JC2 Preliminary
Examination Higher 2

CANDIDATE NAME		**CT GROUP**	**15S ___**
CENTRE NUMBER		**INDEX NUMBER**	

BIOLOGY

9648 / 02

Paper 2 Core Paper

13 September 2016

Additional Materials: Writing Paper

2 hours

INSTRUCTIONS TO CANDIDATES

Write your **name**, **CT group**, **Centre number** and **index number** in the spaces provided at the top of this cover page.

SECTION A

This section contains **eight** questions. Answer **all** questions.
Write your answers on the lines / in the spaces provided.

SECTION B

This section contains **two** questions. Answer any **one** question.
Your answers must be in continuous prose, where appropriate.
Write your answers on the writing paper provided.

BEGIN EACH PART ON A FRESH SHEET OF WRITING PAPER.

A **NIL RETURN** is required for parts not answered.

INFORMATION FOR CANDIDATES

The number of marks is given in brackets [ ] at the end of each question or part question.

The use of an approved scientific calculator is expected, where appropriate. You may lose marks if you do not show your working or if you do not use appropriate units.

You are reminded of the need for good English and clear presentation in your answers.

For Examiners' Use	
Question	**Marks**
1	**/ 8**
2	**/ 8**
3	**/ 11**
4	**/ 8**
5	**/ 10**
6	**/ 9**
7	**/ 12**
8	**/ 14**
9 / 10	**/ 20**
Total	**/ 100**

BOOKLET 1

SECTION A: STRUCTURED QUESTIONS

QUESTION 1

Proteins play an important role in many biological processes.

(a) **(i)** State **two** secondary structures commonly found in proteins.

.....[1]

(ii) Compare the secondary structures stated in **(a)(i)**.

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.....[2]

Fig. 1.1 shows a mammalian DNA polymerase interacting with a DNA molecule. The catalytic and binding amino acid residues of the DNA polymerase are located at different positions on a single polypeptide chain. These residues are brought close together in the active site.

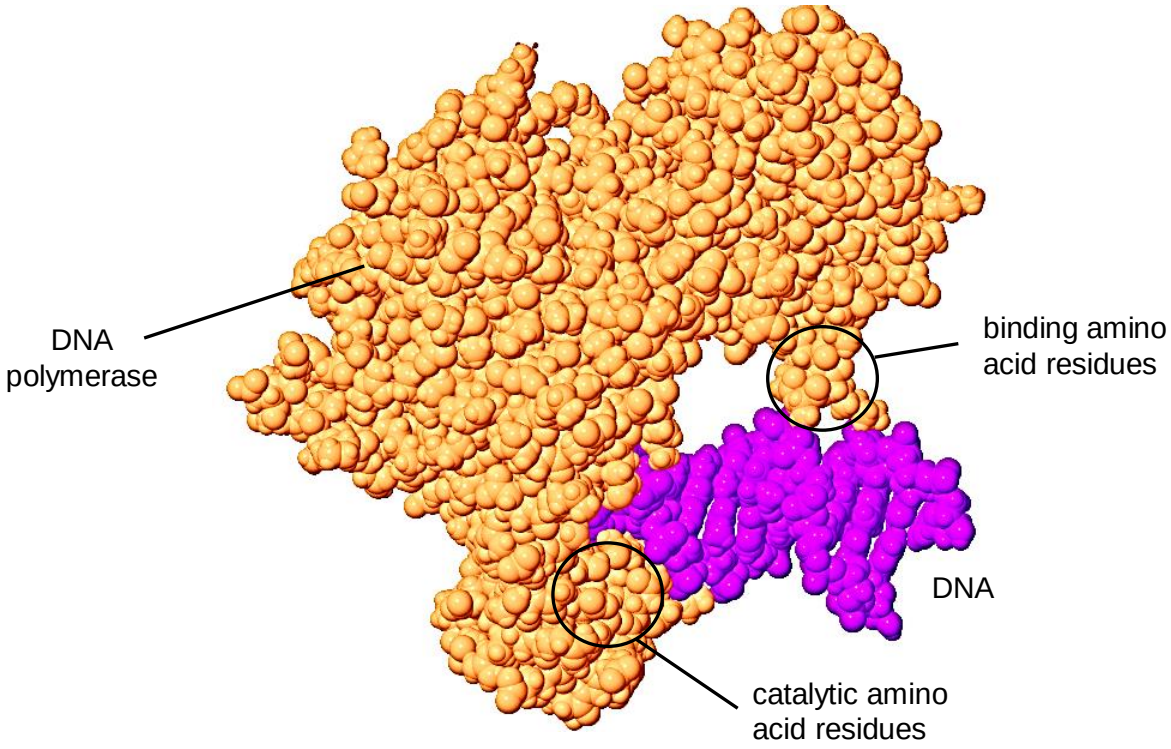

Fig. 1.1

(b) Describe how the catalytic and binding amino acid residues of DNA polymerase located at different positions are brought close together in the active site.

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..... [3]

Taq DNA polymerase has a similar function to the mammalian DNA polymerase. It is involved in polymerase chain reaction (PCR) in the presence of a pH 8.4 buffer.

(c) Explain the significance of the pH 8.4 buffer in PCR.

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..... [2]

[Total: 8]

QUESTION 2

Fig. 2.1 shows information about the movement of chromatids in a cell that has just started metaphase of mitosis.

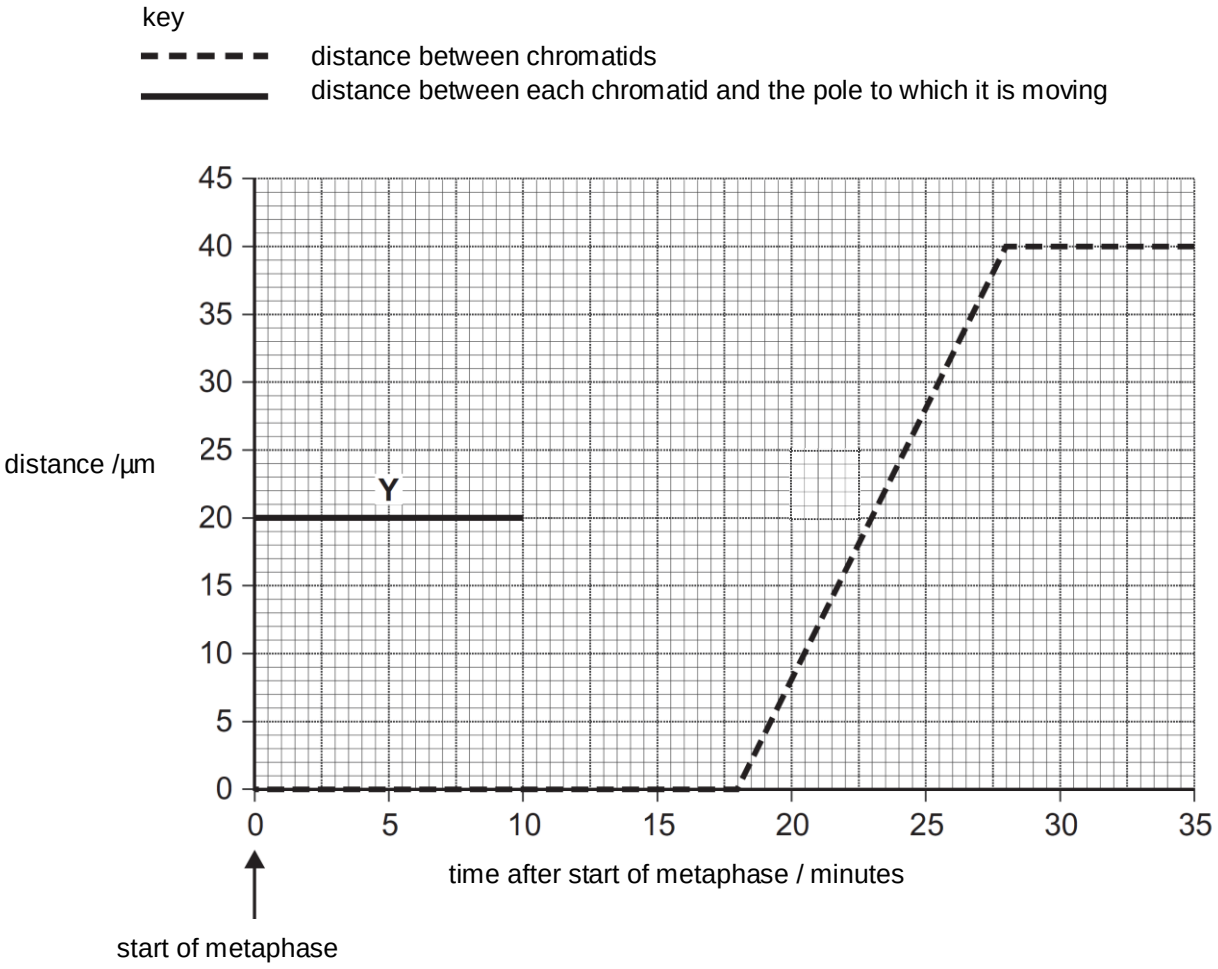

Fig. 2.1

With reference to Fig. 2.1,

(a) **(i)** state the duration of metaphase in the cell.

.....[1]

(ii) complete line **Y** on the graph. [1]

(iii) account for your answer in **(a)(ii)**.

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..... [3]

The movement of chromatids is dependent on spindle fibres, which are made up of many tubulin subunits. Spindle fibres are lengthened at one end during mitosis by the polymerisation of tubulin subunits through GTP hydrolysis.

A drug, eribulin, is known to prevent the polymerisation of the tubulin subunits.

(b) Suggest and explain the effect of eribulin on the behaviour of chromosomes in mitosis.

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..... [3]

[Total: 8]

QUESTION 3

p53 protein is a transcriptional activator that regulates the expression of *Mdm2* gene. Mdm2 protein in turn regulates the activity of p53 protein on genes involved in growth arrest, DNA repair and apoptosis.

Fig. 3.1 shows how p53 protein activates *Mdm2* gene expression.

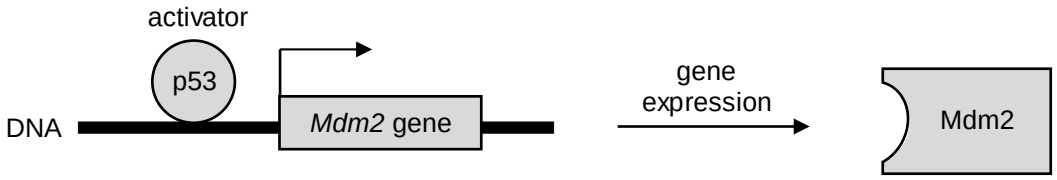

Fig. 3.1

(a) Explain the significance of p53 protein in the regulation of *Mdm2* gene expression.

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..... [3]

The cellular level of p53 protein is tightly regulated by Mdm2 protein.

When no DNA damage is detected in a cell, unphosphorylated p53 protein can bind to Mdm2 protein to trigger degradation of p53 protein via the ubiquitin system as shown in Fig. 3.2a.

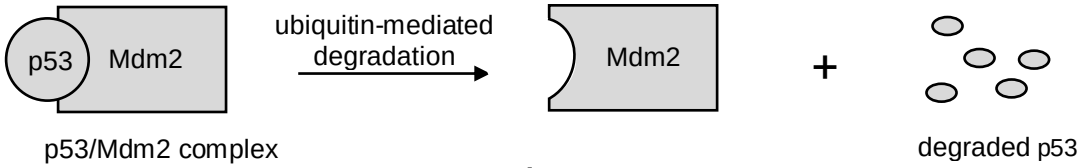

Fig. 3.2a

When DNA damage is detected in a cell, p53 protein will be phosphorylated at Serine15, Threonine18 or Serine20 amino acid residues as shown in Fig. 3.2b. In normal cells, these three amino acid residues are not phosphorylated, and p53 protein is maintained at low levels by Mdm2 protein.

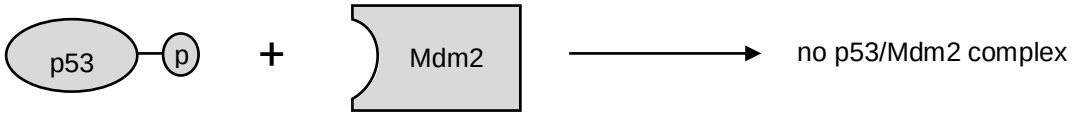

Fig. 3.2b

(b) Account for the high levels of p53 protein when DNA damage is detected in the cell.

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..... [4]

(c) Suggest the need for ubiquitin-mediated degradation of p53 protein to occur.

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..... [1]

(d) Homozygous deletion of *Mdm2* gene in mouse germline cells results in lethality at the blastocyst stage, due to inappropriate apoptosis.

With reference to Fig. 3.2a and Fig. 3.2b, suggest how inappropriate apoptosis occurs.

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..... [3]

[11 marks]

QUESTION 4

The retrovirus, human immunodeficiency virus (HIV), and the influenza virus are two types of enveloped viruses. Both enter the human host cells by adsorption and penetration. Fig. 4.1 shows the entry process of a HIV into a macrophage, which is a type of white blood cell.

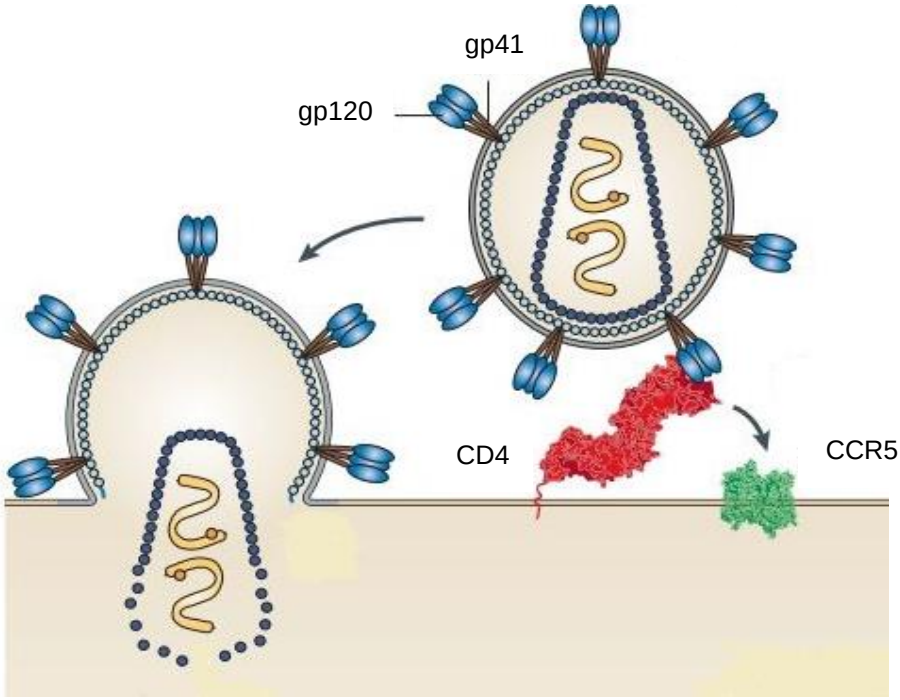

Fig. 4.1

(a) (i) State what is meant by *retrovirus*.

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..... [2]

(ii) Compare the entry processes of the HIV and influenza virus into human host cells.

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..... [3]

(b) Upon completion of the entry process, describe how the genome of HIV is inherited.

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..... [3]

[Total: 8]

QUESTION 5

George Schull, a botanist at Princeton University, conducted a genetic study of a common weed known as shepherd's purse, *Capsella bursa-pastoris*. He studied the shape of its fruit, which could be heart-shaped or narrow respectively, as shown in Fig. 5.1.

Fig. 5.1

When he crossed a pure-breeding plant with heart-shaped fruit to a pure-breeding plant with narrow fruit, the F1 generation all had heart-shaped fruit. When the F1 generation was self-fertilised, the numbers of F2 generation with the respective shaped fruit were recorded as follows:

heart-shaped fruit	2251
narrow fruit	150

Fig. 5.2 shows the possible biochemical pathway that results in the observation made in the F2 generation.

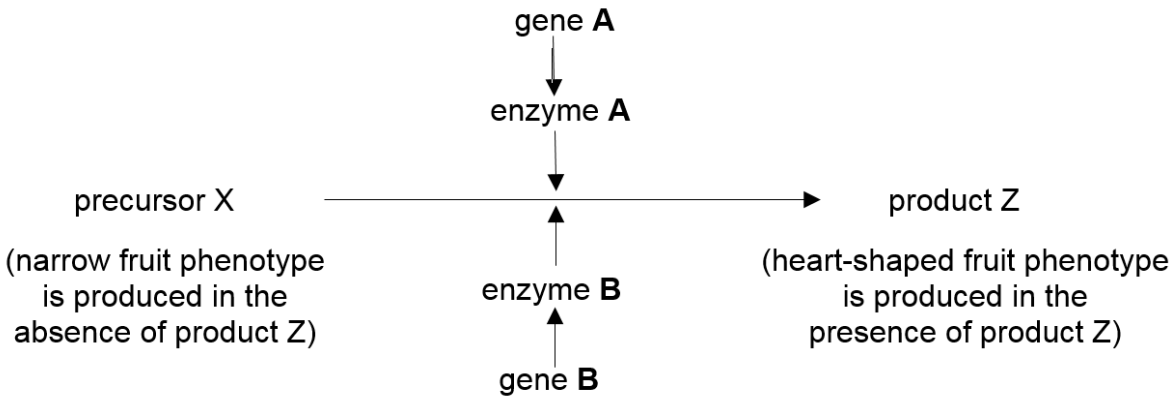

Fig. 5.2

(a) Explain the type of gene interaction observed in this context.

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..... [3]

(b) Using the symbols **A**, **a**, **B** and **b**, draw a genetic diagram to explain the results of the F2 generation in the space provided. [5]

(c) Describe how you could identify *Capsella* sp. plants with heart-shaped fruits, which are homozygous dominant in at least one gene locus.

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..... [2]

[Total: 10]

QUESTION 6

The unicellular green alga, *Chlorella*, a photosynthetic eukaryote is mass produced and harvested by commercial suppliers for use as a health food supplement.

Fig. 6.1 shows the effect of carbon dioxide concentration on the light-independent stage of photosynthesis in *Chlorella*. The following steps were carried out in a study:

- a cell suspension of *Chlorella* was illuminated using a bench lamp.
- the suspension was supplied with carbon dioxide at a concentration of 1% for 200 seconds.
- the concentration of carbon dioxide was then reduced to 0.03% for a further 200 seconds.
- the concentration of RuBP and glycerate-3-phosphate (GP) were measured at regular intervals.
- the temperature of the suspension was maintained at 25 °C throughout the investigation.

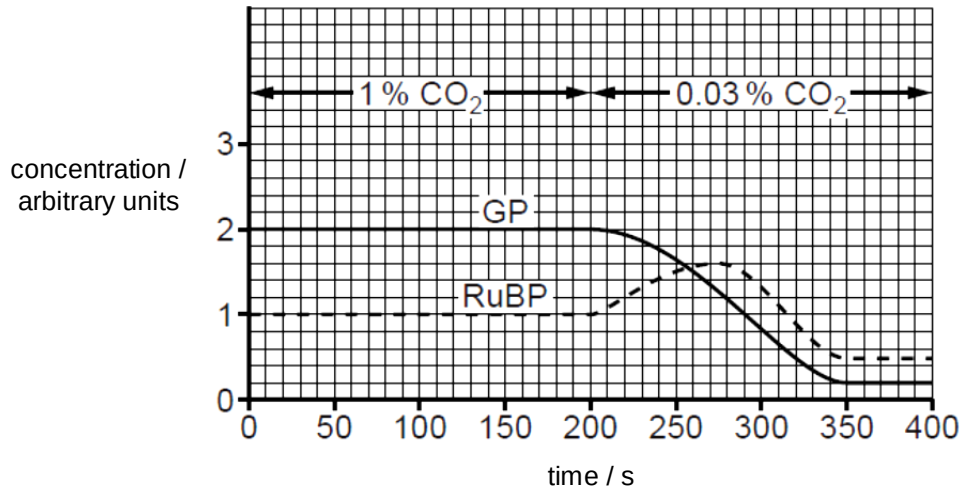

Fig. 6.1

(a) **(i)** State precisely where RuBP and GP are produced in the chloroplast.

.....[1]

(ii) Explain why the concentration of RuBP changed between 200 and 275 seconds.

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.....

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.....[2]

(b) Suggest how the decrease in the concentration of GP leads to a decrease in harvest for commercial suppliers of *Chlorella*.

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..... [2]

(c) In the light dependent stage, illumination of chloroplasts is important for maintaining the high pH in the stroma.

Explain how the illumination of chloroplasts maintains the high pH in the stroma.

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..... [3]

(d) The endosymbiotic theory postulates that the chloroplasts of *Chlorella* evolved from bacteria living within an eukaryotic host cell.

Suggest **one** structural similarity between the chloroplasts and the bacteria that supports this theory.

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..... [1]

[Total: 9]

QUESTION 7

Fig. 7.1 shows part of a myelinated motor neurone. The numbers show the membrane potential, in millivolts (mV), at various points along the axon of the myelinated neurone.

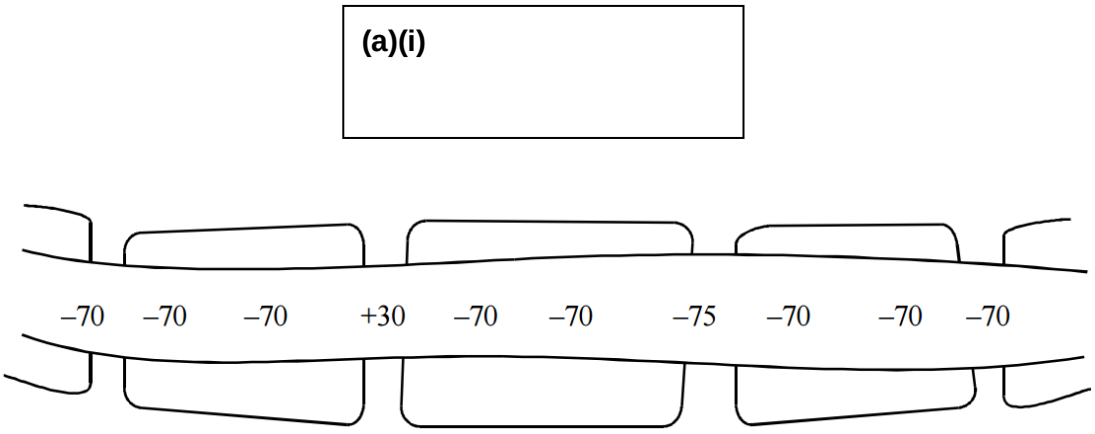

Fig. 7.1

(a) **(i)** Draw an arrow in the box provided on Fig. 7.1 to indicate the direction in which one nerve impulse is being conducted. [1]

(ii) Explain your answer in **(a)(i)**.

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.....

..... [2]

(b) Suggest why transmission of nerve impulses along a myelinated neurone uses less energy in the form of ATP than transmission along an unmyelinated neurone.

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..... [2]

Fig. 7.2 shows the changes in the concentration of sodium ions inside a segment of an axon. The neurone was stimulated at the points indicated on the graph. The neurone was treated with dinitrophenol (DNP) in region **Y**.

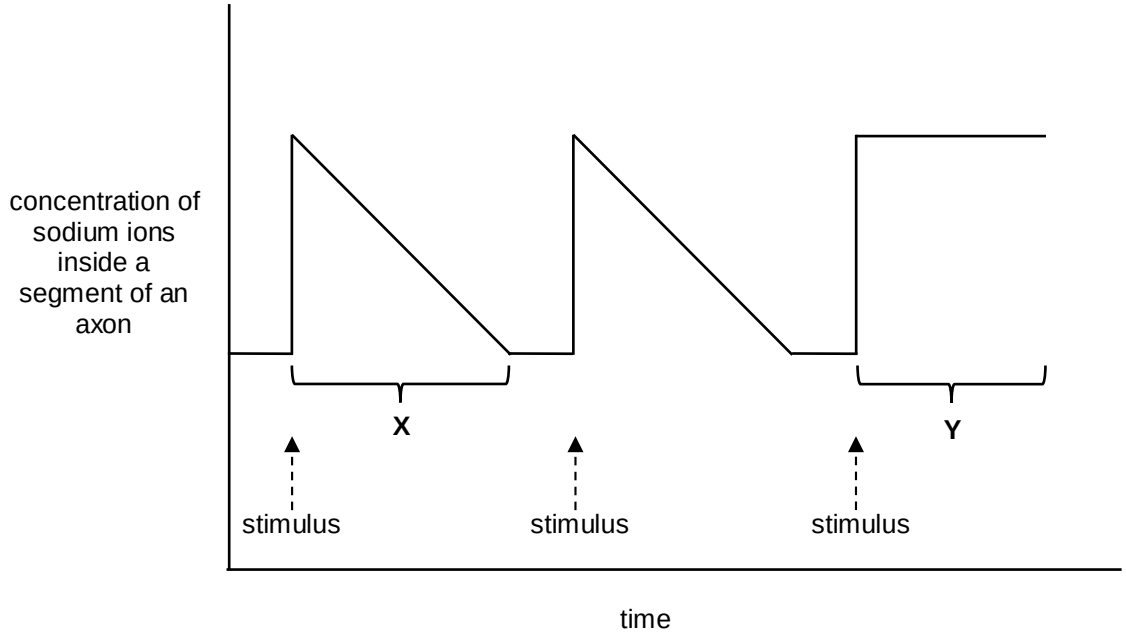

Fig. 7.2

(c) Account for the changes in the concentration of sodium ions at region **X** upon the stimulation of the neurone.

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..... [4]

DNP, a metabolic poison, makes the inner mitochondrial membrane leaky to protons during aerobic respiration.

(d) Suggest and explain why the concentration of sodium ions remain constant at region **Y**.

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..... [3]

[Total: 12]

QUESTION 8

Whales are marine mammals that belong to the order Cetartiodactyla. For a long time, scientists believed that whales are related to pigs and hippopotamuses. To reconstruct the evolutionary relationships between whales, pigs and hippopotamuses, scientists studied their limb and inner-ear bones as shown in Table 8.1.

Table 8.1

type of animal	limb bones	inner ear bones
whales	thick	thick
pigs	thin	thin
hippopotamuses	thick	thin

(a) Explain how the anatomical similarities among the whales, pigs and hippopotamuses support Darwin's theory of natural selection.

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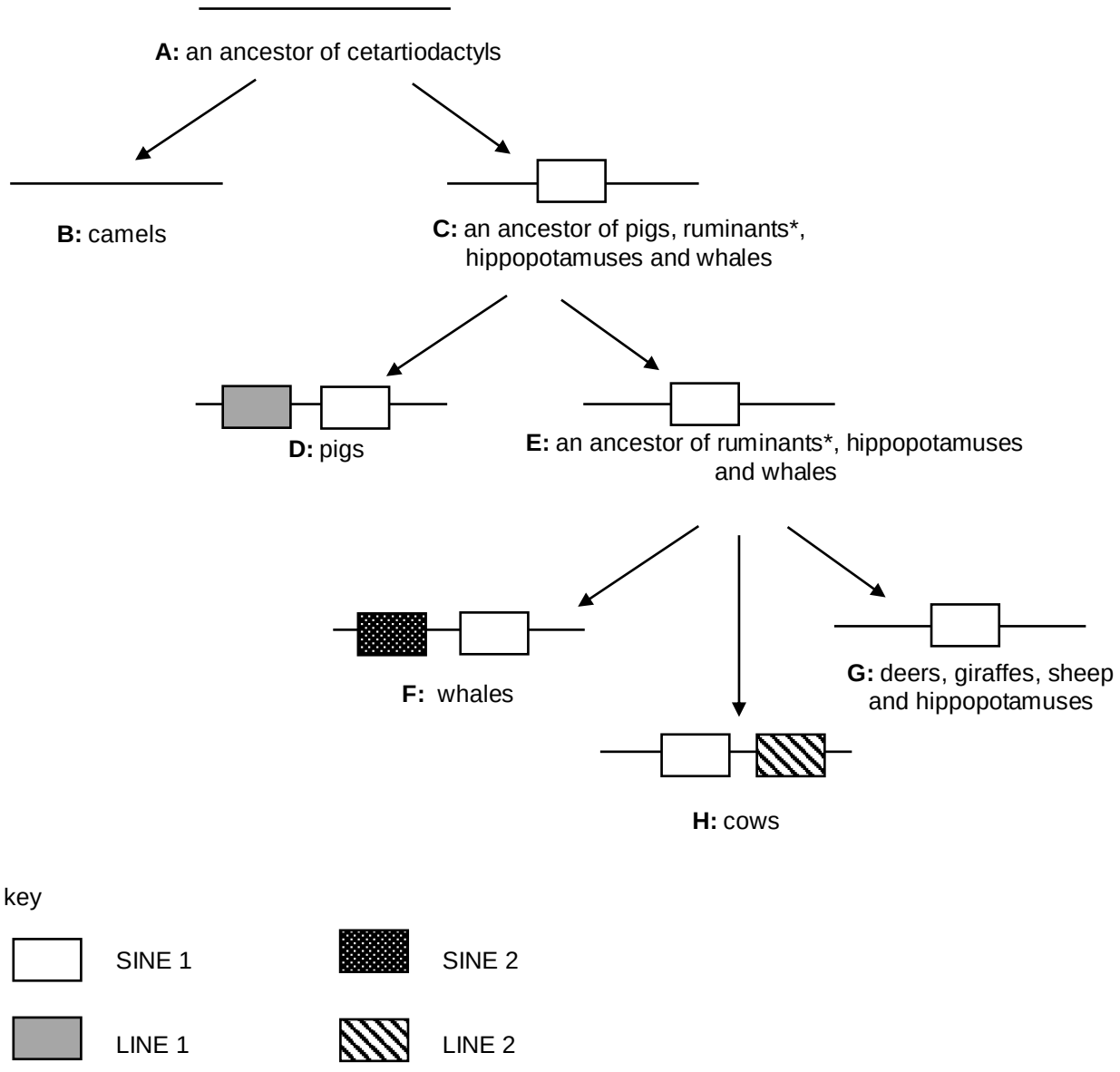
..... [3]

Phylogenetic relationships can be elucidated using molecular data. An important method of analysis involves the study of both Short Interspersed Elements (SINEs) and Long Interspersed Elements (LINEs). SINEs and LINEs are non-coding DNA sequences that have been amplified and inserted into different genomic regions.

Fig 8.1 is a schematic representation of SINEs and LINEs insertions at homologous genomic regions among the subgroups of cetartiodactyls.



*ruminants include deers, giraffes, sheep and cows

Fig. 8.1

(b) **(i)** Explain why camels and whales are of different species according to the phylogenetic species concept.

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..... [3]

(ii) A phylogenetic tree is constructed based on the results of SINEs and LINEs analysis in Fig 8.1. Fill in the blanks at the nodes and end points of the phylogenetic tree with the corresponding letters, **A** to **H** as represented in Fig. 8.1. [2]

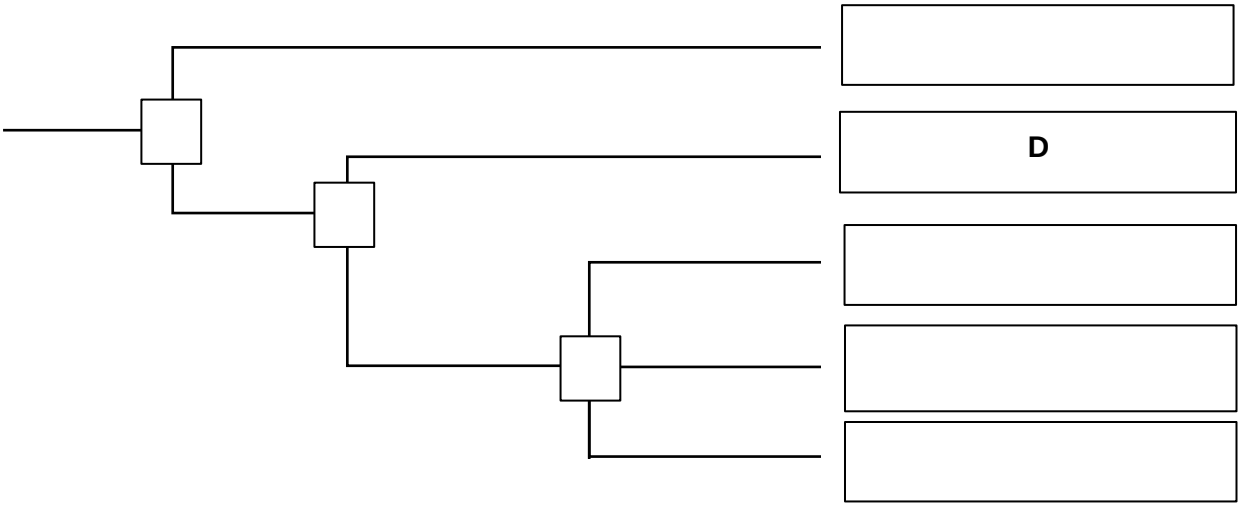

Fig. 8.2

(c) Molecular changes in SINEs and LINEs occur over time due to mutations and these are hypothesised to follow the neutral theory of evolution.

Briefly describe the neutral theory of molecular evolution and suggest how it may be used to study the subgroups of cetartiodactyls.

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..... [4]

(d) The presence of SINEs and LINEs increase the genome size over time. However, this does not contribute to a significant increase in nuclear size.

Outline how the organisation of the genome allows for DNA packaging in a non-dividing cell.

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..... [2]

[Total: 14]

--- End of Section A ---

SECTION B: FREE RESPONSE QUESTION

Answer **one** question.

BEGIN EACH PART ON A FRESH SHEET OF WRITING PAPER.

Your answer should be illustrated by large, clearly labelled diagrams, where appropriate.

Your answer must be in continuous prose, where appropriate.

You answer must be set out in parts **(a)**, **(b)** etc., as indicated in the question

A **NIL RETURN** is required for any parts not answered.

QUESTION 9

(a) Describe how the molecular structure of cellulose is related to its function. [6]

(b) Outline the basis of the selective permeability of the cell membrane with reference to phospholipids, cholesterol and proteins. [8]

(c) Explain why animal cells mainly store lipids instead of carbohydrates. [6]

[Total: 20]

QUESTION 10

(a) Describe how the molecular structure of the G-protein coupled receptor is suited for its role in glucagon-mediated cell signalling. [8]

(b) Outline the concept of negative feedback in regulating glucagon levels in the body. [6]

(c) Explain how signal amplification is illustrated upon the binding of insulin to its receptor. [6]

[Total: 20]

--- End of Section B ---

--- End of Paper ---

MULTIPLE CHOICE QUESTIONS

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

STRUCTURED QUESTIONS

QUESTION 1

(a) (i) State **two** secondary structures commonly found in proteins. [1]

α-helix and β-pleated sheet ;;

(ii) Compare the secondary structures stated in **(a)(i)**. [2]

- Structures are formed as a result of regular/ repetitive, coiling / folding, of the polypeptide chain ;;
- The α-helix and β-pleated sheet are stabilised by intrachain hydrogen bonds between carbonyl (C=O) and amine (–NH) polypeptide backbone ;;
- The α-helix takes the form of an extended spiral spring/ coiled conformation whereas the β-pleated sheet has an extended sheet-like / pleated / zigzag conformation ;;

(b) Describe how the catalytic and binding amino acid residues of DNA polymerase located at different positions are brought close together in the active site. [3]

- Polypeptide chain is first folded and coiled into its secondary structures α-helix and β-pleated sheet ;;
- into specific 3D conformation of active site formed by catalytic and binding amino acid residues;;
- involving various interactions between the R groups of the structural amino acid residues *via* hydrophobic interactions, ionic bonds, hydrogen bonds and disulfide bonds/ covalent bonds (any two);;

(c) Explain the significance of the pH 8.4 buffer in PCR. [2]

- optimum pH ;;
- the rate of reaction is at a maximum/ highest amount of products formed per unit time ;;

QUESTION 2

(a)(i) state the duration of metaphase in the cell. [1]

18min

(ii) complete line Y on the graph. [1]

Horizontal until 18 minutes, then decreases as straight line to 0 μm at 28 minutes

(iii) explain your answer in **(a)(ii)**. [3]

- Chromosomes align singly at the metaphase plate during metaphase of mitosis.
- Sister chromatids separate at the centromere to become daughter chromosomes and migrate towards the opposite poles in anaphase.
- Each chromatid / daughter chromosome did not move / remain at the pole in telophase.

**quoting of data is necessary to support the answers above.*

(b) Suggest and explain the effect of eribulin on the behavior of chromosomes in mitosis. [3]

- Kinetochore microtubules cannot attach to the kinetochores at the centromeres of the chromosomes.
- Cells cannot progress through metaphase, so that chromosomes cannot align singly at the metaphase plate.
- Sister chromatids could not separate / remain attached in anaphase.

QUESTION 3

(a) Explain the significance of p53 protein in the regulation of Mdm2 gene expression. [3]

- As an activator, p53, binds to the enhancer;;
- May interact with mediator proteins;
- To improve recruitment of general transcription factors and RNA polymerase to the promoter;
- to form a stable transcription initiation complex (TIC) at the promoter;
- Ref. to upregulation of transcription of *Mdm 2* gene/ increase rate of transcription of *Mdm2* gene;

(b) Account for the high levels of p53 protein when DNA damage is detected in the cell. [4]

- During DNA damage, p53 protein is activated by phosphorylation of p53 at Ser15, Thr18 or Ser20 amino acid residues;;
- resulting in the 3D conformation of p53 protein to be no longer complementary to the 3D conformation of Mdm2 protein;;
- Hence p53 cannot bind to Mdm2 protein and p53/Mdm2 complex is not formed;;
- Thus, p53 is not degraded via ubiquitin system/ avoiding ubiquitin-mediated degradation to increase levels of p53 protein;;

(c) Suggest the need for ubiquitin-mediated degradation of p53 to occur. [1]

- To remove unwanted p53 protein/ maintain low levels of p53 when no DNA damage is detected;;

(d) Homozygous deletion of *Mdm2* gene in mouse germline cells results in lethality at the blastocyst stage, due to inappropriate apoptosis.
With reference to Fig. 3.2a and Fig. 3.2b, suggest how inappropriate apoptosis occurs. [3]

- With the homozygous deletion of the Mdm2 gene, no functional Mdm2 protein can be produced;;
- In normal cells without DNA damage, p53 has no Mdm2 protein to bind to/ p53/Mdm2 protein complex not formed;
- Resulting in no ubiquitin-mediated degradation of p53/Mdm2 complex/ p53 is not degraded via the ubiquitin system;
- Hence, leading to high levels of unbound p53 which activates apoptosis in cells;;

- despite having no DNA damage;

QUESTION 4

(a) (i) State what is meant by retrovirus. [2]

- Retroviruses are viruses with two identical copies of single stranded RNA;;
- and two molecules of reverse transcriptase ;;

(ii) Compare the entry process between the HIV and influenza virus. [3]

- Adsorption/ attachment of both viruses are by binding to specific cell surface receptors. ;;
- Glycoprotein gp120 on the surface of the HIV binds to CD4, a cell-surface receptor found on white blood cells/ T helper cells / macrophages of the host immune system ;
- Haemagglutinin on the influenza viral membrane binds to sialic acid-containing receptors on the host cell membrane ;
- The influenza virus enters the host cell by receptor-mediated endocytosis;
- The HIV envelope does not enter via receptor-mediated endocytosis. Instead, the HIV envelope fuses with the host cell membrane;
- Upon entry, the influenza virus forms an endosome / endocytic vesicle ;
- whereas the HIV virus does not form an endosome/ endocytic vesicle, the HIV releases the viral contents into the host cell cytoplasm.;

(b) Upon completion of the entry process, describe how the genome of HIV is inherited. [3]

- RNA is reverse transcribed to complementary DNA strand by the enzyme reverse transcriptase ;;
- The enzyme integrase catalyses the integration of the viral DNA into the host chromosome.;;
- The provirus genome is also replicated along with the host cell genome and all daughter cells inherit the HIV genome/ AW ;

QUESTION 5

(a) Explain the type of gene interaction observed in this context. [3]

- The type of gene interaction is (duplicate dominant) epistasis;;.
- Allele A is epistatic to alleles B and b , allele B is epistatic to alleles A and a;;
- The presence of at least 1 dominant allele of either gene A or B causes the conversion of precursor X to product Z hence, allowing the formation of heart-shaped fruits;;.
- If the genotypes at both loci are homozygous, the conversion of precursor X to product Z does not occur. ;;

(c) Using the symbols **A**, **a**, **B** and **b**, draw a genetic diagram to explain the result of the F2 generation in the space provided. [5]

Let A be the dominant allele for the production of heart-shaped fruit
Let a be the recessive allele for the production of narrow fruit;
Let B be the dominant allele for the production of heart-shaped fruit
Let b be the recessive allele for the production of narrow fruit;

Parental phenotype Heart shaped fruit x Narrow fruit

Parental genotype AABB x aabb ;

Parental gametes (AB) (ab) ;

F1 genotype AaBb ;

F1 phenotype Heart-shaped fruit

F1 self-cross AaBb x AaBb

Parental gametes (AB) (Ab) (aB) (ab) x (AB) (Ab) (aB) (ab) ;

Random fertilization

Male gametes \ Female gametes	(AB)	(Ab)	(aB)	(ab)
(AB)	AABB	AABb	AaBB	AaBb
(Ab)	AABb	AAbb	AaBb	Aabb
(aB)	AaBB	AaBb	aaBB	aaBb
(ab)	AaBb	Aabb	aaBb	aabb

;;

F2 genotypic ratio 9 A_B_ : 3 A_bb: : 3 aaB_ : 1 aabb ;

F2 phenotypic ratio 15 Heart-shaped fruit : 1 Narrow fruit ;

F2 observed no 2251 : 150

(d) Describe how you could identify *Capsella* sp. plants with heart-shaped fruits, which are homozygous dominant in at least one gene locus. [2]

- Perform a testcross;;
- If the plants with heart-shaped fruits are homozygous dominant in at least one gene locus, all offspring produced will only have heart-shaped fruits;;.

QUESTION 6

(a) **(i)** State precisely where RuBP and GP are produced in the chloroplast. [1]

stroma

(ii) Explain why the concentration of RuBP changed between 200 and 275 seconds. [2]

- lower CO_2 concentration
- concentration of RuBP increases from 1 au to 1.6 au
- less CO_2 fixed by RuBP

(b) Suggest how the decrease in the concentration of GP leads to a decrease in harvest for commercial suppliers of *Chlorella*. [2]

- less triose phosphate / glyceraldehyde-3-phosphate will be produced
- so less conversion to carbohydrates / lipids / amino acids / proteins

(c) In the light dependent stage, illumination of chloroplasts is important for maintaining the high pH in the stroma. Explain how the illumination of chloroplasts maintains the high pH in the stroma. [3]

- excited electrons leave, special chlorophyll *a*
- electron passed down the electron transport chain
- protons pumped into thylakoid lumen from the stroma
- protons present from photolysis of water

(d) The endosymbiotic theory postulates that the chloroplasts of *Chlorella* evolved from bacteria living within an eukaryotic host cell. Suggest **one** structural similarity between the chloroplasts and the bacteria that supports this theory. [1]

- ref. to chloroplasts having a double membrane / 70S ribosomes

QUESTION 7

(a) (i) Draw an arrow in the box provided on Fig. 7.1 to indicate the direction in which one nerve impulse is being conducted and explain your answer. [1]

Arrow pointing to the left ;;

(ii) Explain you answer in (a)(i). [2]

- Hyperpolarisation occurs during the refractory period;;
- Hence the nerve impulse can only travel towards the left where depolarisation occurs. ;;

(b) Suggest why transmission of nerve impulses along a myelinated neurone uses less energy in the form of ATP than transmission along an unmyelinated neurone. [2]

- For myelinated neurone, charges can only be leaked through the nodes of Ranvier, compared to myelinated neurone, where charges can be leaked throughout the neurone ;;
- Hence, less energy in the form of ATP is required by active transport pumps, Na^+/K^+ pumps to restore the resting membrane potential ;;

(c) Account for the changes in the concentration of sodium ion at region X upon the stimulation of the neurone. [4]

- Upon the stimulation of the neurone above threshold potential, depolarisation occurs at the axon hillock and voltage-gated Na^+ channels open ;
- Na^+ influx / Na^+ enters the neurone ;;
- resulting in sudden increase/ spike in concentration of sodium inside the neurone ;
- subsequently, repolarisation occurs and voltage-gated Na^+ channel close / membrane becomes less permeable to Na^+ ;
- Na^+/K^+ pumps will restore concentration of sodium in the neurone ;;
- leading to gradual decrease in concentration of Na^+ until resting membrane potential is achieved ;

(d) Suggest and explain why the concentration of sodium ions did remain constant at region Y. [3]

- Proton gradient cannot be maintained ;;
- ATP cannot be generated via chemiosmosis ;;
- Ref. to sodium ions entering the neurone but cannot be transported out of the cell;;

QUESTION 8

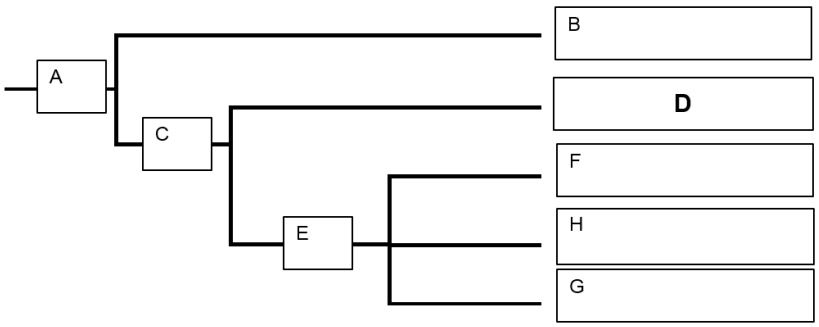
(a) Explain how the anatomical similarities among the whales, pigs and hippopotamuses support Darwin's theory of natural selection. [3]

- Limb and inner ear bones were both present in the ancestor / Cetartiodactyls;
- The presence of homologous structures / anatomical homologies between the pigs, hippopotamus and the whales signify shared / common ancestry / evolutionary relatedness;
- These animals will survive to sexual maturity / survival of the fittest / differential reproduction to pass on the favourable genes / alleles to their offspring;.
- leading to genetic variation that brings about differences in limb and inner ear bone thickness;.
- Hence showing decent with modification;

(b)(i) Explain why camels and whales are of different species according to the phylogenetic species concept. [3]

- In the phylogenetic species concept, a species is defined as the smallest group of individuals that share a common ancestor;;
- Camels and whales do not share a most recent common ancestor;
- The camels are the earliest to diverge from the ancestor of cetartiodactyls ;
- as they do not possess sequences of SINE 1 in their genome / ref made to shared derived characteristics with quotation;
- Because they possess SINE1 / SINE 1 & 2 in their genome / ref made to shared derived characteristics with quotation;

(ii) A phylogenetic tree is constructed based on the results of SINE analysis in Fig 8.1. Fill in the blanks at the branch points and end points of the phylogenetic tree with the corresponding letters, **A** to **H** as represented in Fig. 8.1. [2]



(c) Briefly describe the neutral theory of molecular evolution and suggest how it may be used to study the subgroups of Cetartiodactyls. [4]

- The neutral theory states that vast majority of evolutionary change at the molecular level are caused by random genetic drift of selectively neutral mutations;;
- which do not affect the phenotype / fitness of the organism / not subjected to natural selection / has neither selective advantage or disadvantage.;;
- Since SINE sequences are non-coding, mutations in them are not affected by natural selection;
- The greater the difference in number of mutations;
- the longer time of divergence.;;

(d) Outline how the organization of the genome allows for DNA packing in a non-dividing cell. [2]

- DNA molecule is wound around histone proteins to form the **nucleosome;;**
- Nucleosomes are linked by liner DNA to form **10nm fibers chromatin**;
- nucleosomes are further packed into **30-nm fibres / solenoids;**

FREE RESPONSE QUESTION

QUESTION 9

(a) Describe how the molecular structure of cellulose is related to its support function. [6]

- Alternate inverted β-glucose units linked by β(1,4) glycosidic bonds allow cellulose to form long, unbranched and straight chains;;
- Allow formation of linear chains of polysaccharides that can be packed tightly;;
- Many chains run parallel to each other and their hydroxyl group (OH) project outwards from each chain;;
- Extensive hydrogen bonds form between parallel chains/ Extensive hydrogen bonds form between the protruding OH groups of neighbouring chains;;
- Cross-linked cellulose chains associate in groups to form microfibrils;;
- Microfibrils associate with other, non-cellulose polysaccharides, and are arranged in large bundles to form macrofibrils;;
- Allows formation of a large molecule, resulting in an insoluble material that can be used as structural support;;

(b) Outline the basis of the selective permeability of the cell membrane with reference to phospholipids, cholesterol and proteins. [8]

Phospholipids

- cell membrane is a phospholipid bilayer ;
- it acts as a hydrophobic barrier / ref. to hydrophobic core ;;
- which prevents the diffusion of hydrophilic solutes / polar molecules, charged ions across it ;;
- weak hydrophobic interactions exist between phospholipids / ref. to lateral movement of phospholipids ;
- for small, non-polar, hydrophobic solutes to diffuse across ;;

Cholesterol

- presence of cholesterol in the membrane decreases the permeability of membrane ;;
- it does so by filling in spaces between hydrocarbon chains of phospholipids / plugging transient gaps ;;
- cholesterol regulates membrane fluidity / ref. to higher temperature, cholesterol decreases membrane fluidity / lower temperature, cholesterol increases membrane fluidity ;;

Proteins

- hydrophilic solutes / polar molecules, charged ions can be transported across the membrane ;;
- through transport proteins ;;
- ref. to solute binding and change its 3D conformation ;
- via facilitated diffusion / ref. to down a concentration gradient ;;
- via active transport / ref. to against a concentration gradient ;;
- presence of channel proteins ;;
- ref. to water-filled central pore / hydrophilic channel ;
- via facilitated diffusion / ref. to down a concentration gradient ;;

(c) Explain why animal cells mainly store lipids instead of carbohydrates. [6]

- Triglycerides are a good thermal insulator and hence a layer of fat beneath the skin (subcutaneous fat) insulates the body. This subcutaneous layer is especially thick in whales, seals and most other marine animals living in cold climates and is known as blubber;;
- Upon oxidation, triglycerides release a larger amount of energy per unit / ref. to one gram of fat releases more than twice as much energy (38 kJ / g) as a gram of carbohydrates (17 kJ / g);;
- Being highly reduced molecules, triglycerides release more metabolic water when they are oxidised during cellular respiration compared to carbohydrates, which is extremely important to desert animals like camels;;
- Triglyceride can slide under pressure hence adipose tissue (which contains fats) around the vital organs helps to cushion and protect vital organs against physical impacts;;
- Being less dense than water, fats aid buoyancy of aquatic animals ;;

QUESTION 10

(a) Describe how the molecular structure of the GPCR is suited for its role in glucagon- mediated cell signaling. [8]

- The GPCR is a transmembrane protein comprising an extracellular domain, a transmembrane region and a intracellular domain ;;
- It relays an extracellular signal to a cellular response ;;
- The extracellular domain of GPCR has a ligand binding site that is complementary to the 3D conformation of glucagon;;
- This allows glucagon to bind to its specific GPCR on the target cells in signal reception ;;
- GPCR consists of a single polypeptide chain that has seven α- helices spanning the membrane;;
- The hydrophobic interactions between the non-polar R-groups with the fatty acid tails of phospholipid bilayer allows the GPCR to be embedded in the membrane ;;
- The intracellular domain of GPCR is complementary to the 3D conformation of a G protein;;
- The activated GPCR binds and activates the G protein for signal transduction ;;

(b) Outline the concept of negative feedback in controlling glucagon levels in the body. [6]

- When blood glucose levels falls below set point of 90mg/100ml, the receptor, alpha cells in the islets of Langerhans of the pancreas, detects the change / deviation;;
- This information is integrated at the integrating centre, to initiate a response in the form of signals to act upon target / effector cells;;
- the alpha cells of the islets of Langerhans of the pancreas are then stimulated to release more glucagon;;
- Glucagon binds to GPCR receptors to activate the GPCR receptors;;
- Cellular responses are stimulated when adenylyl cyclase is activated to produce cAMP to activate ;;
- When blood glucose levels are detected by the receptor to return to the set point, negative feedback mechanism is activated to prevent further increase in blood glucose beyond setpoint;;.
- There is decreased stimulation of alpha cells resulting in decreased release of glucagon;;

(c) Explain how signal amplification is illustrated by the binding of insulin to its receptor. [6]

- The binding of small number of insulin to its receptors can lead to the activation of many glycogen synthase / AW ;; to facilitate glucose uptake and storage.
- Cytoplasmic relay proteins specific to the insulin receptor binds to a specific phosphorylated tyrosine on the receptor and undergo conformational changes;.
- These bound relay proteins becomes activated;;.
- The activated relay proteins triggers a signal transduction pathway;;
- whereby activated relay proteins (Accept: protein kinases) activate other protein kinases in phosphorylation cascade;.
- Many activated downstream protein also leads to the activation of glycogen synthase which facilitates glycogenesis / AW;.

JC2 Preliminary
Examinations Higher 2

CANDIDATE NAME		**CT GROUP**	**15S ___**
CENTRE NUMBER		**INDEX NUMBER**	

BIOLOGY

9648 / 03

Paper 3 Applications Paper and Planning Question

16 September 2016

Additional Materials: Writing Paper

2 hours

INSTRUCTIONS TO CANDIDATES

Write your **name**, **CT group**, **Centre number** and **index number** in the spaces provided at the top of this cover page.

STRUCTURED QUESTIONS

Answer **all** three questions.

Write your answers on the lines / in the spaces provided.

PLANNING QUESTION

Answer the question in booklet **4**.

Write your answers on the lines / in the spaces provided.

FREE RESPONSE QUESTION

Answer the question.

Your answers must be in continuous prose, where appropriate.

Write your answers on the writing paper provided.

BEGIN EACH PART ON A FRESH SHEET OF WRITING PAPER.

A **NIL RETURN** is required for parts not answered.

INFORMATION FOR CANDIDATES

The number of marks is given in brackets [ ] at the end of each question or part question.

The use of an approved scientific calculator is expected, where appropriate. You may lose marks if you do not show your working or if you do not use appropriate units.

You are reminded of the need for good English and clear presentation in your answers.

For Examiners' Use	
Question	**Marks**
1	**/ 13**
2	**/ 12**
3	**/ 15**
4	**/ 12**
5	**/ 20**
Total	**/ 72**

BOOKLET 1

STRUCTURED QUESTIONS

QUESTION 1

Bacteria can be genetically modified to produce insulin for human use. To achieve this, human insulin genes are transferred into bacteria. Plasmids containing two antibiotic resistance genes, one coding for resistance to tetracycline and one for resistance to ampicillin, are used to carry out this transfer.

A restriction enzyme was used to cut the human DNA and plasmids. Fig. 1.1 shows the different human DNA fragments and the linearised plasmid that was produced.

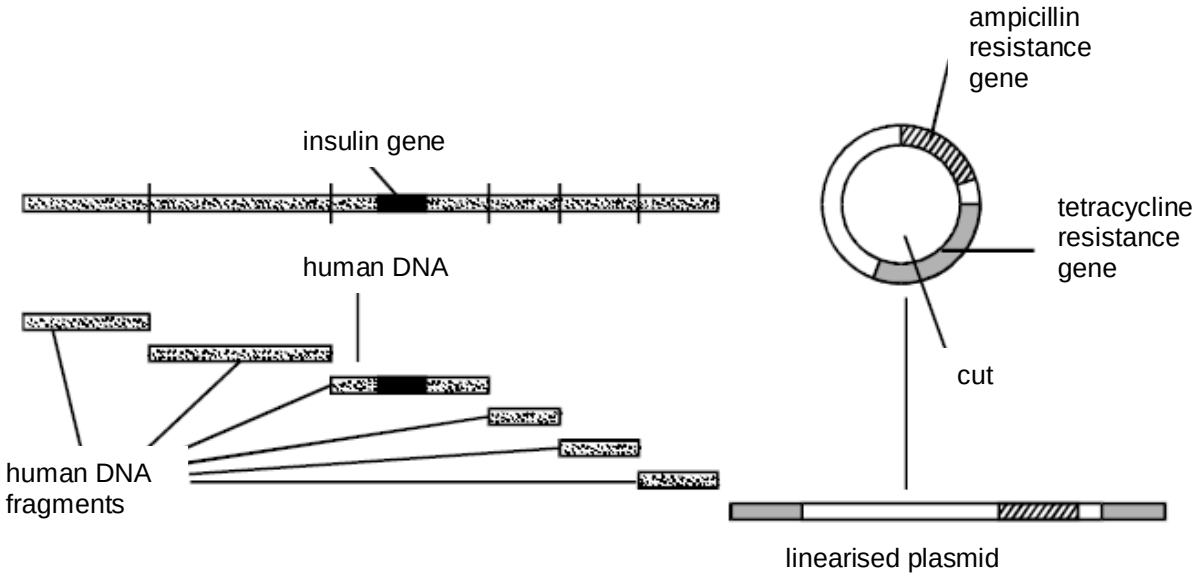


Fig. 1.1

(a) Describe how the restriction enzyme cuts the human DNA and plasmid.

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..... [2]

The human DNA fragments and linearised plasmids were mixed together with DNA ligase. The linearised plasmids are then mixed with bacteria. Some of the bacteria take up the plasmids.

(b) **(i)** Explain how it is possible to distinguish between bacteria which have taken up a plasmid containing a human DNA fragment and those which have taken a plasmid without any extra DNA.

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..... [4]

(ii) Suggest why genes for antibiotic resistance are now rarely used as selectable markers in genetic engineering.

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..... [2]

Unlike diabetes, Huntington's disease (HD), an autosomal dominant disorder, has a clear genetic basis. The gene involved contains a section of DNA with many repeats of the base sequence CAG. The number of repeats found in an allele of this gene may determine how early an individual develops HD. In genetic testing for HD, fragments of DNA are cut from an individual's alleles and separated by gel electrophoresis to determine their length.

Four members of a family affected by HD were tested:

- **A** is the father of **B** and developed symptoms of HD in old age.
- **B** is the mother of **D** and developed symptoms of HD in middle age.
- **C** is the **unaffected** father of **D**.
- **D** is the son of **B** and **C** and developed symptoms of HD as a child.

The results of the genetic test are shown in Fig. 1.2.

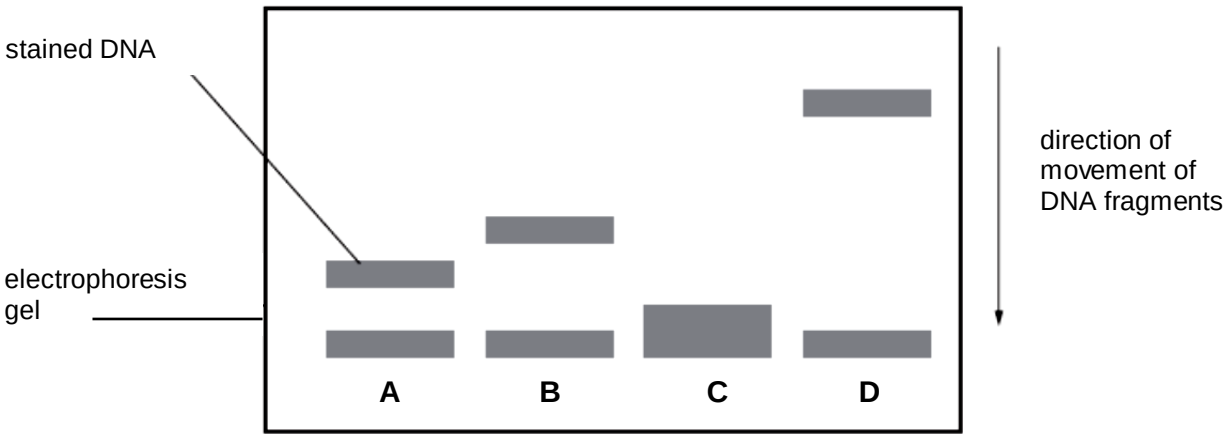

Fig. 1.2

(c) With reference to Fig. 1.2,

(i) describe the relationship between length of DNA fragment and age of onset of HD.

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..... [2]

(ii) explain the difference in positions of DNA fragments from the different members of the family in the test.

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..... [3]

[Total: 13]

QUESTION 2

Duchenne Muscular Dystrophy (DMD) is a progressive muscle wasting disease caused by mutation in the *dmd* gene. The *dmd* gene is the largest gene in humans and it encodes for the dystrophin protein. Mutations in the *dmd* gene result in a nonfunctional dystrophin protein, hence resulting in the disease.

Since the discovery of the *dmd* gene, investigations have been made to exploit gene therapy as a possible cure for DMD. In past studies, DNA library was produced by splicing together essential exons. This reduces the size of the *dmd* gene, allowing viral vectors to carry it.

(a) Identify the type DNA library and outline how the DNA library is produced.

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..... [3]

(b) Explain the limitations of using viruses in the gene transfer of the *dmd* gene.

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..... [2]

(c) Normal muscle fibres can be generated if adult mesenchymal stem cells, without the genetic defect that causes DMD, is delivered into the patients' muscles. Mesenchymal stem cells can give rise to many types of cells including bone, cartilage, lung and muscle cells.

Describe the unique features of adult stem cells.

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..... [2]

Fig. 2.1 shows a possible approach for DMD gene therapy in humans.
The following were used in the study:

- fibroblasts, which are differentiated cells.
- induced Pluripotent Stem (iPS) cells, which are cells that have been genetically reprogrammed from fibroblast cells.
- an artificial vector that carries the normal *dmd* gene (ART). Using an artificial vector overcomes the size limitations encountered with viral vectors and consequently, allows for the expression of full-length dystrophin protein.

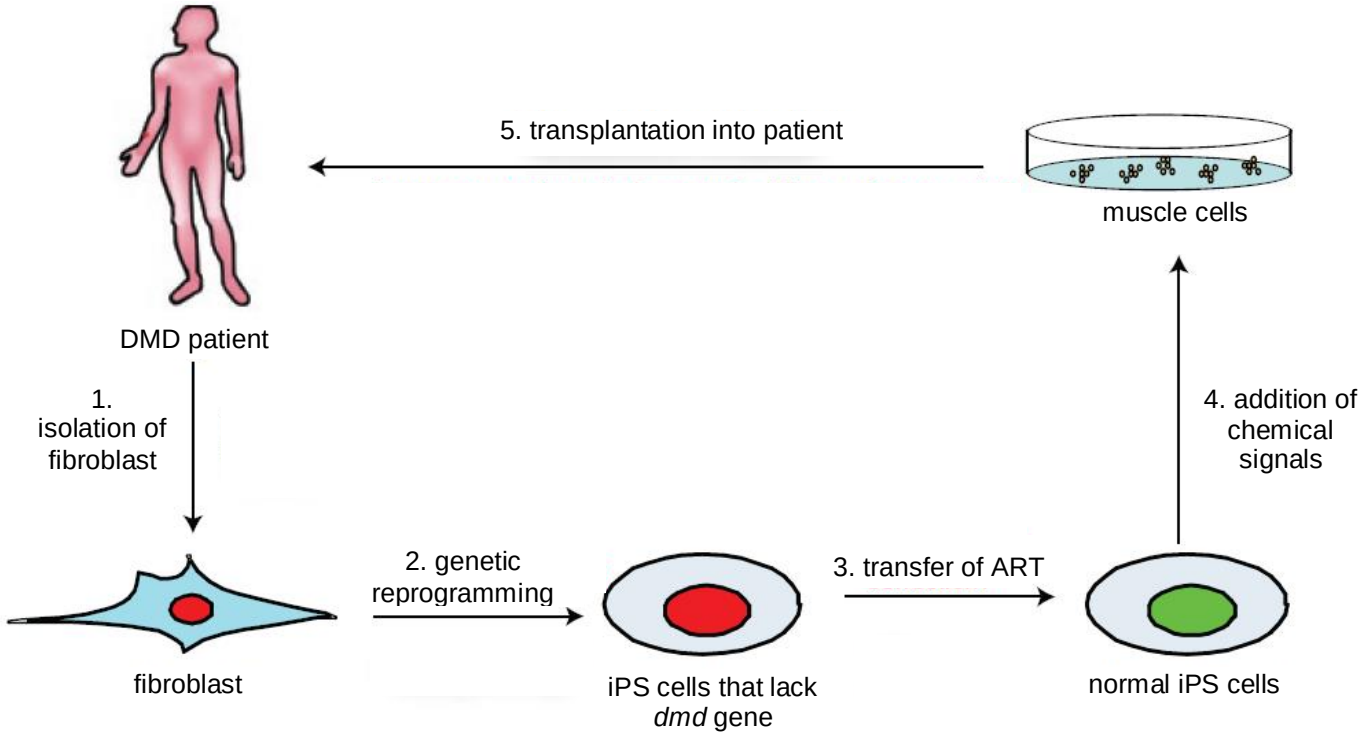

Fig. 2.1

(d) Explain why it is preferable to isolate fibroblast from the the DMD patient.

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..... [2]

(e) Suggest the purpose of adding chemical signals (step 4).

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..... [1]

(f) Upon transplantation into the patient, suggest two disadvantages of this approach that is similar to conventional gene therapy.

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..... [2]

[Total: 12]

QUESTION 3

Many of the original genetically modified crops contained only one modification. An example is Bt Corn A, which produces only one type of Bt protein. To delay Bt resistance in pest populations, such as corn rootworm, modern crops such as Bt Corn B produces two types of Bt proteins against the same pest. Fig. 3.1 shows the survival of corn rootworm larvae on the two types of Bt corn.

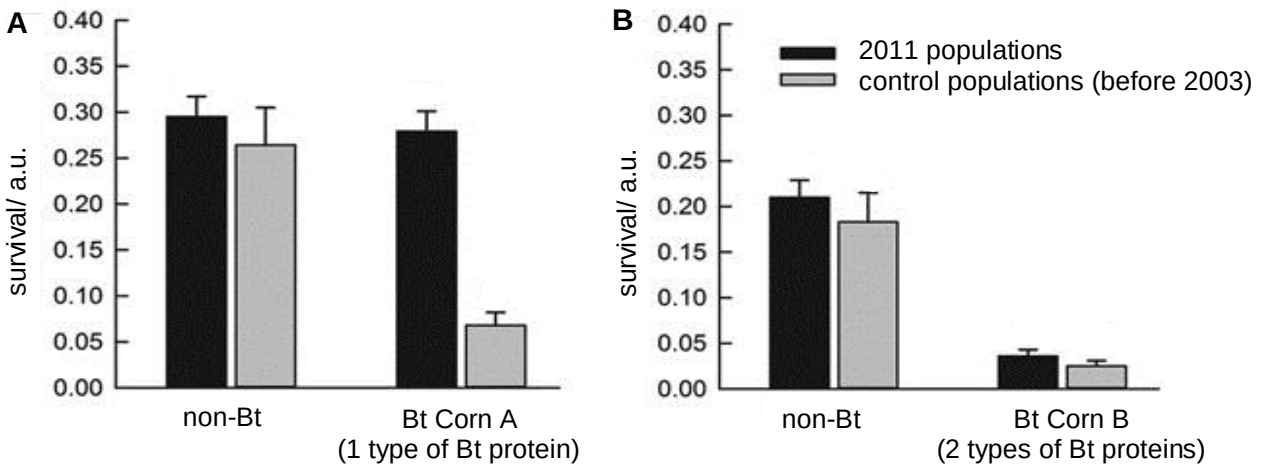

Fig. 3.1

(a) **(i)** With reference to Fig. 3.1A, suggest if there is any significant difference between the 2011 and control populations (before 2003) for non-Bt corn.

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..... [2]

(ii) With reference to Fig. 3.1B, suggest why two types of controls were used.

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..... [2]

(iii) Explain whether expressing one type or two types of Bt proteins is more effective in delaying Bt resistance in corn rootworm.

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..... [3]

Different Bt proteins have varying effects on particular pests. Some Bt proteins are toxic to caterpillars, such as the European corn borer, while other Bt proteins are toxic to rootworms, such as the corn rootworm. Bt genes coding for Bt proteins could be introduced in various combinations, with or without herbicide tolerance genes.

Table 2.1

type of Bt Corn	type of Bt protein	target pest	herbicide tolerance gene
A	Cry1Ab	European corn borer	-
B	Cry3Bb	corn rootworm	-
C	Cry1Ab Cry3Bb	European corn borer corn rootworm	-
D	Cry1Ab Cry3Bb	European corn borer corn rootworm	present

(b) Explain which type of Bt Corn will best increase crop yield.

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..... [2]

Glufosinate ammonium, is a broad-spectrum herbicide, effective in killing a wide range of weed species. Researchers isolated a gene (*bar* gene) derived from soil bacterium *Streptomyces hygroscopicus*, encoding for a herbicide tolerant protein. This gene is introduced to Corn **D** via a plasmid (pCIB3064), resulting in the herbicide tolerant corn.

(c) Outline how herbicide tolerant Corn **D** can be cultured by using calli of Corn **C**.

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(d) Suggest one social issue related to an increase in the use of Corn **D**.

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..... [1]

[Total: 15]

QUESTION 4

Enzymes, such as proteases, are key components found in contact lens cleaning solution. Proteases remove protein deposits from the surfaces of contact lenses.

Two essential contents of such contact lens cleaning solution are:

- pH 7 buffer containing disodium EDTA, a preservative and
- subtilisin A, a protease.

A student researched online and found that there is a range of concentrations of subtilisin A in different commercially available contact lens cleaning solutions. This range of concentration of subtilisin A was between 20 μg cm^{-3} and 100 μg cm^{-3}.

Fig. 4.1 shows how the student simulated a dirty contact lens using protein gelatin and a thin transparent plastic sheet. One side of the plastic sheet was dipped into melted gelatin containing a red dye. The gelatin was then allowed to set and solidify on the plastic sheet, which acts as a support for the gelatin. Subsequently, this simulated dirty contact lens was added to the cleaning solution.

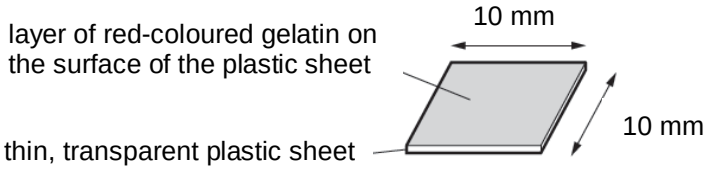

Fig. 4.1

The student tested the activity of subtilisin A by recording the intensity of the red dye in the cleaning solution as shown in Fig. 4.2. This was possible because the cleaning solution was able to remove the red-coloured gelatin from the surface of the transparent plastic sheet.

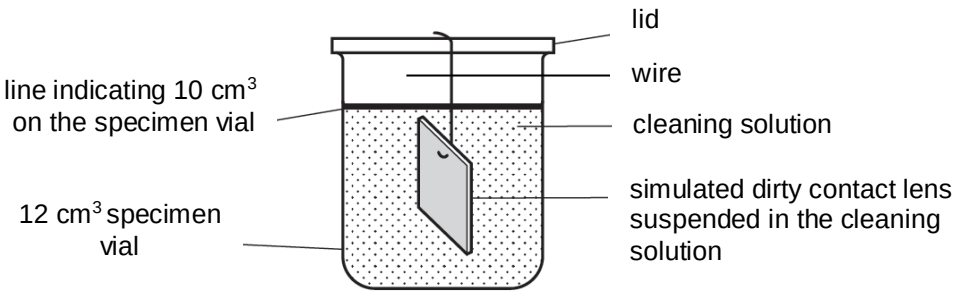

Fig. 4.2

Using this information and your own knowledge, design an experiment to investigate the effect of different concentrations of subtilisin A in commercially available contact lens cleaning solutions on the removal of protein deposits from the simulated dirty contact lenses.

You must use:

- 100 μg cm^{-3} subtilisin A 

- pH 7 buffer containing disodium EDTA, for dilution purpose
- 12 cm^3 specimen vials, with a wire attached to its lid
- 50 mm x 50 mm thin, transparent plastic sheet coated with red-coloured gelatin
- thermostatically-controlled water bath
- colourimeter and cuvettes

You may select from the following apparatus:

- any normal laboratory glassware e.g. test-tubes, beakers, measuring cylinders, graduated pipettes, glass rods etc
- syringes
- white tile
- scalpel
- 15 cm ruler
- timer e.g. stopwatch or stopclock

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it,
- 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]

FREE RESPONSE QUESTION

Your answers must be in continuous prose, where appropriate.
Your answers should be illustrated by large, clearly labelled diagrams, where appropriate.
Write your answers in the writing paper provided.
BEGIN EACH PART ON A FRESH SHEET OF WRITING PAPER.
A **NIL RETURN** is required.

QUESTION 5

(a) Describe the process of polymerase chain reaction. [6]

(b) Explain the advantages and limitations of polymerase chain reaction. [6]

(c) Explain the principles of restriction fragment length polymorphism analysis and how it can be used to study genetic variation within a species of organism. [8]

[Total: 20]

--- END OF PAPER---

(COLLEGE SECTION)
2016 JC2 H2 BIOLOGY
PRELIMINARY EXAMINTION PAPER 1 & PAPER 3 MARK SCHEME

MULTIPLE CHOICE QUESTIONS

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

Question 1

(a) Describe how the restriction enzyme cuts the human DNA and plasmid. [2]

- restriction enzyme cuts at restriction site
- by cleaving phosphodiester bond
- human DNA five restriction sites
- only one restriction site in plasmid

(b)(i) Explain how it is possible to distinguish between bacteria which have taken up a plasmid with human DNA and those which have taken a plasmid without any extra DNA. [4]

- use of sterile velvet to transfer bacteria from master plate of nutrient agar containing ampicillin press velvet onto replica plate of nutrient agar containing tetracycline
- bacteria carrying plasmid with human DNA, the tetracycline resistance gene is disrupted
- bacteria carrying plasmid with human DNA will be killed
- while bacteria with no extra DNA in plasmid will have intact tetracycline resistance gene
- bacteria carrying plasmid with no extra DNA will not killed

(ii) Suggest why genes for antibiotic resistance are now rarely used as markers in genetic engineering. [2]

- risk spread of resistance to other bacteria / ref. to being resistant to antibiotics
- spread of resistance via, conjugation / transformation / incorporation into the genome of bacteria

(c) With reference to Fig. 3.2,

(i) describe the relationship between length of DNA fragment and age of onset of HD. [2]

- the longer the fragment length the earlier the onset
- ref. to shorter fragments moving further / longer fragments moving slower

(ii) explain the difference in positions of DNA fragments from the different members of the family in the test. [3]

- C has recessive, alleles / ref. to homozygote
- A, B and D have one normal and dominant allele / ref. to heterozygotes
- dominant allele gets longer / ref. to increasing CAG repeats from A to B to D

Question 2

a. Identify the type DNA library and outline how the DNA library is produced. [3]

- cDNA library ;;
- Reverse transcriptase catalyses the synthesis of single stranded cDNA using the mature mRNA template of the *dmd* gene;
- DNA polymerase uses the single stranded cDNA as a template to synthesise the complementary/ second DNA strand ;
- Formation of recombinant plasmid/ vector containing the double stranded cDNA and plasmid ;
- The recombinant plasmid/ vector is transformed into bacteria cells ;

b. Explain the limitations of using viruses in the gene transfer of the *dmd* gene. [2]

- There is a limit on the size of the dystrophin gene that can be inserted into a viral vector that results in unsuccessful *dmd* gene expression / AW ;;
- The viral vector may regain virulence and trigger an immune response / AW;;
- There is a possibility of triggering the immune system leading to the host rejecting the *dmd* transgene ;;
- There is random insertion of the *dmd* transgene within the host cells' genome that may induce oncogene activation / AW ;;

c. Describe the unique features of adult mesenchymal stem cells. [2]

- Multipotent stem cells ;
- Mesenchymal stem cells are unspecialised cells ;
- capable of long term self-renewal via mitosis reproducing itself for long periods of time;
- Mesenchymal stem cells can differentiate into specialised cell types under appropriate conditions ;

d. Explain why it is preferable to isolate fibroblast from the the DMD patient. [2]

- Cells are obtained from the same individual and hence ;;
- it will not induce immune rejection when transplanted back into patients ;;

.

e. Suggest the purpose of adding chemical signals (step 4). [1]

1 Differential gene expression to induce the differentiation of iPS to muscle cells ;;

f. Upon transplantation into the patient, suggest two disadvantages of this approach that is similar to conventional gene therapy. [2]

1. Efficiency of transfer of muscle cells into patient maybe low ;;
2. Cells may not produce enough DMD protein ;;
3. The risk of stimulating the immune system leads to rejection by the host ;;

QUESTION 3

(a) (i) With reference to Fig. 3.1A, suggest if there is any significant difference between the 2011 and control populations (before 2003) for non-Bt corn. [2]

1. No significance difference between the control and 2011 populations
2. As the error bars overlap with each other

(ii) With reference to Fig. 3.1B, suggest why two types of controls were used. [2]

1. Non-Bt corn is used to determine the effectiveness of Bt protein in decreasing the survival of corn rootworm larvae
2. Control population (before 2003) is used to determine the effectiveness in delaying resistance of Bt protein over the years

(iii) Explain whether expressing one type or two types of Bt proteins is more effective in delaying Bt resistance in corn rootworm.

1. Expressing two types of Bt proteins is more effective
2. In Bt Corn A, survival of larvae increase greatly from 0.075 a.u to 0.275 a.u from control to 2011 populations
3. While in Bt corn B, survival of larvae increase very slightly, almost insignificant**;;**
4. AVP

(b) Explain which type of Bt Corn will best increase crop yield.

1. Corn D
2. Not only can Corn D protect against both the European Corn borer and the corn rootworm;
3. It is also resistant to herbicide

(c) Outline how herbicide tolerant Corn D can be cultured by using calli of Corn C.

1. Cut the region flanking the *bar* gene and the plasmid (pCIB3064) with a restriction enzyme to form a recombinant plasmid
2. Which contains the transgene, the promoter and a genetic marker, such as an antibiotic resistance gene

3. a.The callus culture of Corn C can be treated to produce a protoplast culture
   b. The recombinant plasmid can be introduced to the protoplast culture/ ref. to electroporation/heat shock/gene/ virus-mediated delivery

4. Calli grown in a medium containing the antibiotic
5. Cells who have successfully taken up the recombinant plasmid will be resistant to the antibiotic and will survive, while those who did not take up the recombinant plasmid will die
6. Surviving colonies can be transferred to an agar medium to induce shoot development/ ref. to cytokinin
7. Then transfer to another agar medium after 2-4 weeks to induce root development/ ref. to auxin
8. Ref. to acclimatization of the plantlet

d) Suggest one social issue related to an increase in the use of Corn **D**.

1. After long usage of Corn D, weeds may gain resistance to herbicide
2. Ref. to concerns associated with Bt corn/ pests gain resistance to Bt proteins

Question 4

- Enzyme subtilisin A is a protease that can be used to digest proteins (gelatin) coated on the simulated dirty contact lenses.
- The higher the concentration of subtilisin A in the cleaning solution, higher the rate of gelatin digestion.
- Cut transparent plastic sheet coated with red-coloured gelatin into squares of constant dimensions.
- Place these plastic squares coated with red-coloured gelatin into the specimen vial containing the cleaning solution.
- Start reaction in a thermostatically-controlled water bath for fixed period of time.
- Pour a fixed volume of the cleaning solution into a cuvette.
- Measure the absorbance of the cleaning solution using a colourimeter.
- Carry out a control.
- Perform replicates, repeats and statistical test.
- Calculate the average absorbance of the cleaning solution and obtain rate of gelatin digestion.
- Tabulate data in an appropriate manner.
- Draw sketch of expected trend.

Question 5

(a) Describe the process of polymerase chain reaction. [6]

- DNA denatures when hydrogen bonds are broken, resulting in separation of DNA strands;;
- by heating to 95^{0}C;;. (accept: 90-100 ^{0}C)

- A pair of primers anneal via complementary base pairing to the 3'ends of the single-stranded DNA template;;
- by cooling to 54 ^{0}C;;. (accept: 50-65 ^{0}C)

- New strands of DNA are synthesised from the position of the primers in the 5' to 3' direction;;
- by *Taq* DNA polymerase;; and
- by heating to 72 ^{0}C;;. (accept: 60-75 ^{0}C)

(b) Explain the advantages and limitations of polymerase chain reaction. [6]

Advantages

- The PCR method is extremely sensitive
- it can amplify sequences from trace amounts of DNA

- PCR is rapid
- A single PCR cycle takes less than 5 minutes / 20 – 30 cycles typically required takes only 2 – 3 hours

- PCR is robust
- PCR can permit amplification of specific sequences from material in which the DNA is badly degraded or embedded in a medium from which conventional DNA isolation is difficult

Limitations

- PCR is prone to contamination
- Due to the extreme sensitivity of PCR, any contamination of the reaction by non-template nucleic acids in the environment could be easily amplified

- PCR may result in infidelity of DNA replication
- *Taq* polymerase used in PCR lacks 3' to 5' exonuclease activity

- Prior knowledge on the DNA sequence is required for the PCR
- Designing specific oligonucleotide primers is required for selective amplification of a particular DNA sequence

(c) Explain the principles of restriction fragment length polymorphism analysis and how it can be used to study genetic variation within a species of organism. [8]

- RFLP are variations in the number / length of restriction fragments generated upon digestion with restriction enzymes;;.
- VNTR resulting in changes in the distance between two restriction sites;;.
- DNA samples of different individuals within a species are isolated and cut/cleaved with the same restriction enzyme for RFLP analysis. ;;
- The digested DNA fragments are subjected to gel electrophoresis, the DNA fragments separate by size ;;.
- Subsequently, Southern blotting was performed to transfer the size-fractionated DNA fragments from the gel onto nitrocellulose membrane ;;.
- Use of radioactively-labelled, single stranded probes complementary to the VNTR / DNA sequence of interest that hybridize to the probes by complementary base-pairing ;;
- Carry out autoradiography to visualise the DNA bands ;;.
- When cut with the same restriction enzyme, genetic variation within a species can be shown by the unique/different DNA fingerprint / banding patterns generated. ;;