading 2	Reading 3	Average
20				
16				
12				
8				
4				
0 (Control)				

$$\text{Average} = \frac{\text{reading 1 + reading 2 + reading 3}}{3}$$

12. Plot a graph of diameter of clear zone /mm produced against the concentration of garlic solution /%.

Graph of diameter of clear zone /mm produced against the concentration of garlic /%.



The increase in the diameter of the clear zones should be directly proportional to the concentration of garlic solution, followed by plateau after a certain high concentration.

Derive the lowest concentration of garlic solution to get the largest clear zone from the graph.

Risk and safety precautions

To prevent infection or growth of harmful microorganisms:

- Cover all cut or broken skin with a waterproof dressing
- Wear tightly fitting disposable gloves and clean laboratory coat
- Clean the bench surface with bactericidal disinfectant and use a Bunsen burner which creates a sterile environment. Students should work as close as possible to the flame.
- Swap any spillages with bactericidal disinfectant.
- Proper disposal / treatment of contaminated materials or equipment using sterilizer/autoclave

Examiner's comments
Most candidates used the information provided to plan a valid method for the collection of results relevant to the investigation, making use of the available apparatus and materials. The majority were able to identify the dependent and independent variables and describe how other variables could be controlled or standardised.

Many correctly described relevant practical details such as how to prepare a suitable range of concentrations of garlic extract and how to improve confidence in results by carrying out repeats.

Some provided additional details of how to refine the method in follow-up experiments to obtain more precise estimates of the lowest concentration of garlic extract that was fully effective. Fewer considered aspects relating to safe practices when working with microorganisms, including the disposal of cultures and plates. Most candidates described how results could be recorded and presented appropriately in tables and graphs and included appropriate units.

QUESTION 5a: OUT OF SYLLABUS

QUESTION 5b, c

(b) Outline the process of nucleic acid hybridization. [10]

1 Nucleic acid hybridization can be used when analyzing RFLP, criminal investigations and paternal testing.
2 Restriction enzymes are used to cut DNA strands into fragments.
3 Agarose gel electrophoresis is performed to separate the DNA fragments based on size.
4 The gel is treated with an sodium hydroxide (an alkaline solution) to cause the double-stranded DNA to denature, separating it into single strands.
5 Denaturation is necessary so that the DNA will stick to the membrane and be hybridized by the single stranded DNA probe.
6 DNA fragments are transferred onto nitrocellulose membrane by placing a sheet of nitrocellulose membrane on top of the gel and applying pressure evenly to the gel (either using suction, or by placing a stack of paper towels and a weight on top of the membrane and gel).
7 This causes the DNA to move from the gel onto the nitrocellulose membrane by capillary action, where it sticks.
8 The membrane is then baked to permanently crosslink the DNA to the membrane.
9 The membrane is now treated with a single-stranded radioactive DNA probe which is complementary to the target sequence.
10 After hybridization, excess probe is washed from the membrane
11 The pattern of hybridization is visualized on X-ray film by autoradiography.

(c) Explain how RFLP analysis facilitates the process of DNA fingerprinting. [5]

1 DNA fingerprints make use of polymorphisms that consist of repeated sequences of DNA (eg. STRs).
2 The number of repeating segments varies greatly from individual to individual.
3 STRs found mainly in intergenic (non-gene) regions of chromosomes, especially near the centromeres.
STRs regions are flanked by restriction sites on either side
4 Homologous regions of a given STRs differ from each other in the size of the segment cut by the restriction enzyme, giving rise to unique RFLP patterns.
5 To visualize a fingerprint pattern for a specific STR site, the DNA fragments corresponding to the STR is detected by DNA hybridization with a radioactive probe.
6 X-ray autoradiography is then performed, and the pattern of bands appearing on the resulting autoradiogram is the DNA fingerprint for that individual.
7 A combination of 13 STRs are used to create unique DNA fingerprints for different individuals.

Examiner's comments:

Question 5

(a) Most candidates demonstrated an understanding of the nature of restriction fragment length polymorphism. Fewer provided explanations that considered DNA sequences, restriction sites and the role of mutation.

(b) Candidates were able to describe nucleic acid hybridisation and provide relevant examples of its use in applications such as RFLP analysis and some other contexts. Most accounts referred to several of the steps in the process and many candidates correctly identified the underlying principles. Candidates were less confident in describing how the results of nucleic acid hybridisation were visualised.

(c) Candidates were aware of the principles of DNA fingerprinting and many were able to provide specific details about the nature of the probes used. Some candidates repeated details of RFLP analysis from previous answers that were not directly relevant to this question.