

CANDIDATE NAME: \_\_\_\_\_

INDEX NUMBER \_\_\_\_\_



SERANGOON JUNIOR COLLEGE  
JC2 PRELIMINARY EXAMINATION 2017

BIOLOGY 9744  
Higher 2

CG \_\_\_\_\_

PAPER 1

Thursday  
21 September 2017

1 hour

Additional materials:  
Optical Mark Sheet

### INSTRUCTIONS TO CANDIDATES

Write your name and CG in the spaces at the top of this page.

On the Optimal Mark Sheet, enter your name, subject title, test name, class. For your index number, enter your full NRIC number. Shade the corresponding lozenges on the OMS according to the instructions given by the invigilators.

**AT THE END OF THE EXAMINATION, HAND IN BOTH THE OMS AND QUESTION PAPER.**

### INFORMATION FOR CANDIDATES

There are **thirty (30) questions** in this paper. Answer **all** questions. For each question, there are four possible answers, **A, B, C, D**. Choose the **one** you consider correct and record your choice in **soft pencil** on the OMS.

Each correct answer will score one mark. A mark will not be deducted for a wrong answer. Any rough working should be done on the question paper.

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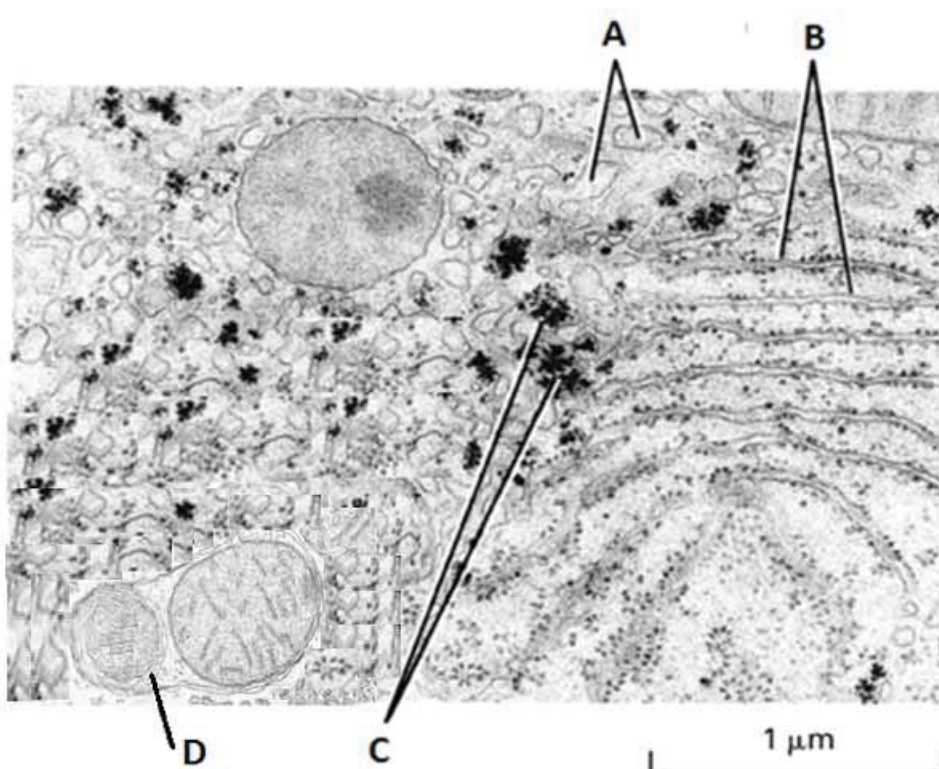
**This question paper consists of 16 printed pages.**

**Answer all questions on the OTAS provided.**

- 1 EDTA is used extensively as an anticoagulant for stored blood in blood banks. Thrombokinase plays a major role in the clotting of blood. EDTA decreases the reaction rate of thrombokinase by binding to calcium ions.

Which of the following describes the role of calcium ions?

- A Allosteric inhibitors
  - B Coenzymes
  - C Cofactors
  - D Competitive inhibitors
- 2 A cell in the G1 phase has two homologous pairs of chromosomes. It then undergoes two mitotic divisions. At the end of the second mitotic division, what is the total number of chromosomes and gene loci found in all the daughter cells formed?
- A 8 chromosomes and 4 times as many gene loci as the original parent cell.
  - B 8 chromosomes and 8 times as many gene loci as the original parent cell.
  - C 16 chromosomes and 4 times as many gene loci as the original parent cell.
  - D 16 chromosomes and 8 times as many gene loci as the original parent cell.
- 3 The electron micrograph below shows a liver cell.

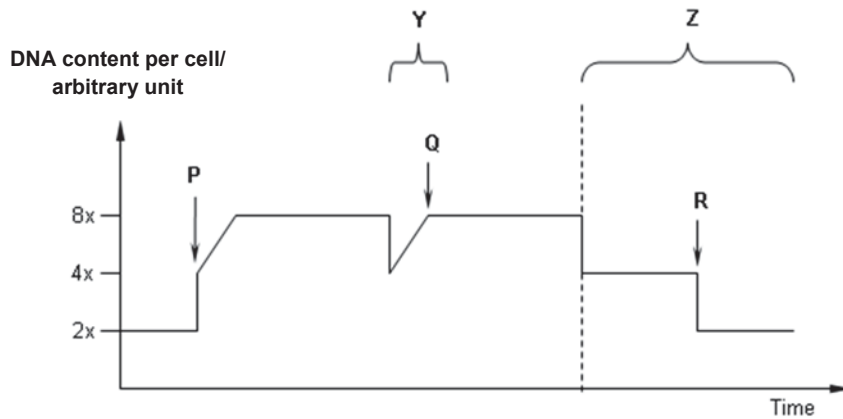


Which statement(s) correctly describe(s) the labelled structures?

- 1 Structure **A** transports proteins from Structure **B** to Golgi Apparatus.
- 2 Proteins enter the lumen of Structure **B**, where they undergo chemical modifications such as glycosylation.
- 3 Structure **C** is starch grain.
- 4 The process shown in structure **D** is autolysis.

**A** 2 only      **B** 1 and 2 only      **C** 2 and 3 only      **D** 2, 3 and 4 only

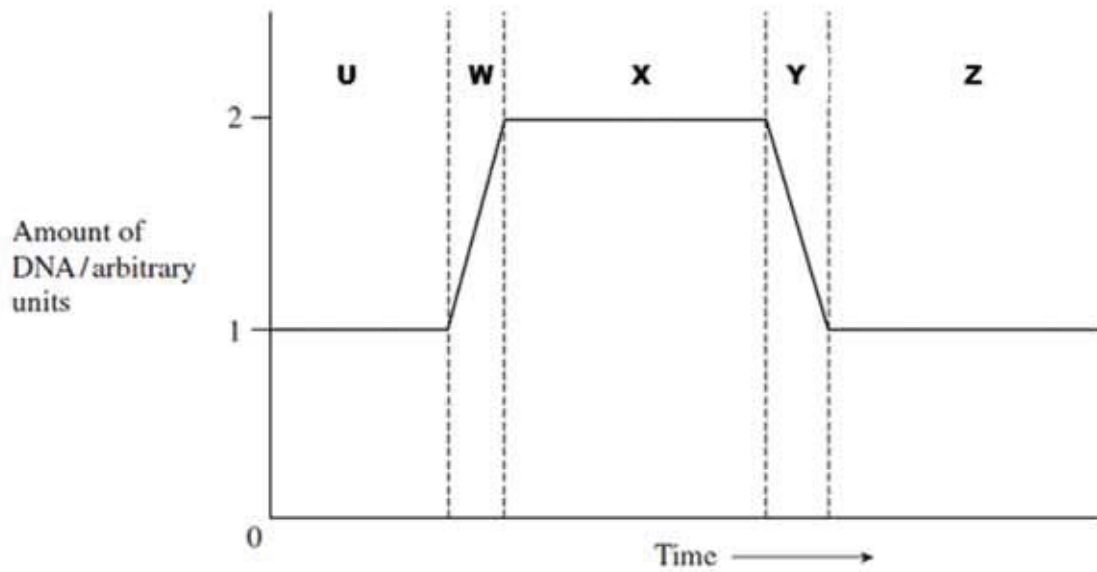
- 4 The graph represents the changes in the DNA content within a cell at different stages in the cell cycle.



Name the events occurring at **P**, **Q** and **R**, and identify the stage where meiosis is occurring.

	<b>P</b>	<b>Q</b>	<b>R</b>	<b>Meiosis occurring at</b>
<b>A</b>	S phase	Fertilisation	Cytokinesis	<b>Y</b>
<b>B</b>	Fertilisation	Interphase	Cytokinesis	<b>Z</b>
<b>C</b>	S phase	Prophase	Telophase	<b>Y</b>
<b>D</b>	Fertilisation	Metaphase	Telophase	<b>Z</b>

- 5 The graph shows changes in the amount of DNA in a cell during one cell cycle. The letters U – Z marks out the different phases in the cell cycle.



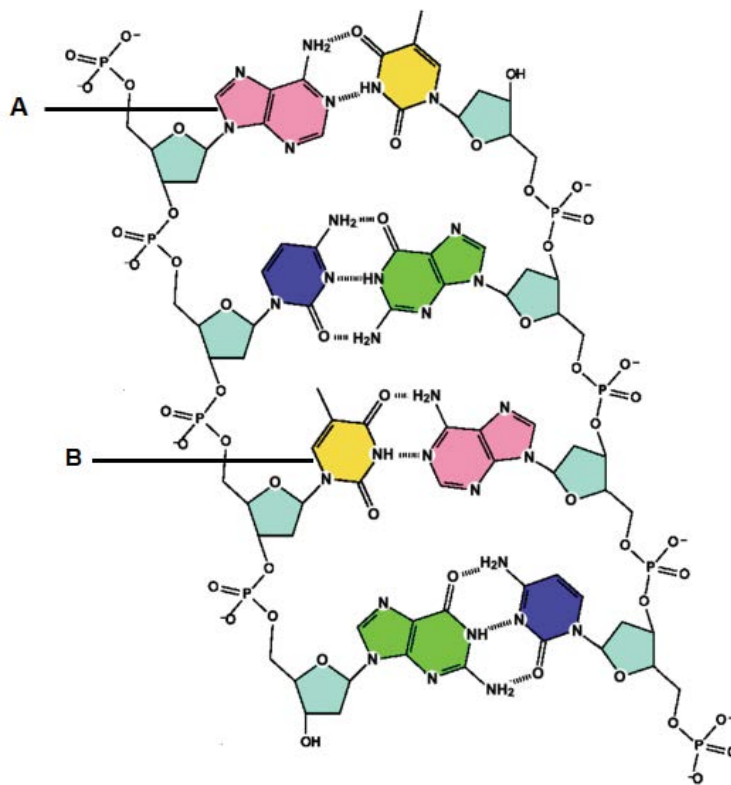
Many drugs that are used to treat cancer work at different time periods during the cell cycle.

- (i) Cisplatin binds to DNA and stops free DNA nucleotides from joining together.
- (ii) Drug B stops spindle fibres from shortening.

With reference to the cell cycle above, determine where these 2 drugs work.

	<b>Cisplatin</b>	<b>Drug B</b>
<b>A</b>	<b>W</b>	<b>X</b>
<b>B</b>	<b>W</b>	<b>Y</b>
<b>C</b>	<b>U</b>	<b>X</b>
<b>D</b>	<b>U</b>	<b>Z</b>

6 The figure below shows a DNA molecule.



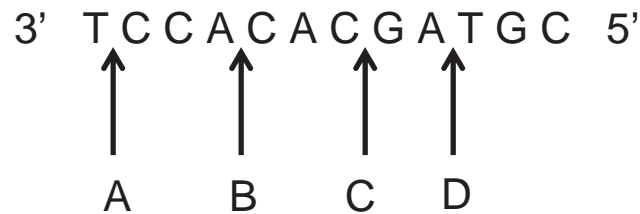
Which statement(s) correctly describe the polynucleotide?

- 1 The structure labelled **A** corresponds to that of a purine, while the structure labelled **B** corresponds to that of a pyrimidine.
- 2 The antiparallel nature of DNA double helix allows phosphodiester bonds to form between the nitrogenous bases of opposite strands.
- 3 Distance between adjacent deoxyribonucleotides is 3.4  $\text{\AA}$  and one turn consists of 10 deoxyribonucleotides. (Note: 10  $\text{\AA}$  = 1 nm)
- 4 The wound DNA double helix consists of alternating major grooves and minor grooves along its axis which are essential for the binding with proteins.

- A** 1 only  
**B** 1 and 2 only  
**C** 3 and 4 only  
**D** 1, 3 and 4 only

- 7 The RNA triplet UAG acts as a stop codon terminating the synthesis of a polypeptide. The diagram shows a template strand of DNA which codes for four amino acids.

Where would a mutation, introducing a thymine nucleotide, result in the termination of translation?



- 8 Which of the following is **not** a feature of eukaryotic gene expression?

- A Polycistronic mRNAs are very rare.
- B Many genes are interrupted by noncoding DNA sequences.
- C RNA synthesis and protein synthesis are coupled.
- D mRNA is often extensively modified before translation.

- 9 Human telomeres consist of repeating TTAGGG sequences which extend from the ends of the chromosomal DNA. When cells undergo mitotic division, some of these repeating sequences are lost. This results in a shortening of the telomeric DNA.

What is a consequence of the loss of repeating DNA sequences from the telomeres?

- A The cell will begin the synthesis of different proteins.
- B The cell will begin to differentiate as a result of the altered DNA.
- C The number of mitotic divisions the cell can make will be limited.
- D The production of mRNA will be reduced.

- 10** The translation mixture contains a polynucleotide that directs the synthesis of Met-Gly-Gly-Phe-Leu-Ala. In the presence of Azithromycin, this polymer directs the synthesis of Met-Gly only.

From the information given, which of the following deductions could you make about Azithromycin?

	<b>Control Stage</b>	<b>Conclusion</b>
<b>A</b>	Translational	It prevents formation of the initiation complex, which contains the initiator tRNA and both ribosomal subunits.
<b>B</b>	Post translational	It inhibits binding of aminoacyl- tRNAs to the A site in the ribosome.
<b>C</b>	Translational	It blocks translocation of peptidyl transferase-rRNA from the A site to the P site of the ribosome.
<b>D</b>	Post translational	It interferes with chain termination and release of the peptide.

- 11** Which of the following statement(s) about cancer is / are true?

- I Individuals who inherit one mutant tumour suppressor gene are more likely to develop cancer than individuals with two non-mutant copies.
- II Cancer is a result of increased cell division which promotes the mutation of a proto-oncogene.
- III Mutagenic activation of a single oncogene is sufficient to cause a normal cell to develop into a cancerous cell.

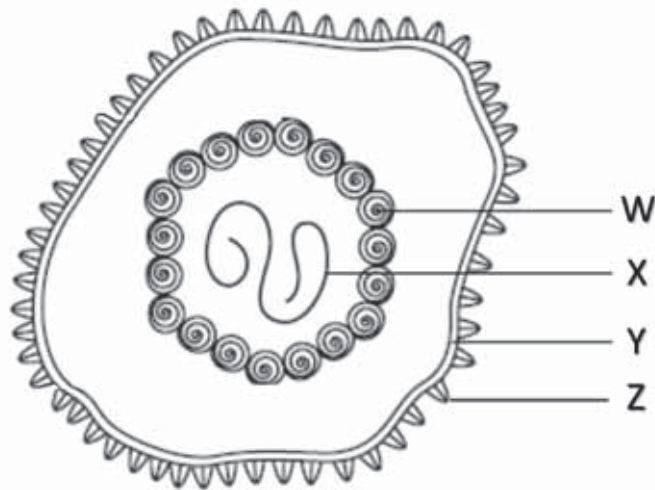
- A** I only
- B** I and II only
- C** I and III only
- D** I, II and III

- 12** To date, more than 10 different strains of influenza virus (e.g. H1N1, H2N3, H5N1, H7N9 and so on) have been documented.

Which of the following structural characteristic of influenza virus makes this possible?

- A** Single-stranded RNA as its genetic material
- B** Presence of an envelope that is derived from the host cell
- C** Eight separate segments of genetic material
- D** Presence of error-prone reverse transcriptase within the virus

13 The figure below shows the structure of a virus.



Which of the following matches the functions of structures W – Z?

	W	X	Y	Z
A	Ensures the integrity of the viral genome is maintained	Entry of virus into host cell	Specificity of host cell	Assembly of viruses
B	Ensures the integrity of the viral genome is maintained	Assembly of viruses	Entry of virus into host cell	Specificity of host cell
C	Specificity of host cell	Assembly of viruses	Ensures the integrity of the viral genome is maintained.	Entry of virus into host cell
D	Assembly of viruses	Ensures the integrity of the viral genome is maintained.	Entry of virus into host cell	Specificity of host cell

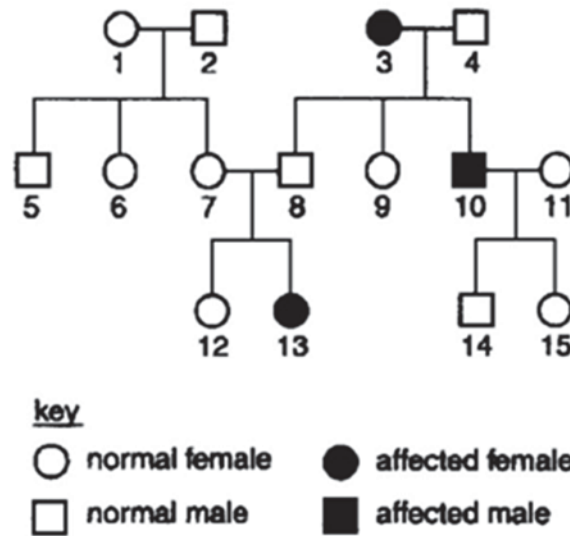
14 When the *lac* operon for lactose metabolism is switched off, which of the following genes would still be expressed?

- I  $\beta$ -galactosidase gene
- II RNA polymerase gene
- III CAP gene
- IV Repressor gene

- A I and II
- B I and III
- C II, III and IV
- D All of the above



- 15 The pedigree chart below shows the inheritance of a recessive condition known as human albinism. Only homozygous recessive individuals are albinos.



What is the probability of individual 9 being a heterozygous carrier?

- A 0.00
- B 0.25
- C 0.50
- D 1.00
- 16 Which of the following regarding embryonic stem cells and hematopoietic stem cells is true?
- A As embryonic stem cells develop, they turned into hematopoietic stem cells as they lose their ability to differentiate into all types of cells.
- B Embryonic stem cells have more genes than hematopoietic stem cells and thus are able to form more types of cells.
- C Under normal conditions, embryonic stem cells express more of their genes compared to the hematopoietic stem cells.
- D Both stem cells are derived from the zygotic stem cells with the hematopoietic stem cells having a lowered telomerase activity compared to the embryonic stem cells.

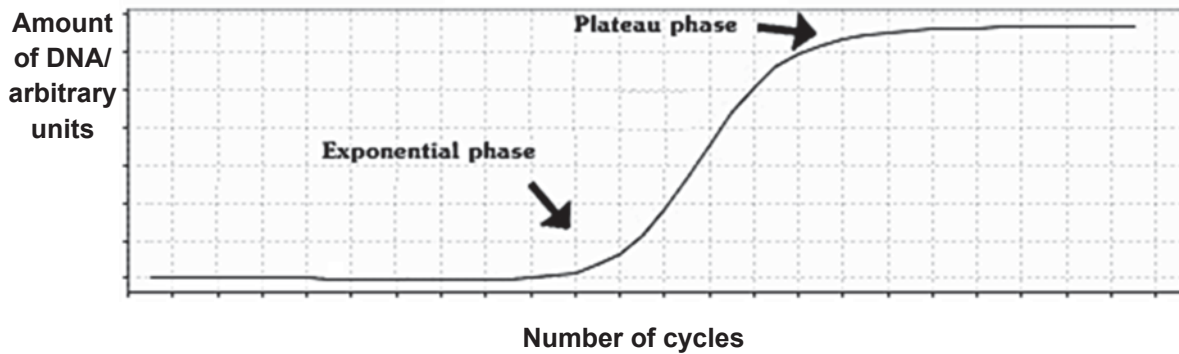
- 17 A plant researcher tried to investigate a cross between two heterozygous Snapdragon plants that produced red flowers. She predicted three possible phenotypic outcomes, namely plants with white flowers, pink flowers and red flowers, with a phenotypic ratio of 4:3:9 respectively. When the cross was performed, she found 50 plants with white flowers only, 41 plants with pink flowers, and 85 plants with red flowers. A chi-squared test was performed, and the chi-squared value was calculated to be 4.74

Degree of freedom	Probability, P				
	0.10	0.05	0.02	0.01	0.001
1	2.71	3.84	5.41	6.64	10.83
2	4.61	5.99	7.82	9.21	13.82
3	6.25	7.82	9.84	11.35	16.27
4	7.78	9.49	11.67	13.28	18.47

Which of the following statements is correct?

- A** The degree of freedom is 3.
- B** The calculated chi-squared value is greater than the critical chi-squared value.
- C** There is a high probability that the difference between the observed and expected values is due to chance.
- D** The probability that the difference between observed and expected values is due to chance is less than 5%.
- 18 Recent advances in the field of stem cell research have shown that induced pluripotent stem cells (iPS cells) can be artificially derived from adult somatic cells. iPS cells are mostly similar to natural pluripotent cells. This implies that iPS cells can
- A** theoretically differentiate into all cell types.
- B** theoretically differentiate into any of the three germ layers.
- C** theoretically differentiate into gametes.
- D** theoretically capable of transdifferentiation.

- 19 During the process of polymerase chain reaction (PCR), the amount of DNA synthesised can be traced using fluorescent probes and the measurements are shown in the following plot. The process initially goes through an exponential phase followed by a plateau phase eventually.



Which of the following statements is **true**?

- A During the exponential phase, the number of DNA molecules synthesized after 15 cycles is  $15^2$ .
  - B During the exponential phase, the temperature is always maintained at the optimum temperature of  $72^\circ\text{C}$  hence there is rapid amplification.
  - C During the plateau phase, the reaction mixture is being depleted of ribonucleotides.
  - D During the plateau phase, *Taq* polymerase may be denatured.
- 20 The dashed lines in the template sequence represent a long sequence of bases to be amplified.

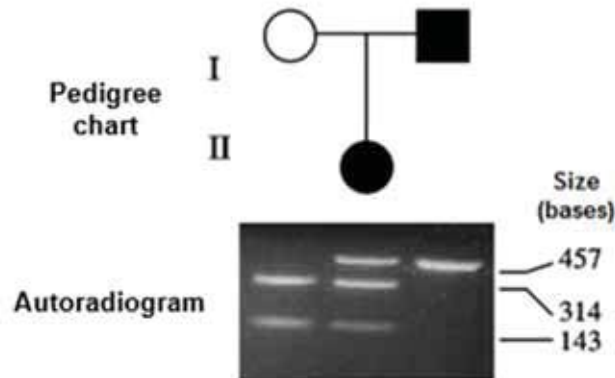
**Template**



Which of the following sets of primers can be used in the PCR for the amplification of the following DNA sequence?

- A 5' GTCCAGC 3' & 5' CCTGAAC 3'
- B 5' ATTCGGA 3' & 5' CCTCTAG 3'
- C 5' GGAAGTTG 3' & 5' GCTGGAC 3'
- D 5' AUUCGGA 3' & 5' GAUCUCC 3'

- 21 A family with a history of a genetic disease is studied using restriction digestion of the DNA samples containing the gene responsible for the disease. The pedigree chart of the family is aligned with the autoradiogram obtained from Southern blotting. (Shaded symbols in the pedigree chart indicate individuals affected by disease.)



Based on the information given, which of the following can be deduced?

- A The disease allele is dominant to the normal allele.  
 B The mutation creates a new restriction site in the affected gene.  
 C One of the parents in generation I is a carrier.  
 D The offspring in generation II is a carrier.
- 22 Which of the following statements correctly compares oxidative phosphorylation and non-cyclic photophosphorylation?
- A Both types of phosphorylation produce ATP and oxygen as end products.  
 B Both types of phosphorylation produce ATP and the reduced form of a redox reagent.  
 C Oxidative phosphorylation is involved in the conversion of one form of chemical energy to another while non-cyclic photophosphorylation is involved in converting light energy to chemical energy.  
 D Water is an electron donor in non-cyclic photophosphorylation while it is an electron acceptor in oxidative phosphorylation.
- 23 What happens to most of the reduced NAD molecules in cell metabolism?
- A They act as oxidising agents in glycolysis.  
 B They are oxidised in inner mitochondrial membrane for ATP formation.  
 C They are oxidised in the Calvin cycle.  
 D They combine with succinic acid as part of Krebs cycle.

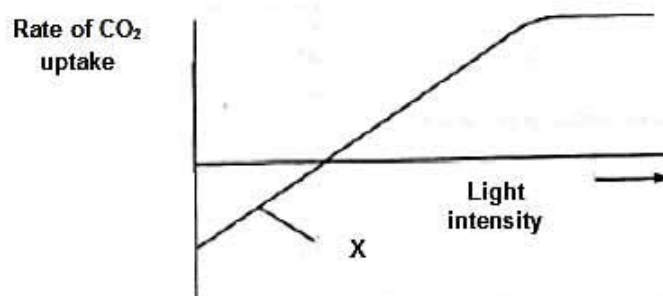
- 24 Rotene and oligomycin are two metabolic poisons which affect cellular respiration. The effects of rotene and oligomycin on aerobic respiration are summarised in the table.

	Ability to use glucose	Ability to use oxygen	ATP yield
<b>Rotene</b>	Yes	No	Decreases
<b>Oligomycin</b>	Yes	Yes	Decreases

Which of the following correctly identifies the specific functions of these two metabolic poisons?

	<b>Rotene</b>	<b>Oligomycin</b>
<b>A</b>	Electron transport inhibitor	Inhibits ATP synthase
<b>B</b>	Inhibits ATP synthase	Electron transport inhibitor
<b>C</b>	Dissipate proton gradient	Inhibits ATP synthase
<b>D</b>	Inhibits ATP synthase	Dissipate proton gradient

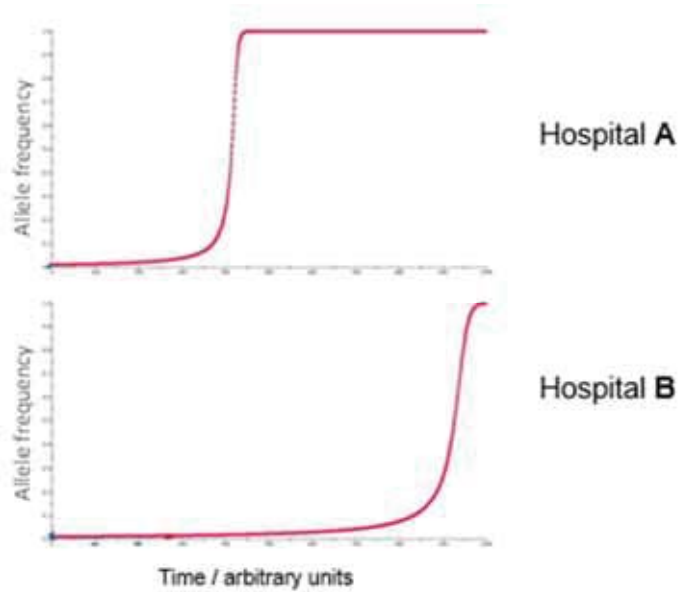
- 25 In the graph below, the rate of CO<sub>2</sub> uptake by plant cells is shown to vary with increasing light intensity.



Which of the following is true at point X?

- A** The plant is photosynthesizing.
- B** Rate of respiration equals rate of photosynthesis.
- C** CO<sub>2</sub> is a limiting factor.
- D** There is not enough light for photosynthesis to have commenced.

- 26 The two graphs below show the allele frequency of an antibiotic resistance gene *Neo* in the gene pool of *Streptococcus pneumoniae*, a bacteria that causes pneumonia.



Which of the following statements can be concluded from the graphs?

- A There is more genetic variation in the gene pool of *Streptococcus pneumoniae* in hospital **A** than hospital **B**.
  - B Patients in hospital **A** were treated with antibiotic Neomycin more frequently than patients in hospital **B**.
  - C The rate of mutation in the genome of *Streptococcus pneumoniae* in hospital **B** occurs more slowly than that in hospital **A**.
  - D Patients in hospital **A** has a stronger immune system than patients in hospital **B**.
- 27 Which sequence of events correctly describes evolution?

- 1 Differential reproduction of the spiders occurs.
- 2 A new selection pressure occurs.
- 3 Allele frequencies within the spider population change.
- 4 Poorly adapted spiders have decreased survivorship.

- A 2 4 1 3
- B 2 4 3 1
- C 4 1 3 2
- D 4 3 1 2

**28** The following statements are some findings of scientists in an attempt to investigate the evolutionary relationship between the anteater, armadillo and pangolin.

- I** Anteater, armadillo and pangolin feed primarily on insects such as ants.
- II** Anteater, armadillo and pangolin have long tongue and strong digging limbs.
- III** The tongues of the anteater and armadillo are connected to the hyoid bone while the tongue of pangolin is not.
- IV** There is a higher percentage similarity between the DNA sequences of Anteater and armadillo than with the pangolin.
- V** There is very low percentage similarity between the DNA sequences of anteater and pangolin as well as between the armadillo and pangolin.

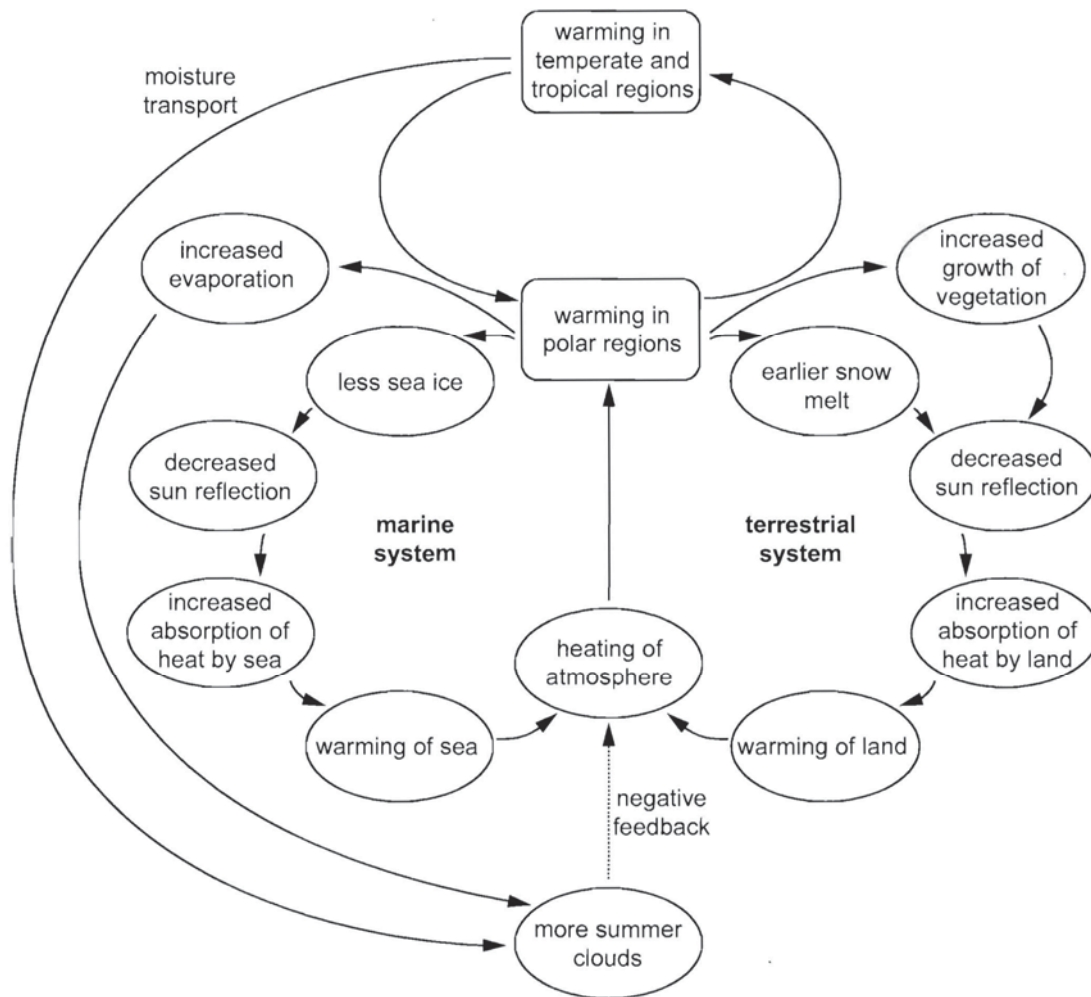
Which of the following conclusions can be drawn from the statements given above?

- A** The anteater and pangolin have experienced divergent evolution as shown by homologous structures between their hyoid bones and tongues.
- B** The anteater and pangolin have experienced convergent evolution as shown by homologous structures in their hyoid bones and tongues.
- C** The armadillo and pangolin have experienced divergent evolution as shown by the low similarity between their DNA sequences.
- D** The anteater and armadillo have experienced divergent evolution as shown by similarities in their DNA sequences and homologous anatomical structures.

**29** In order to initiate an adaptive immune response, antigenic peptide must be presented to antigen-specific T cells. Which one type of cell presents this antigen to T cells?

- A** Dendritic cell
- B** Epithelial cell
- C** Neutrophil
- D** Plasma cell

- 30 The diagram shows the effect of increasing temperatures on the ice and snow cover at the polar regions.



Which effect of higher temperatures in the polar regions could increase global warming?

- A Increased evaporation leads to more rainfall, which absorbs heat from the land and sea.
- B Melting of ice and snow results in less reflection of sunlight and more heat absorption by the Earth.
- C Melting of sea ice causes more cloud formation, which increases absorption of heat in the atmosphere.
- D Earlier melting of snow allows vegetation cover to increase faster, reducing loss of heat from the surface of the Earth.

**End of Paper**



CANDIDATE NAME: \_\_\_\_\_

INDEX NUMBER \_\_\_\_\_

CG \_\_\_\_\_



SERANGOON JUNIOR COLLEGE  
JC2 PRELIMINARY EXAMINATION 2017

BIOLOGY  
Higher 2  
9744

Tuesday  
18 September 2017

2 hours

Additional materials:  
Writing paper

### READ THESE INSTRUCTIONS FIRST

Write your name and index number in the spaces at the top of this page and on all the work you hand in.

Write in dark blue or black pen.

You may use a soft pencil for any diagrams, graphs or rough working.

Do not use staples, paper clips, highlighters, glue or correction fluid.

Answer **all** questions in all sections.

### INFORMATION FOR CANDIDATES

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

FOR EXAMINER'S USE	
Paper 1 (MCQ)	/30
Paper 2	
1	/15
2	/20
3	/15
4	/15
5	/18
6	/17
P2 Total	/100
Paper 3	/75
Paper 4	/55
TOTAL (100%)	

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This question paper consists of 19 printed pages.

Answer **all questions** in the spaces provided.

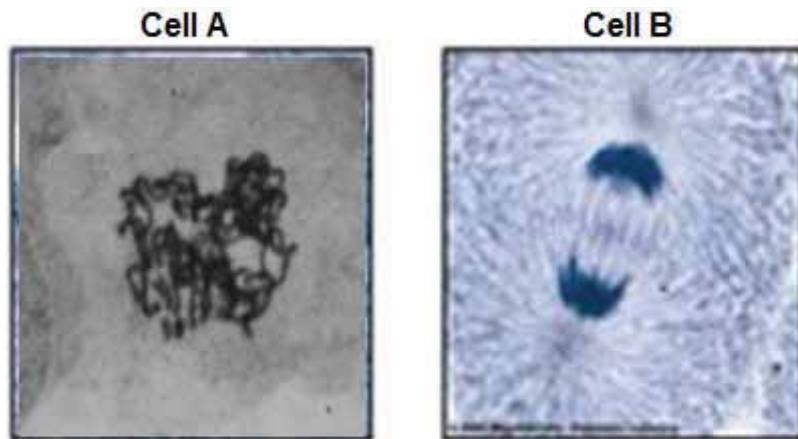
**Question 1**

There have been many breakthroughs in stem cell research in recent years. It has been discovered that stem cells are involved in the replacement of worn-out cells and repair of damaged tissues. Further research is being conducted to better understand the mechanism involved in controlling the behaviour of stem cells in order to better manipulate them to treat various diseases and disorders.

- (a) State the type of stem cells involved in the replacement of worn-out cells and repair of damaged tissues, and describe the unique properties of this type of stem cells. [2]

.....  
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.....

Stem cells undergo cell division to produce genetically identical daughter cells. **Fig. 1.1** shows two cells, each at a different stage of cell division.



**Fig. 1.1**

- (b)(i) With reference to **Fig. 1.1**, state the stages of cell division in **Cell A** and **Cell B**. [1]

**Cell A:** .....

**Cell B:** .....

- (ii) The dysregulation of cell cycle can result in cancer. Outline the checkpoints that are present in normal cells to prevent this from occurring. [2]

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Fig. 1.2 shows information about the movement of chromatids in a cell that has just started metaphase of mitosis.

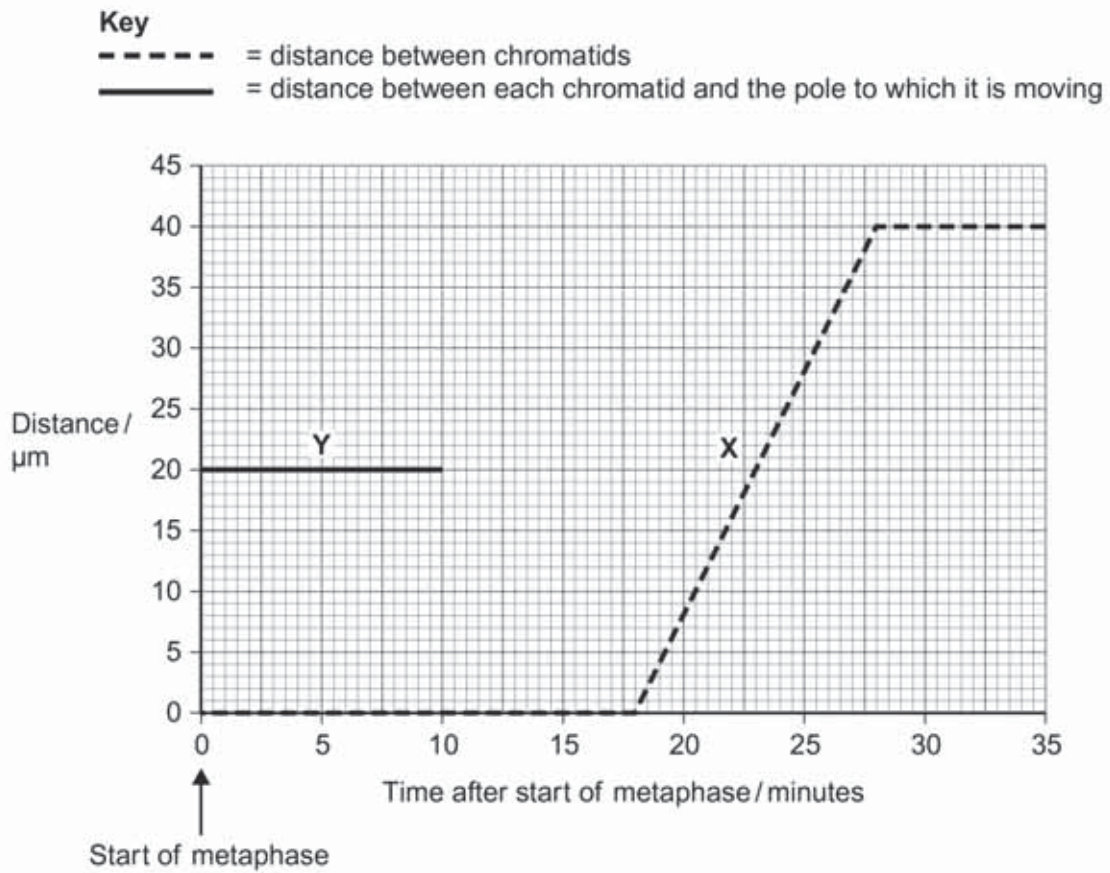


Fig. 1.2

- (c)(i) With reference to Fig. 1.2, state the duration of metaphase in the cell. [1]

.....

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

**(iii)** Account for your answer in **(c)(ii)**. [3]

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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.

**(d)(i)** Contrast between the structure of tubulin with that of DNA. [2]

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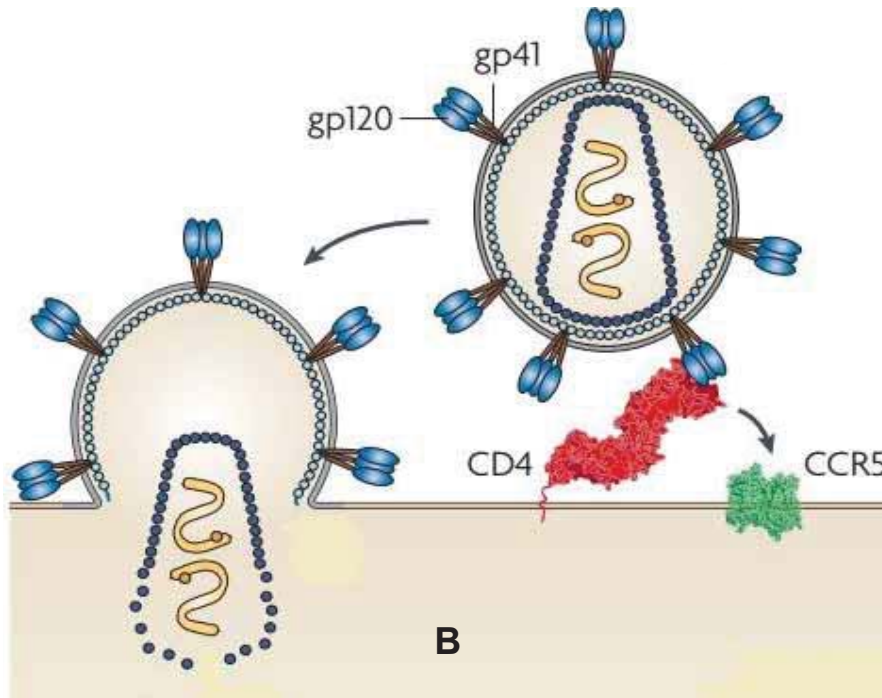
**(ii)** Suggest how eribulin work to prevent tubulin polymerisation and explain its effect on the behaviour of chromosomes in mitosis. [3]

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**[Total: 15]**

**Question 2**

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. 2.1** shows the entry process of a HIV into a macrophage, which is a type of white blood cell.



**Fig. 2.1**

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

.....

.....

.....

**(ii)** Compare the entry processes of the HIV and influenza virus into human host cells. [3]

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**(iii)** Upon completion of the entry process, describe how the HIV genome is inherited by macrophage daughter cells. [3]

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**(iv)** 'HIV-positive patients usually develop weak immunity.'

Explain what is meant by this statement. [4]

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Another example of an enveloped virus is the herpes simplex virus. Viral DNA enters the nucleus of the host cell via nuclear pores and *directs synthesis of viral RNA and DNA*. The virus is able to grow in non-dividing cells because its genome encodes enzymes such as viral DNA polymerase and thymidine kinase. Thymidine kinase is involved in synthesising deoxyribonucleotides required for DNA replication.

**(b)(i)** Describe the normal function of nuclear pores. [2]

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.....  
.....

**(ii)** State the process that is involved in the [1]

*Synthesis of viral RNA:* .....

*Synthesis of viral DNA:* .....

(iii) Describe two ways in which the named processes in (ii) are different. [2]

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Acyclovir is an anti-viral drug which is used to treat herpes. It prevents the complete replication of viral DNA by viral DNA polymerase. The structure of acyclovir is shown below in Fig. 2.2.

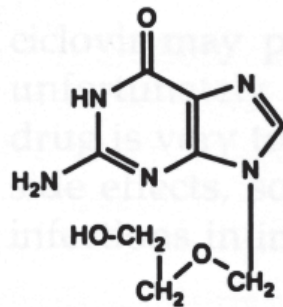


Fig. 2.2

When acyclovir enters the infected cell, it is first phosphorylated by thymidine kinase and subsequently becomes further phosphorylated by host cell kinases. When this phosphorylated acyclovir is incorporated into the newly-synthesised DNA strand, it prevents further elongation of the DNA strand.

(c)(i) With reference to Fig. 2.2, suggest how the incorporation of the phosphorylated acyclovir prevents further elongation of the DNA strand. [2]

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(ii) Some strains of herpes simplex virus are now resistant to acyclovir. Suggest how the virus has gained resistance to this anti-viral drug. [1]

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[Total: 20]

### Question 3

In a particular variety of tomato plant, the allele for red fruit colour (**A**) is dominant over the allele for orange fruit (**a**) and the allele for green base when ripe (**B**) is dominant over the allele for no green base when ripe (**b**).

Two students, Faiz and Jacob crossed plant with red fruit and green base when ripe with pure bred plant with orange fruit and no green bases when ripe. The phenotypes of 50 offspring of each of Faiz's and Jacob's crosses were recorded and are shown in **Table 3.1**.

**Table 3.1**

	Phenotypes of offspring of test crosses			
	Red fruit with green base	Red fruit with no green base	Orange fruit with green base	Orange fruit with no green base
Faiz's cross	23	4	3	20
Jacob's cross	3	21	23	3

degrees of freedom	probability, $p$				
	0.10	0.05	0.02	0.01	0.001
1	2.71	3.84	5.41	6.64	10.83
2	4.61	5.99	7.82	9.21	13.82
3	6.25	7.82	9.84	11.35	16.27
4	7.78	9.49	11.67	13.28	18.47

- (a)(i) With the aid of the table of probabilities as shown above, carry out a  $\chi^2$  test on the results of Faiz's cross and provide a brief explanation for the results obtained. [6]



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**(ii)** State the probability that the results of Faiz's cross depart significantly by chance from the expected ratio. [1]

.....

**(b)** Draw a genetic diagram to explain the results of Jacob's cross. [5]

(c) Explain the difference in results between Faiz's and Jacob's cross when the parental genotypes are the same. [3]

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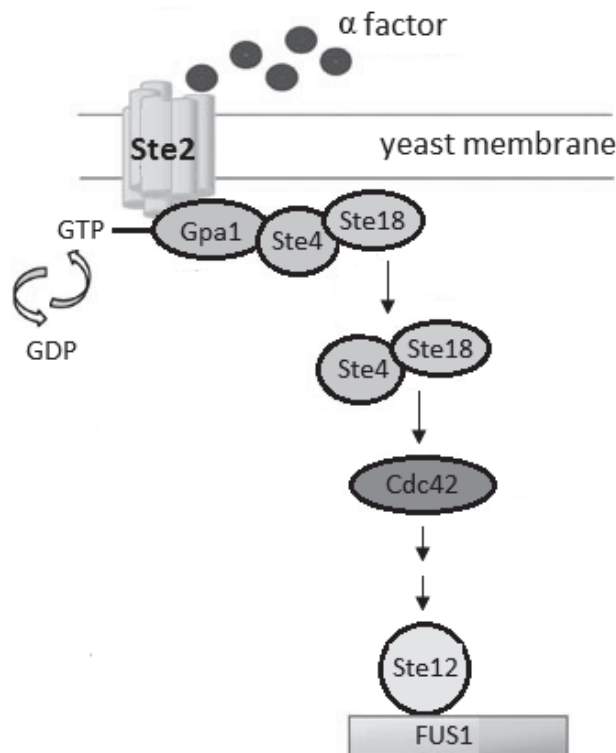
.....

[Total: 15]

#### Question 4

Yeast haploid cells secrete  $\alpha$  factor to signal mating, and respond by growing a mating projection towards a potential mate. Upon contact of the two partner cells, these fuse to form a diploid zygote.

**Fig. 4.1** shows the  $\alpha$  factor signaling pathway mediated by yeast G-protein coupled receptor, Ste2. The activation of the pathway induces the expression of *FUS1* gene which is required for yeast mating.



**Fig. 4.1**

**(a)(i)** With reference to **Fig. 4.1**, describe how  $\alpha$  factor triggers the activation of Cdc42. [4]

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**(ii)** Briefly explain why  $\alpha$  factor cannot enter the yeast cell directly. [2]

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**(iii)** Explain the possible role of Ste12 in the expression of FUS1 gene. [2]

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**(iv)** It has been observed that the binding of a protein Y to a region upstream of the promoter results in the mating projection to be produced at a rate higher than normal in the yeast cells.

Provide a reason for this observation. [3]

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Fig. 4.2 shows the Ste2 receptor on another yeast cell membrane.

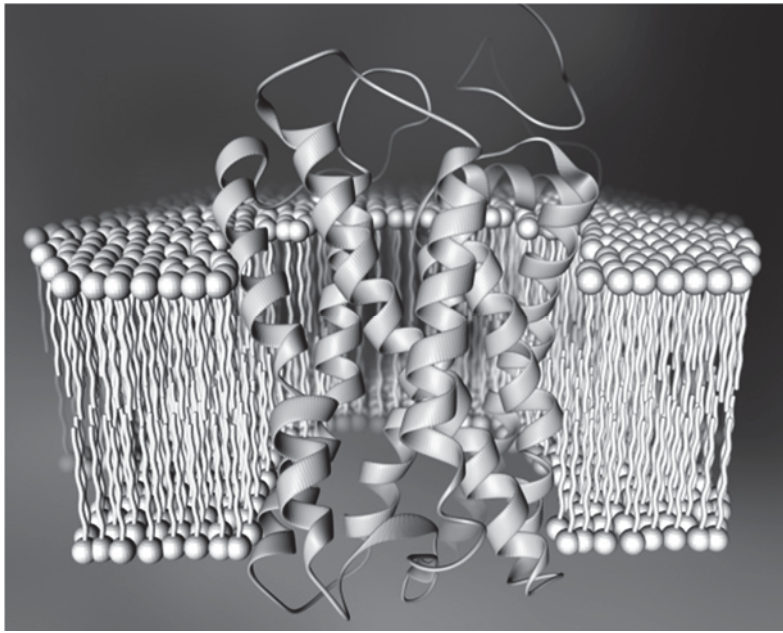


Fig. 4.2

(b)(i) Describe the structure of Ste2 receptor. [2]

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(ii) Explain how Ste2 receptor remains embedded in the yeast cell membrane. [2]

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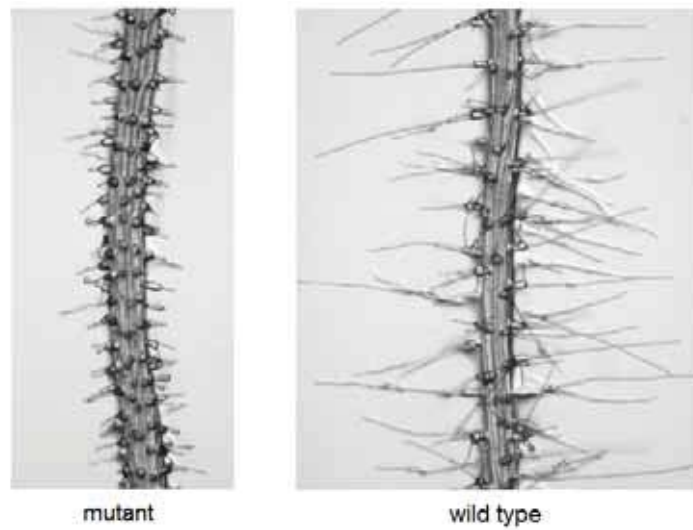
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[Total: 15]

**Question 5**

*Arabidopsis thaliana* is a small flowering plant native to Asia. A mutation in the gene coding for NADP oxidase results in plants with short root hairs. NADP oxidase is an enzyme that converts NADPH to NADP<sup>+</sup>.

**Fig. 5.1** shows the root hairs in the two variant of *A. thaliana*.



**Fig. 5.1**

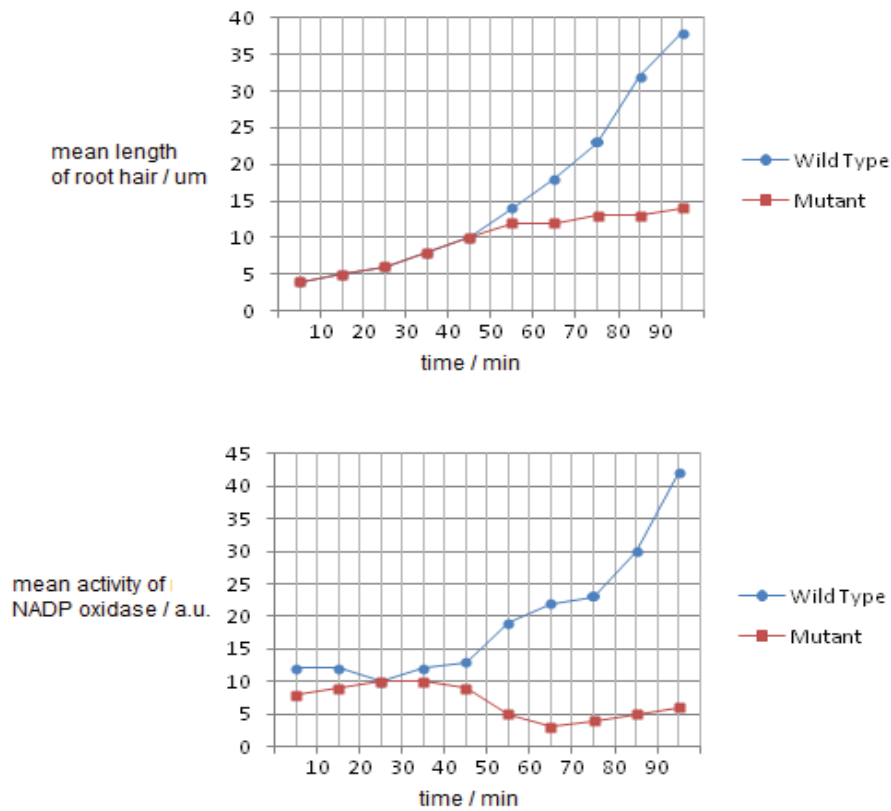
(a) Explain the role of NADPH in photosynthesis. [2]

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In a separate experiment, activity of NADP oxidase in the tips of the root hair cells in wild type and mutant *A. thaliana* were measured at intervals. Changes in mean length of the root hair cell was also measured to track the rate of growth, which is known to be an energy-requiring process. Both sets of results are shown in **Fig. 5.2**.



**Fig. 5.2**

(b) Using your knowledge of photosynthesis, provide an explanation for the difference in the growth of root hairs in the two types of *A. thaliana*. [4]

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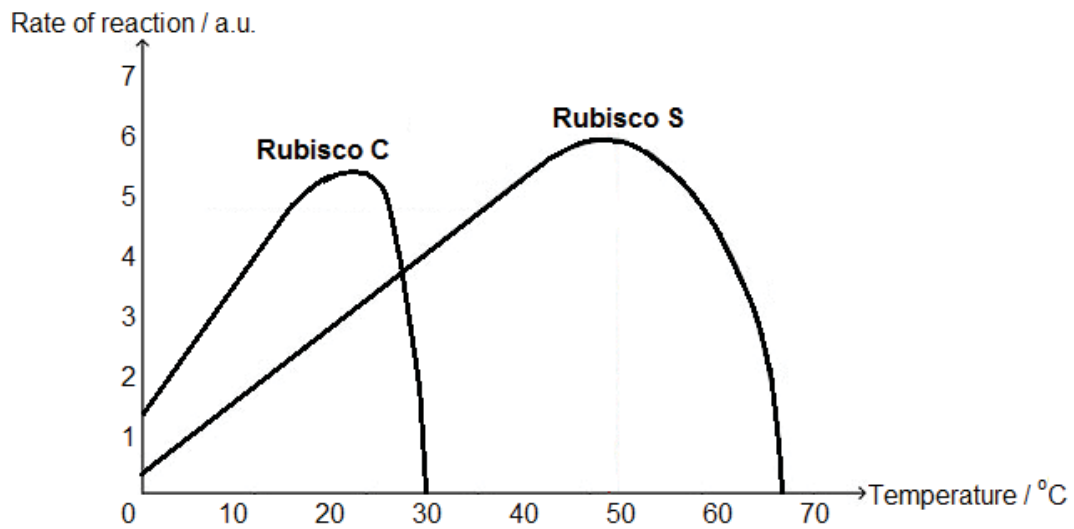
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Rubisco is an enzyme required in the light-independent stage of photosynthesis. **Fig. 5.3** shows the effect of increasing temperature on the activity of two variations of Rubisco, **Rubisco C** and **Rubisco S**.



**Fig. 5.3**

(c) With reference to **Fig. 5.3**, compare the effect of temperature on the two enzymes. [3]

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(d) It is known that **Rubisco C** is obtained from a species of coniferous tree found in Canada, while **Rubisco S** is obtained from a species of cactus found in the Sahara Desert.

(i) Explain how different alleles give rise to different Rubisco structure. [3]

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(ii) It has been predicted that **Rubisco S** will be found in more plant species in view of climate change. Explain how Darwin's theory of evolution supports this observation. [4]

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(iii) Suggest two other ways plants can adapt to the changing climate. [2]

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**[Total: 18]**

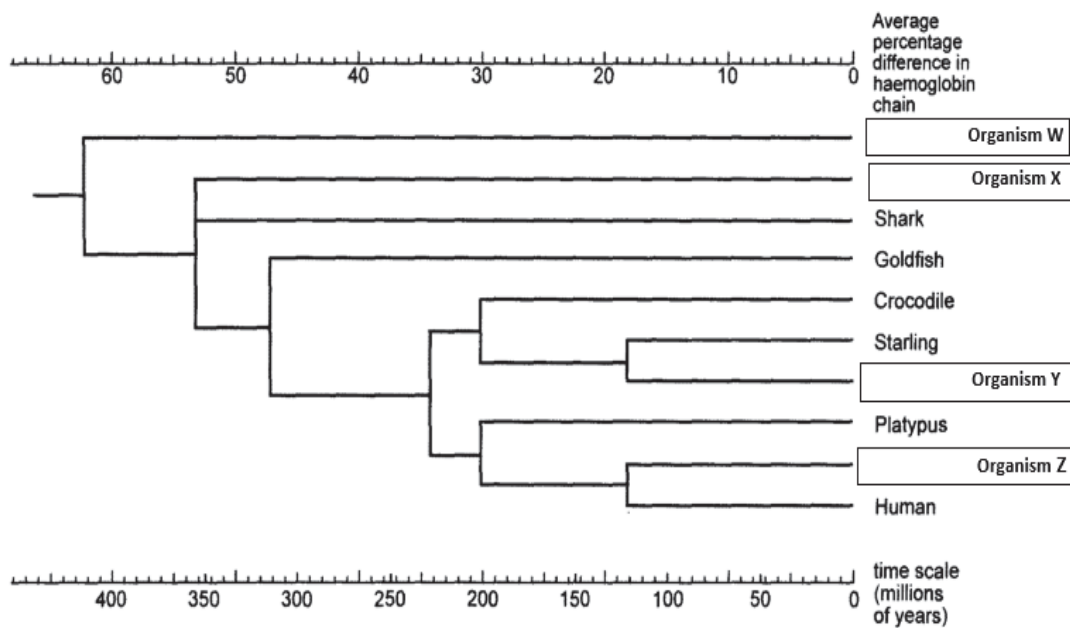


**Question 6**

The table below shows the amino acid differences in the cytochrome b protein between various vertebrates.

	Human	Elephant	Platypus	Ostrich	Starling	Crocodile	Lungfish	Coelacanth	Goldfish	Shark
Human		26	40	43	41	47	83	70	68	71
Elephant			45	45	48	50	84	72	63	74
Platypus				54	52	51	89	74	70	76
Ostrich					26	36	91	75	68	73
Starling						47	91	77	67	70
Crocodile							85	78	70	77
Lungfish								90	94	86
Coelacanth									83	78
Goldfish										88
Shark										

**Fig 6.1** shows the phylogenetic tree based on differences between the cytochrome b proteins.



**Fig. 6.1**

(a) Using information from the table and **Fig. 6.1**, identify organisms **W** to **Z**. [2]

Organism **W**: .....

Organism **X**: .....

Organism **Y**: .....

Organism **Z**: .....

(b) Distinguish between classification and phylogeny. [2]

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(c) Explain how differences in amino acid sequences in the cytochrome b chain allow the establishment of the phylogenetic tree. [2]

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(d) Suggest why homology still features prominently in evolutionary studies despite the advantages that molecular evidence can confer. [1]

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Brown adipocytes were one of the cells isolated for the above investigation. **Fig. 6.2** shows the schematic representation of a series of protein complexes found on the inner membrane of organelle **X** present in brown adipocytes.

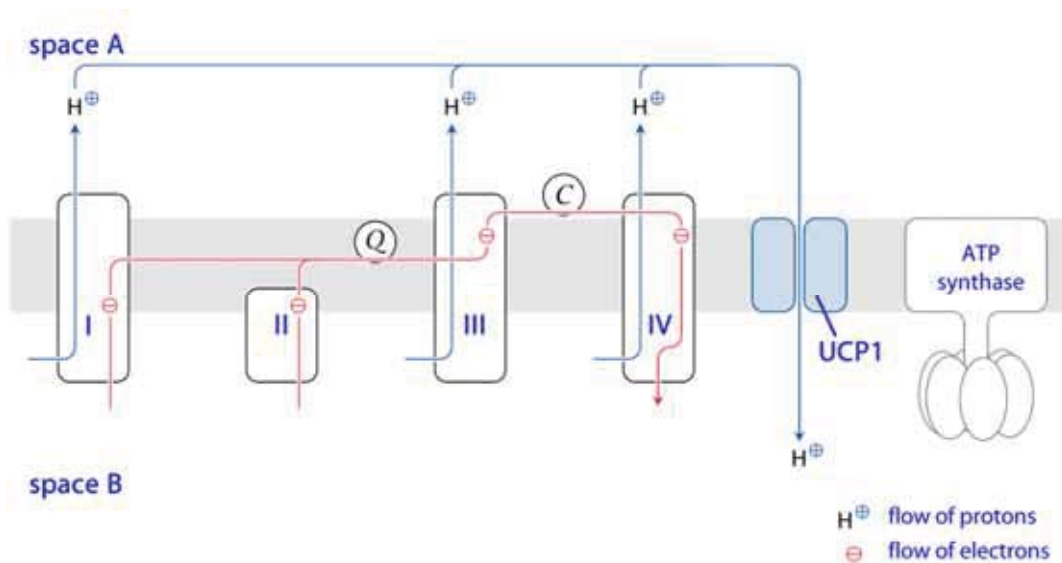


Fig. 6.2

**(e)(i)** State the identity of organelle **X**. [1]

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**(ii)** Outline how ATP is usually synthesised in the inner membrane of organelle **X**. [4]

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**(f)** Brown adipocytes contain a unique protein, UCP1, which is not found in organelle **X** in any other cell type.

Evaluate the impact of UCP1 on ATP synthesis and suggest the physiological significance of brown adipose tissue. [3]

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**(g)** In other cell types, NADH and FADH<sub>2</sub> are used to drive ATP synthesis by ATP synthase. Using relevant information from **Fig. 6.2**, suggest and explain why more ATP is produced from NADH. [2]

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**[Total: 17]**

**END OF PAPER**

CANDIDATE NAME: \_\_\_\_\_

INDEX NUMBER \_\_\_\_\_



SERANGOON JUNIOR COLLEGE  
JC2 PRELIMINARY EXAMINATION 2017

BIOLOGY 9744  
Higher 2

CG \_\_\_\_\_

PAPER 3

Friday  
15 September 2017

2 hours

Additional materials:  
Optical Mark Sheet

**READ THESE INSTRUCTIONS FIRST**

Write your name and CG in the spaces at the top of this page.

Write in dark blue or black pen.

Do not use staples, paper clips, glue or correction fluid.

Do NOT write in any barcodes.

**Section A**

Answer all the questions in the spaces provided in the question paper.

**Section B**

Answer any **one** question in the spaces provided in the question paper.

FOR EXAMINER'S USE	
Paper 3	
1	/29
2	/21
3 or 4	/25
<b>P3 Total</b>	<b>/75</b>

At the end of the examination, fasten all your work securely together.

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

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This question paper consists of 14 printed pages.

## Section A

Answer all the questions in this section.

### Question 1

#### Repeat droughts may cause permanent damage to forests

By: [Alex Whiting](#)

ROME, Aug 9 (Thomson Reuters Foundation) - Trees and their environment need as long as two years to recover from drought in some places, and if a second dry spell hits before then, it may cause permanent damage to the landscape, researchers said on Wednesday.

With climate change expected to bring more frequent and intense droughts, the implications for areas that do not have time to bounce back fully could be severe, the researchers said in a paper to be published in *Nature* journal this week. "That could have a double whammy effect," said co-author William Anderegg, assistant professor of biology at the University of Utah. "A second drought could be harder on an ecosystem and have the potential to push it off a cliff."

In practice, that means affected areas could eventually turn from lush forest to a land of grass and shrubs. Boreal forests in northern parts of Europe, Russia and Canada can take up to two years to recover from drought, partly because they do not have a wide variety of plants, Anderegg told the Thomson Reuters Foundation. Forests in the tropics of South America and Southeast Asia have also taken the same amount of time to rebound.

**"That's worrisome because those regions store the largest chunks of carbon in ecosystems across the globe," Anderegg said.** Forests help tackle climate change by sucking carbon out of the air, reducing levels of planet-warming carbon dioxide, the main greenhouse gas. But when trees die, most of the carbon they have absorbed is released back into the atmosphere.

The Amazon rainforest suffered a double drought in the first decade of this century when dry spells, both of a once-in-a-100-years severity, hit the region. "Satellites showed that forests hadn't recovered from the 2005 drought by the time the 2010 drought struck," Anderegg said.

#### Adapted from a Reuters Article

- (a) Discuss how anthropogenic climate change has affected the growth of forests in the world. [4]

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(b) The article talks about how forests may not be able to recover if a second drought soon after a first. Suggest how forests may recover from a drought and explain why a second drought soon after a first impedes its ability to recover. [4]

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(c) The article talks about forests storing carbon as shown by the following quote "*That's worrisome because those regions store the largest chunks of carbon in ecosystems across the globe,*" Anderegg said. Define the role of forests in storing carbon and explain briefly how they are able to do this. [4]

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Climate change does not just affect our forests, it also affects our farms. The following article elaborates on this.

*Aug. 4 (UPI) -- Scientists from Lancaster University suggest major changes in agricultural practices are needed to offset increases in nutrient losses due to climate change. The study, shows that phosphorus losses will continue to increase due to climate change unless major changes in agricultural practices are made.*

*"Although farmers are already doing what they can to prevent these losses, the currently adopted measures are not likely to be enough to offset the increase expected under climate change. This paper should alert policy makers and government to the help and support that farmers will need to achieve the scale of agricultural change that may be necessary to keep up with the increase in pollution due to climate change."*

*Although phosphorus and nitrogen are essential to crop and animal growth, too much of it can cause algae blooms in rivers and lakes.*

(d) Phosphorus and nitrogen are essential nutrients for plant growth and are typically found in large concentrations within the fertilizers used on farms. Suggest how climate change can lead to phosphorus and nitrogen from fertilizers ending up in rivers and water bodies. [2]

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(e) The article talks about nitrogen and phosphorus causing algal blooms in rivers. Using a specific example of a plant or animal, discuss the possible impact of such blooms on the natural ecosystem in these rivers. [4]

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- (f) The article suggests that farmers need to change their agricultural practices to limit the impact of phosphorus used in fertilizer on nearby aquatic bodies. Describe two changes they could make and suggest why it may be difficult for them to make such changes. [3]

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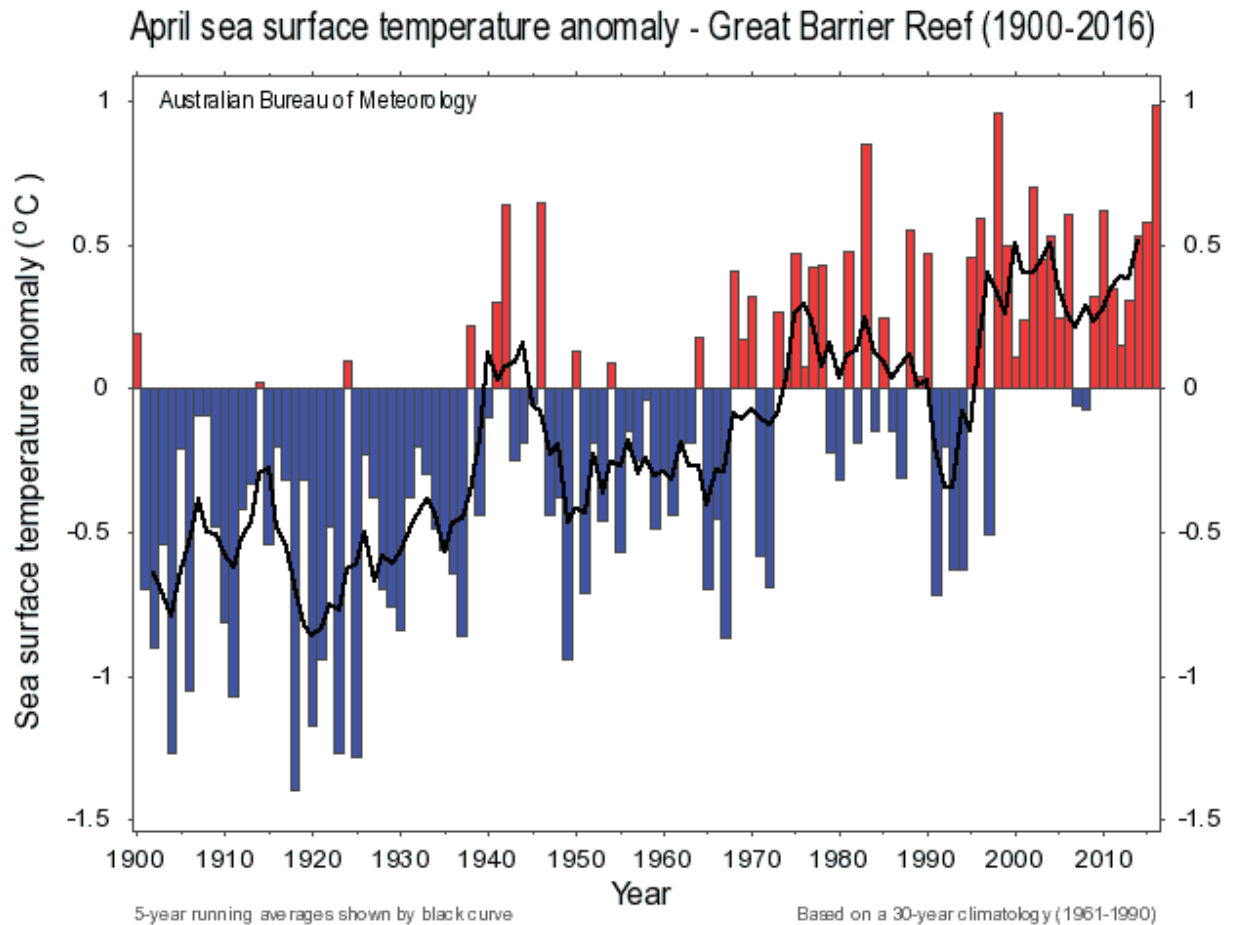
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Another aquatic ecosystem affected by climate change are coral reefs. The following graph, **Figure 1**, shows how sea water temperatures have varied over the last century or so at the Great Barrier Reef. 0°C is considered the average sea water temperature.

Rising sea level temperatures have been suggested as a reason for more frequent coral bleaching events. **Figure 2** shows the trend for coral bleaching events globally since 1980.



**Figure 1**

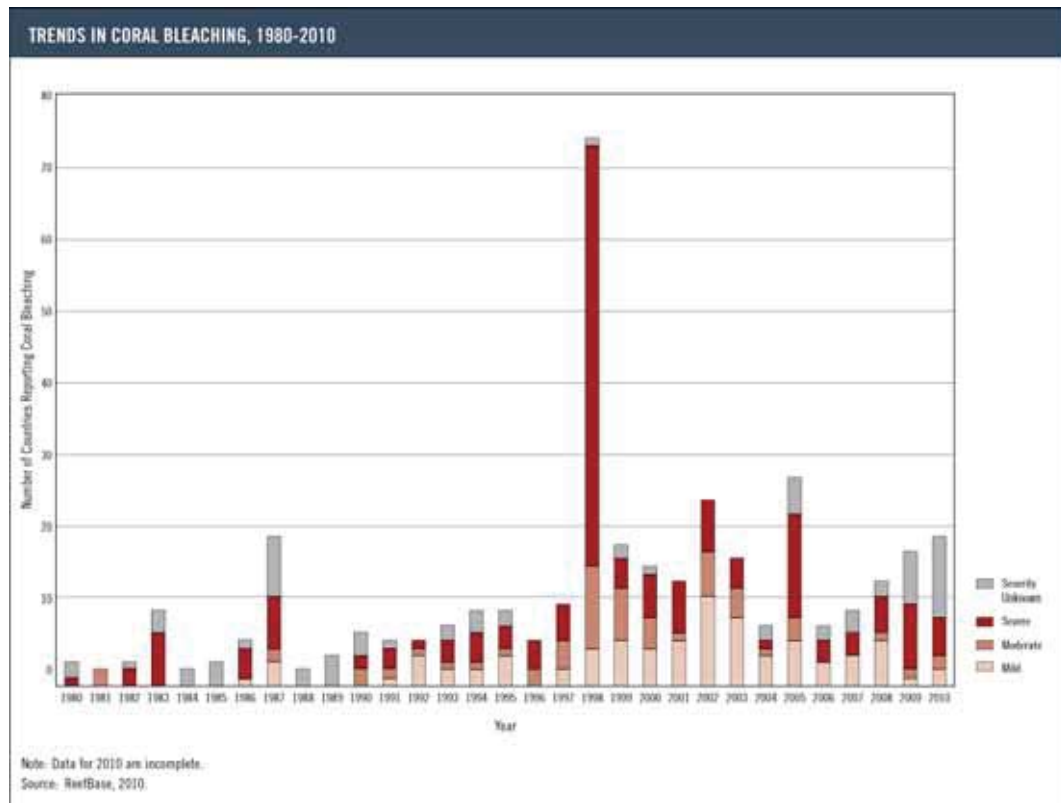


Figure 2

(g) With reference to **Figures 1 & 2**, discuss if the data provides evidence that the more frequent bleaching events seen in coral reef ecosystems over the last 15 years are due to rising water temperatures. [6]

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(h) Other than warming water temperatures, describe two other ways anthropogenic climate change has impacted coral reefs. [2]

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**[Total: 29 marks]**

## Question 2

A method that has been used to sequence DNA is called Sanger Sequencing, named after the scientist that invented the process. Sequencing DNA essentially allows the sequence of bases on a DNA strand to be read. During Sanger sequencing, DNA polymerases copy single-stranded DNA templates by adding nucleotides to a growing chain (**extension product**). Chain elongation occurs at the 3' end of a primer, an oligonucleotide that anneals to the template. The extension product grows by the formation of a phosphodiester bridge between the 3'-hydroxyl group on the primer and the 5'-phosphate group of the incoming deoxynucleotide.

DNA polymerases can also incorporate analogues of nucleotide bases. The dideoxy method of DNA sequencing developed by Sanger et al. 1977 takes advantage of this characteristic by using 2',3'-dideoxynucleotides (ddNTPs) as substrates. When dideoxynucleotides are incorporated at the 3' end of the growing chain, chain elongation is terminated selectively at A, C, G, or T. This leads to the production of a DNA fragment with the ddNTP at its end.

The ddNTPs are usually labelled using fluorescent tags. Many fragments of the original DNA strand terminated in the manner shown in **Figure 3** are created using this processes. When all these fragments are separated on a gel using a form of gel electrophoresis, the sequence of bases can be read by reading the labelled ddNTPs from the shortest to the largest fragment.

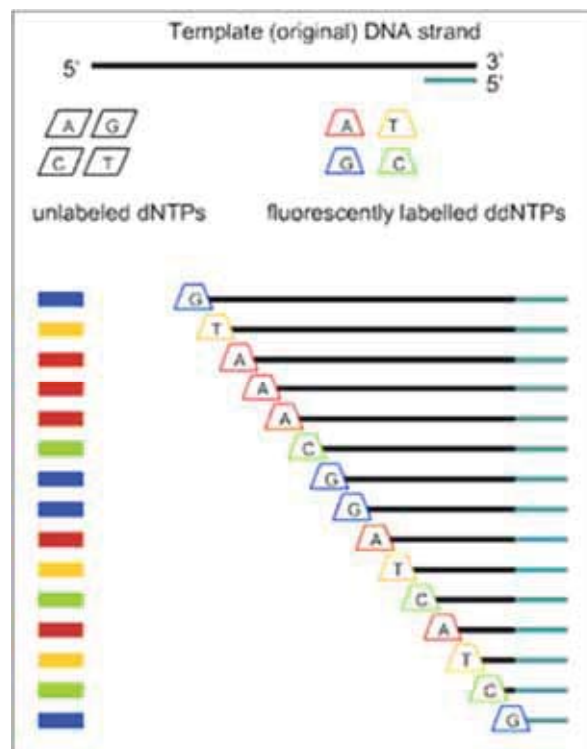


Figure 3

(a) Suggest how the labelling of the ddNTPs allows for the identification of the base that normally occupies that position. [2]

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(b) Describe how the extension product (line 4) is created and explain the role of the template DNA strand to this process. [5]

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(c) Explain how, when separated using gel electrophoresis, the strands produced by Sanger Sequencing end up in different positions on the gel. [3]

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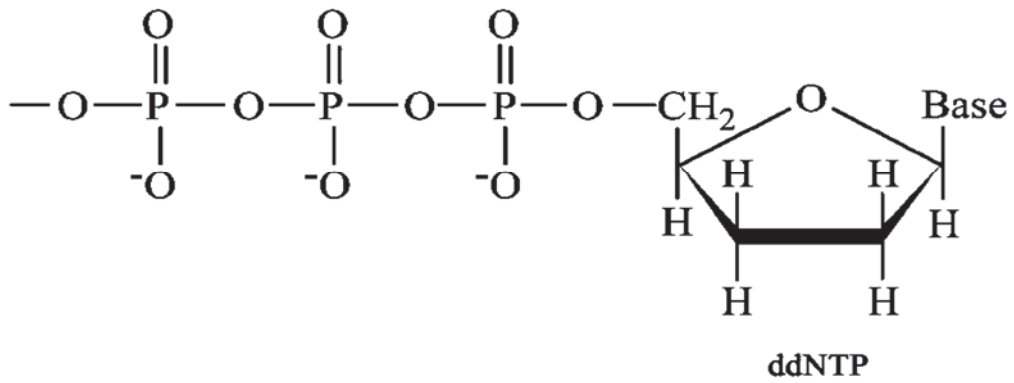
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The following **Figure 4** shows an example of dideoxynucleotide (ddNTP).



**Figure 4**

(d) Explain why dideoxynucleotides, such as the one shown in Figure 4, leads to the formation of fragmented DNA. [3]

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(e) Methods such as Sanger Sequencing has enabled the sequencing of the human genome. This has opened up the possibility of detecting specific alleles in human genomes. Discuss the possible advantages of this and its possible ethical ramifications. [4]

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Human genomes include the DNA found in the mitochondria. Similar to the nuclear genome, the mitochondrial genome is made up of double-stranded DNA, and it encodes genes. However, the mitochondrial genome differs from the nuclear genome in several ways

(f) Suggest two ways in which mitochondrial DNA may differ from the nuclear genome. [2]

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(g) With reference to specific genes, explain the role of mitochondrial DNA. [2]

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**[Total: 21 marks]**

**Section B (25 Marks)**

Answer **only one** question

Write your answers in the space provided.

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

Your answers must be in continuous prose where appropriate.

Your answers must be set out in sections (a), (b), etc as indicated in the question.

**Question 3**

- (a) All organisms need to replicate and transcribe/translate their DNA in the process of growth and development. Even viruses need to do so. Using the example of a dengue virus, compare and contrast the replication and protein synthesis process in a virus with the similar processes in a typical eukaryotic cell. [13]
- (b) Describe how viruses such as the influenza virus is able to create genetic variation and explain how this makes it difficult for us to eradicate harmful viruses with modern medicine. [12]

**Question 4**

- (a) All organisms need to control the expression of their DNA in the process of growth and development. Even bacteria need to do so. Using the example of *E. coli*, compare and contrast the control of gene expression in a bacteria with the similar processes in a typical eukaryotic cell. [13]
- (b) Describe how bacterial cells are able to create genetic variation and explain how this makes it difficult for us to eradicate harmful bacteria with modern medicine. [12]

**End of Paper**



CANDIDATE NAME \_\_\_\_\_

CG \_\_\_\_\_



**SERANGOON JUNIOR COLLEGE**  
**JC2 PRELIM PRACTICAL EXAMINATION 2017**

**H2 BIOLOGY**

**9744**  
**2.5 hours**

**INSTRUCTIONS TO CANDIDATES**

Write your name, CG and index number in the spaces at the top of this page.

Write in dark blue or black pen.

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.

Answer **all** questions in the spaces provided on the Question Paper.

<b>FOR EXAMINER'S USE</b>	
<b>Question</b>	
<b>1</b>	<b>/13</b>
<b>2</b>	<b>/14</b>
<b>3</b>	<b>/14</b>
<b>4</b>	<b>/14</b>
<b>TOTAL</b>	<b>/55</b>

**INFORMATION FOR CANDIDATES**

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

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**This question paper consists of 14 printed pages.**

## Question 1

You are provided with a quantity of vitamin C solution and a dye called DCPIP.

You are also provided with three test-tubes containing respectively lemon juice, orange juice, grapefruit juice, and labelled as such. These juices contain natural vitamin C and the dye DCPIP can be used to determine the concentration of this vitamin in the juices.

**Apparatus:** 6 test-tubes and a test-tube rack  
4 plastic teat pipettes  
Plastic ruler

**Material:** 30 cm<sup>3</sup> DCPIP solution, labelled '**DCPIP**'  
50 cm<sup>3</sup> vitamin C solution, labelled '**Vitamin C solution**'  
50 cm<sup>3</sup> lemon juice  
50 cm<sup>3</sup> orange juice  
50 cm<sup>3</sup> grapefruit juice

Proceed as follows:

1. Into a clean test-tube, transfer a quantity of the dye DCPIP to a depth of 0.5 cm. Take note its colour.
2. Fill a teat pipette with vitamin C solution. Add one drop of vitamin C solution to the DCPIP solution in the test-tube and shake gently. Continue to add the drops, counting the number of drops which are needed to bring about a colour change. Shake gently after each drop, refilling the pipette if necessary.
3. Record the initial colour of DCPIP (from step 1) and the first colour change after vitamin C is added as well as the number of drops counted to bring about this colour change in a suitable table after step 9.
4. After the first colour change, continue adding drops of vitamin C and counting the drops until the DCPIP solution becomes colourless/or consistent pale yellow. (Ignore any coloured granules that might form.). Record the number of drops counted in the same table from step 3.
5. Repeat steps 1 to 4 adequately to obtain enough data for analysis, cleaning all apparatus before use.
6. Place the DCPIP solution into each of three clean test-tubes to a depth of 0.5 cm. (The amount of DCPIP solution must be exactly the same in each of the tubes). Label the tubes A, B and C.
7. Fill a clean teat pipette with lemon juice and drop by drop add this to the contents of tube **A**, shaking the tube gently after each drop. Count the number of drops needed to turn the DCPIP solution colourless. Repeat this step adequately to obtain enough data for analysis.
8. Repeat the step 7 with orange juice and grapefruit juice, using a clean pipette each time to add the juice to the DCPIP solution in tubes **B** and **C** respectively.

9. Record the results for the three juices and the earlier results for DCPIP and Vitamin C in an appropriate table. [5]

10. What conclusions can you draw from your results? [2]

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11. Comment on the main source(s) of error and the limitations of the measurements or experimental procedure. [3]

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12. What improvements could you make to the experimental procedures to overcome these sources of error? [3]

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[Total: 13 marks]

## Question 2

You are provided with a leaf labelled Q. In this question you will be required to investigate the number of stomata present on the lower surface of leaf Q.

Proceed as follows:

1. Use nail varnish to cover a small area of the lower epidermis of leaf Q. Apply a thin layer over an area about  $1\text{cm}^2$ . Avoid any large veins that may be present.
2. Repeat this process for three different areas of the lower epidermis.
3. Allow the nail varnish about 20 minutes to dry. (Proceed to question 3 while the leaf is drying)
4. After 20 minutes, use a razor blade or a fine scalpel to lift one edge of the layer of nail varnish. Use forceps to then gently peel a layer of nail varnish off the leaf.
5. Transfer this layer of nail varnish to a slide and cover with a cover slip.
6. Examine the slide using a microscope and count the number of stomata you can observe in the field of view.
7. State which objective you chose to make this stomatal count and explain your choice. [1]

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8. Repeat the counting of stomata in the field of view for a second piece of peeled nail varnish. Calculate the mean number of stomata per field of view in the space below. [2]

9. Make a high power drawing of three adjacent stomata from either of your stomatal peels. [3]

10. Using the eyepiece graticule in your microscope and the provided stage micrometer, find the actual length, in  $\mu\text{m}$ , of one of the guard cells that you have drawn. [3]

A researcher obtained leaves from two different plants, Plant A and Plant B. From the same forest. He found that the number of stomata on the underside of the both leaves differed. He ensured that he calculated the number of stomata based on per unit area and ensured he had sufficient replicates. However, the numbers still did not match. The mean density of stomata per  $1\text{cm}^2$  for leaves A and B were as follows:

Leaf A – 234  
Leaf B – 297

11. While the researcher expected a difference in the number of stomata, he could not be sure if the difference seen was significant. Suggest a statistical test he could use to confirm if the difference was significant. [1]

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12. When carried out this test, the probability value he obtained was less than 0.05. Comment on what these results show and suggest an explanation for the pattern seen in Leaves A and B collected by the researcher. [4]

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[Total: 14 marks]

### Question 3

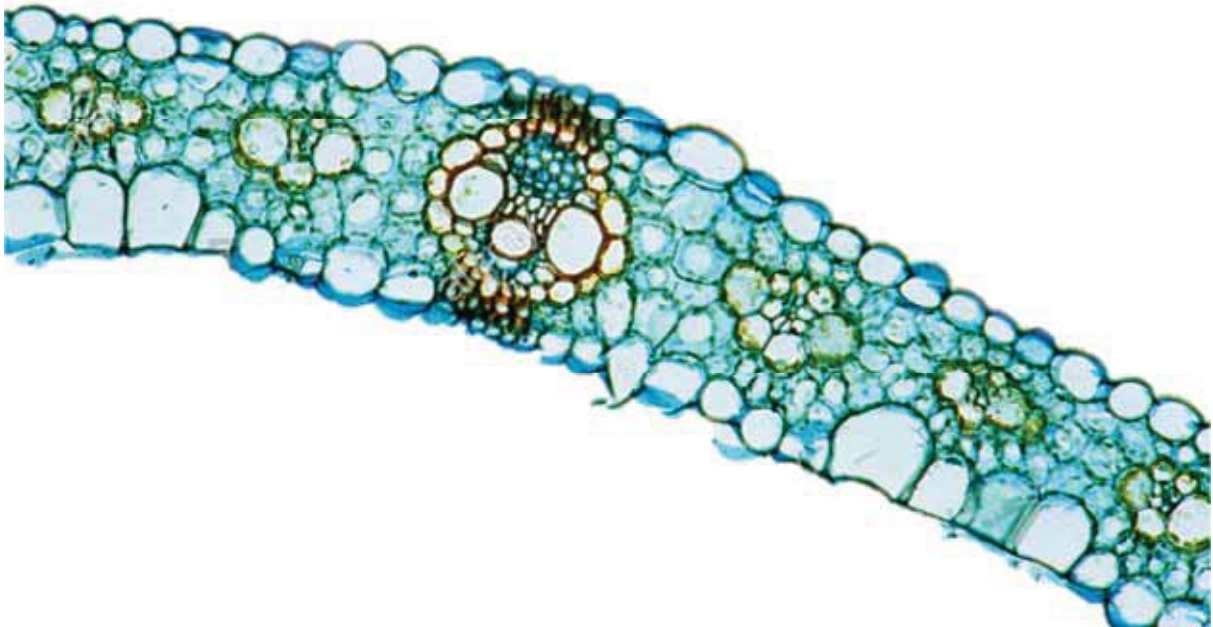
Slide S1 is a transverse section from a leaf of the *Amophilia* plant.

(a) In the space below, draw a labelled plan drawing of this leaf. [6]

(b) Using the provided **slide graticule**, measure the diameter of the leaf. You may assume that the slide graticule can be used as you would use a ruler. [1]

Diameter of leaf: \_\_\_\_\_

The following **Figure 2.1** is a microscope image of a section of a typical monocot leaf.



*From alamy.com*

**Figure 2.1**

(c) Given that the magnification of Figure 2.1 is 50X, calculate the actual width of the specimen. [2]



(d) Identify three differences between a typical monocot leaf and the *Amophilia* leaf. [3]

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(e) Suggest reasons for the differences you have identified. [2]

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[Total: 14 marks]

#### Question 4

The Tasmanian Tiger or Thylacine (*Thylacinus cynocephalus*), the world's largest carnivorous marsupial, was once common throughout Australia and Papua New Guinea. The Thylacine resembled a large, short-haired dog with a stiff tail which smoothly extended from the body in a way similar to that of a kangaroo. The female Thylacine had a pouch with four teats, but unlike many other marsupials, the pouch opened to the rear of its body.

An example of convergent evolution, the Thylacine showed many similarities to the members of the Canidae (dog) family of the Northern Hemisphere: sharp teeth, powerful jaws, raised heels and the same general body form.

Due to human activities, the Thylacine was hunted to extinction by early 1930. A Thylacine specimen with soft tissue remaining is found in the Australian Museum in Sydney.

Imagine that you are a researcher in the Australian Rare Fauna Research Association. Recently it was reported that locals near Mount Carstensz in Western New Guinea had sighted creatures that resemble Thylacines. Some members of the Association believe that the creatures sighted may be descendents of the Thylacine, while other members believe that the creatures may be a new species. If the former were true, then the Thylacine is not extinct and conservation effects may revive the species.

Plan an investigation to investigate if these creatures found in Western New Guinea are descendents of the Thylacine that was thought to be extinct.

Your planning must be based on the assumption that you have been provided with the following equipment and materials.

- Tissue sample from the museum specimen and from the Western New Guinea creatures under investigation
- Pestle and mortar
- DNA extraction buffer solution
- Microcentrifuge tubes
- Centrifuge
- Restriction enzymes
- Agarose or polyacrylamide gel plate
- Suitable source of electric current
- Radioactive probe
- Nitrocellulose membrane
- Autoradiography equipment

Your plan should have a clear and helpful structure to include:

- An explanation of the theory to support your practical procedure
- A description of the method used including scientific reasoning behind the method
- The type of data generated by the experiment
- How the results will be analysed including how the origin of the organism can be determined

[Total: 14]

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**End of Paper**



CANDIDATE NAME: \_\_\_\_\_

INDEX NUMBER \_\_\_\_\_



SERANGOON JUNIOR COLLEGE  
JC2 PRELIMINARY EXAMINATION 2017

BIOLOGY 9744  
Higher 2

CG \_\_\_\_\_

PAPER 1

Thursday  
21 September 2017

1 hour

Additional materials:  
Optical Mark Sheet

### INSTRUCTIONS TO CANDIDATES

Write your name and CG in the spaces at the top of this page.

On the Optimal Mark Sheet, enter your name, subject title, test name, class. For your index number, enter your full NRIC number. Shade the corresponding lozenges on the OMS according to the instructions given by the invigilators.

**AT THE END OF THE EXAMINATION, HAND IN BOTH THE OMS AND QUESTION PAPER.**

### INFORMATION FOR CANDIDATES

There are **thirty (30) questions** in this paper. Answer **all** questions. For each question, there are four possible answers, **A, B, C, D**. Choose the **one** you consider correct and record your choice in **soft pencil** on the OMS.

Each correct answer will score one mark. A mark will not be deducted for a wrong answer. Any rough working should be done on the question paper.

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**This question paper consists of 16 printed pages.**

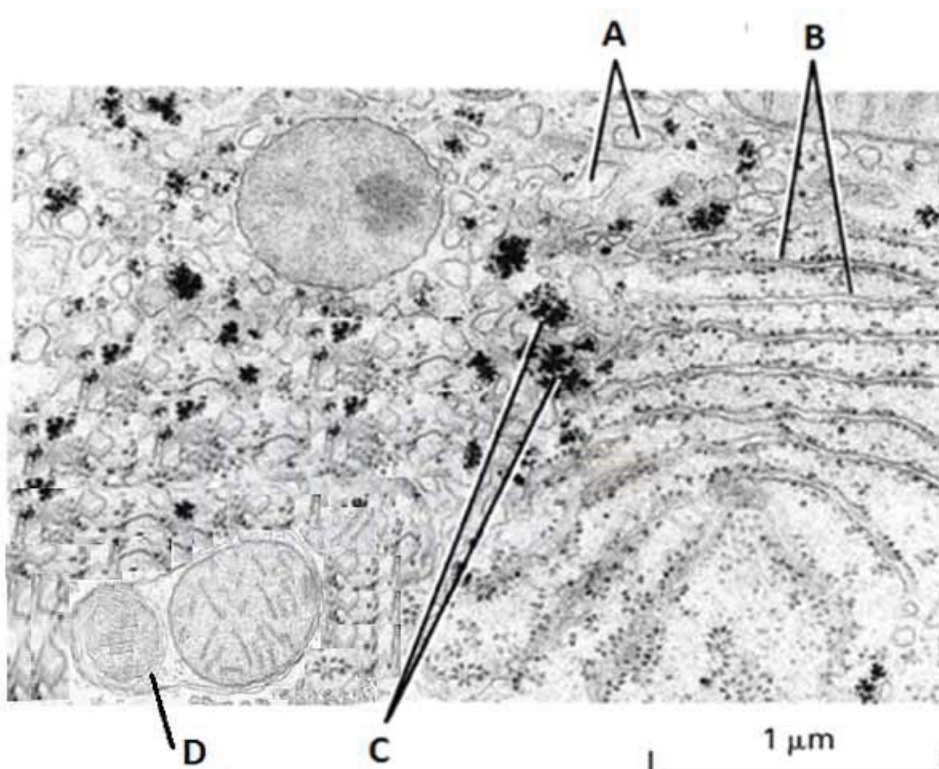


**Answer all questions on the OTAS provided.**

- 1 EDTA is used extensively as an anticoagulant for stored blood in blood banks. Thrombokinase plays a major role in the clotting of blood. EDTA decreases the reaction rate of thrombokinase by binding to calcium ions.

Which of the following describes the role of calcium ions?

- A Allosteric inhibitors
  - B Coenzymes
  - C Cofactors**
  - D Competitive inhibitors
- 2 A cell in the G1 phase has two homologous pairs of chromosomes. It then undergoes two mitotic divisions. At the end of the second mitotic division, what is the total number of chromosomes and gene loci found in all the daughter cells formed?
- A 8 chromosomes and 4 times as many gene loci as the original parent cell.
  - B 8 chromosomes and 8 times as many gene loci as the original parent cell.
  - C 16 chromosomes and 4 times as many gene loci as the original parent cell.**
  - D 16 chromosomes and 8 times as many gene loci as the original parent cell.
- 3 The electron micrograph below shows a liver cell.

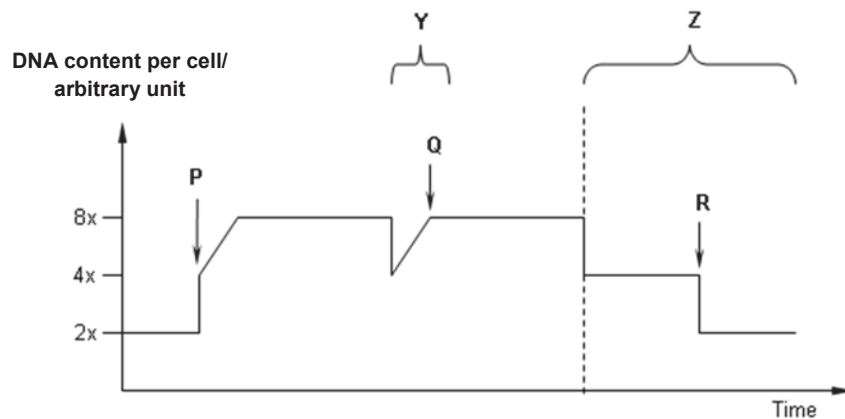


Which statement(s) correctly describe(s) the labelled structures?

- 1 Structure **A** transports proteins from Structure **B** to Golgi Apparatus.
- 2 Proteins enter the lumen of Structure **B**, where they undergo chemical modifications such as glycosylation.
- 3 Structure **C** is starch grain.
- 4 The process shown in structure **D** is autolysis.

**A** 2 only      **B** 1 and 2 only      **C** 2 and 3 only      **D** 2, 3 and 4 only

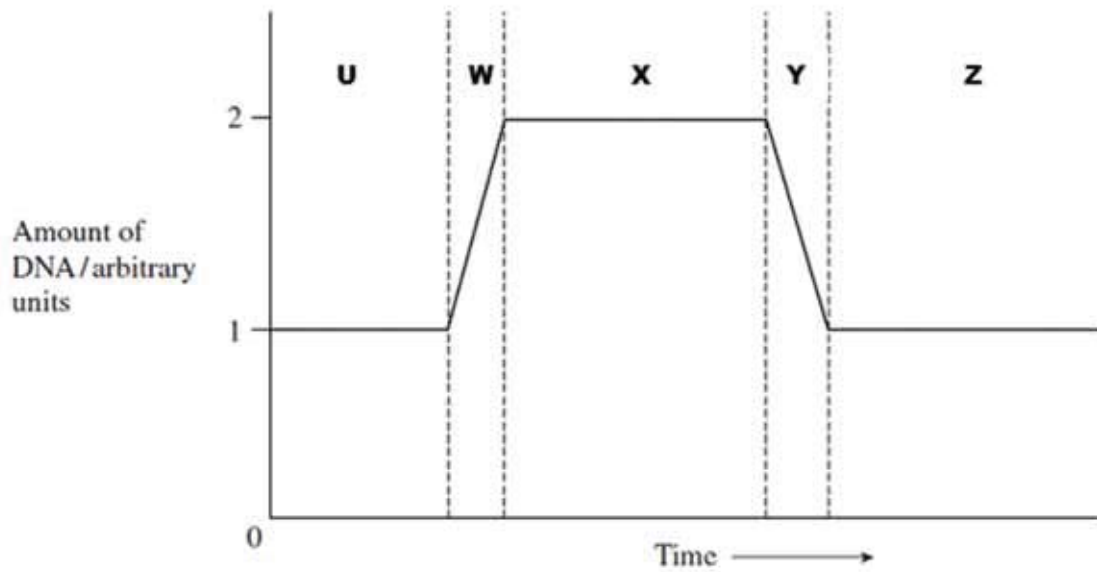
- 4 The graph represents the changes in the DNA content within a cell at different stages in the cell cycle.



Name the events occurring at **P**, **Q** and **R**, and identify the stage where meiosis is occurring.

	<b>P</b>	<b>Q</b>	<b>R</b>	<b>Meiosis occurring at</b>
<b>A</b>	S phase	Fertilisation	Cytokinesis	<b>Y</b>
<b>B</b>	<b>Fertilisation</b>	<b>Interphase</b>	<b>Cytokinesis</b>	<b>Z</b>
<b>C</b>	S phase	Prophase	Telophase	<b>Y</b>
<b>D</b>	Fertilisation	Metaphase	Telophase	<b>Z</b>

- 5 The graph shows changes in the amount of DNA in a cell during one cell cycle. The letters U – Z marks out the different phases in the cell cycle.



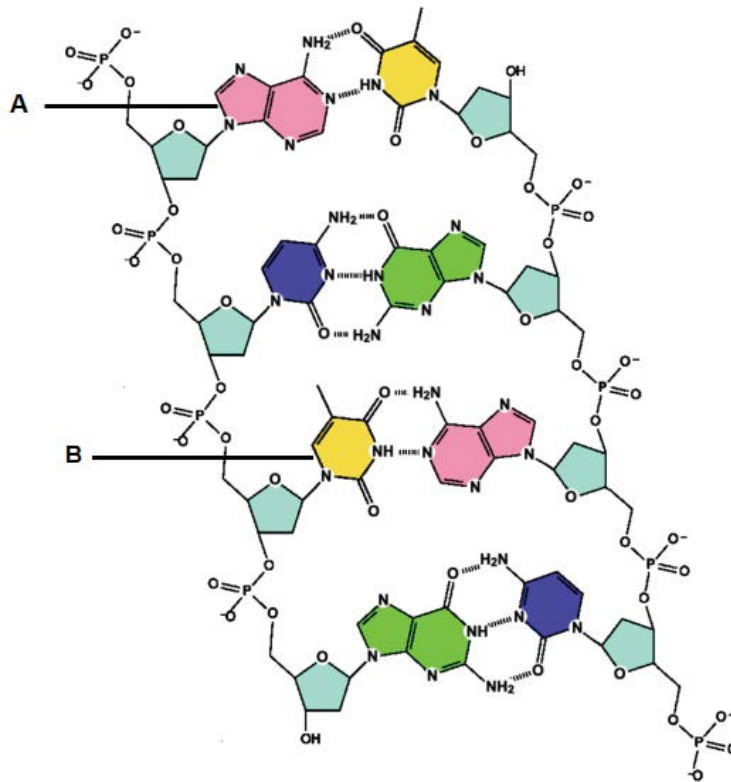
Many drugs that are used to treat cancer work at different time periods during the cell cycle.

- (i) Cisplatin binds to DNA and stops free DNA nucleotides from joining together.
- (ii) Drug B stops spindle fibres from shortening.

With reference to the cell cycle above, determine where these 2 drugs work.

	Cisplatin	Drug B
<b>A</b>	<b>W</b>	<b>X</b>
<b>B</b>	<b>W</b>	<b>Y</b>
<b>C</b>	<b>U</b>	<b>X</b>
<b>D</b>	<b>U</b>	<b>Z</b>

6 The figure below shows a DNA molecule.



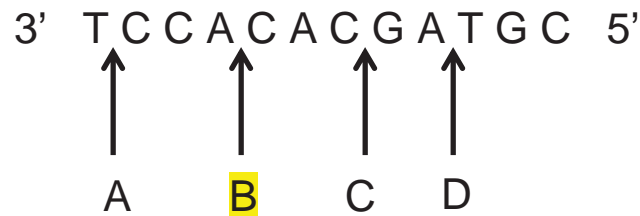
Which statement(s) correctly describe the polynucleotide?

- 1 The structure labelled **A** corresponds to that of a purine, while the structure labelled **B** corresponds to that of a pyrimidine.
- 2 The antiparallel nature of DNA double helix allows phosphodiester bonds to form between the nitrogenous bases of opposite strands.
- 3 Distance between adjacent deoxyribonucleotides is 3.4  $\text{\AA}$  and one turn consists of 10 deoxyribonucleotides. (Note: 10  $\text{\AA}$  = 1 nm)
- 4 The wound DNA double helix consists of alternating major grooves and minor grooves along its axis which are essential for the binding with proteins.

- A** 1 only  
**B** 1 and 2 only  
**C** 3 and 4 only  
**D** 1, 3 and 4 only

- 7 The RNA triplet UAG acts as a stop codon terminating the synthesis of a polypeptide. The diagram shows a template strand of DNA which codes for four amino acids.

Where would a mutation, introducing a thymine nucleotide, result in the termination of translation?



- 8 Which of the following is **not** a feature of eukaryotic gene expression?

- A Polycistronic mRNAs are very rare.
- B Many genes are interrupted by noncoding DNA sequences.
- C RNA synthesis and protein synthesis are coupled.**
- D mRNA is often extensively modified before translation.

- 9 Human telomeres consist of repeating TTAGGG sequences which extend from the ends of the chromosomal DNA. When cells undergo mitotic division, some of these repeating sequences are lost. This results in a shortening of the telomeric DNA.

What is a consequence of the loss of repeating DNA sequences from the telomeres?

- A The cell will begin the synthesis of different proteins.
- B The cell will begin to differentiate as a result of the altered DNA.
- C The number of mitotic divisions the cell can make will be limited.**
- D The production of mRNA will be reduced.

- 10 The translation mixture contains a polynucleotide that directs the synthesis of Met-Gly-Gly-Phe-Leu-Ala. In the presence of Azithromycin, this polymer directs the synthesis of Met-Gly only.

From the information given, which of the following deductions could you make about Azithromycin?

	Control Stage	Conclusion
A	Translational	It prevents formation of the initiation complex, which contains the initiator tRNA and both ribosomal subunits.
B	Post translational	It inhibits binding of aminoacyl- tRNAs to the A site in the ribosome.
C	Translational	It blocks translocation of peptidyl transferase-rRNA from the A site to the P site of the ribosome.
D	Post translational	It interferes with chain termination and release of the peptide.

- 11 Which of the following statement(s) about cancer is / are true?

- I Individuals who inherit one mutant tumour suppressor gene are more likely to develop cancer than individuals with two non-mutant copies.
- II Cancer is a result of increased cell division which promotes the mutation of a proto-oncogene.
- III Mutagenic activation of a single oncogene is sufficient to cause a normal cell to develop into a cancerous cell.

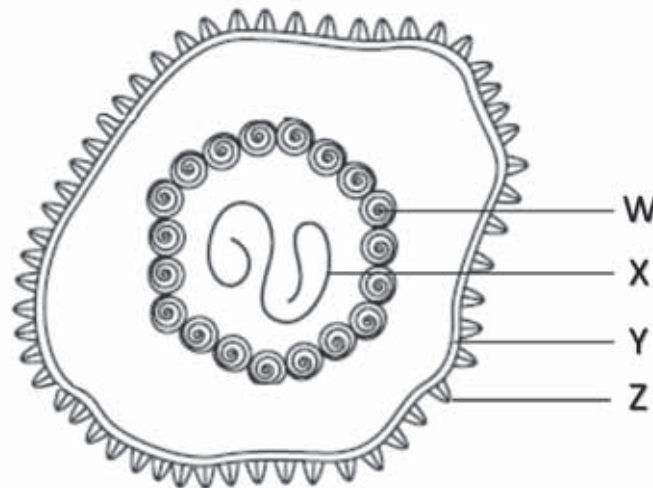
- A I only
- B I and II only
- C I and III only
- D I, II and III

- 12 To date, more than 10 different strains of influenza virus (e.g. H1N1, H2N3, H5N1, H7N9 and so on) have been documented.

Which of the following structural characteristic of influenza virus makes this possible?

- A Single-stranded RNA as its genetic material
- B Presence of an envelope that is derived from the host cell
- C Eight separate segments of genetic material
- D Presence of error-prone reverse transcriptase within the virus

13 The figure below shows the structure of a virus.



Which of the following matches the functions of structures W – Z?

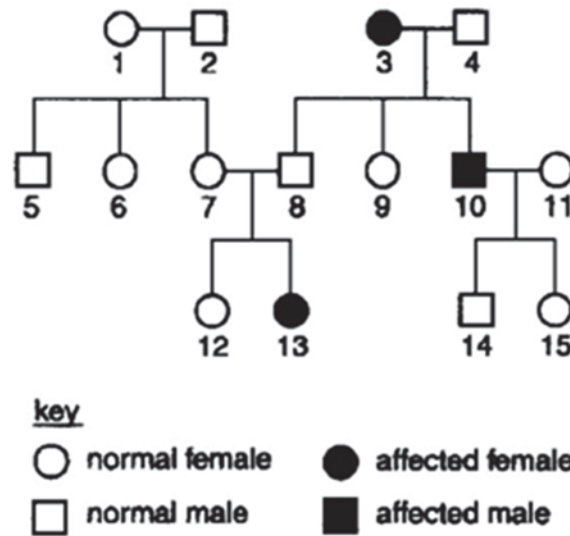
	W	X	Y	Z
A	Ensures the integrity of the viral genome is maintained	Entry of virus into host cell	Specificity of host cell	Assembly of viruses
B	Ensures the integrity of the viral genome is maintained	Assembly of viruses	Entry of virus into host cell	Specificity of host cell
C	Specificity of host cell	Assembly of viruses	Ensures the integrity of the viral genome is maintained.	Entry of virus into host cell
D	Assembly of viruses	Ensures the integrity of the viral genome is maintained.	Entry of virus into host cell	Specificity of host cell

14 When the *lac* operon for lactose metabolism is switched off, which of the following genes would still be expressed?

- I  $\beta$ -galactosidase gene
- II RNA polymerase gene
- III CAP gene
- IV Repressor gene

- A I and II
- B I and III
- C II, III and IV
- D All of the above

- 15 The pedigree chart below shows the inheritance of a recessive condition known as human albinism. Only homozygous recessive individuals are albinos.



What is the probability of individual 9 being a heterozygous carrier?

- A 0.00
- B 0.25
- C 0.50
- D 1.00**
- 16 Which of the following regarding embryonic stem cells and hematopoietic stem cells is true?
- A As embryonic stem cells develop, they turned into hematopoietic stem cells as they lose their ability to differentiate into all types of cells.
- B Embryonic stem cells have more genes than hematopoietic stem cells and thus are able to form more types of cells.
- C Under normal conditions, embryonic stem cells express more of their genes compared to the hematopoietic stem cells.
- D Both stem cells are derived from the zygotic stem cells with the hematopoietic stem cells having a lowered telomerase activity compared to the embryonic stem cells.**



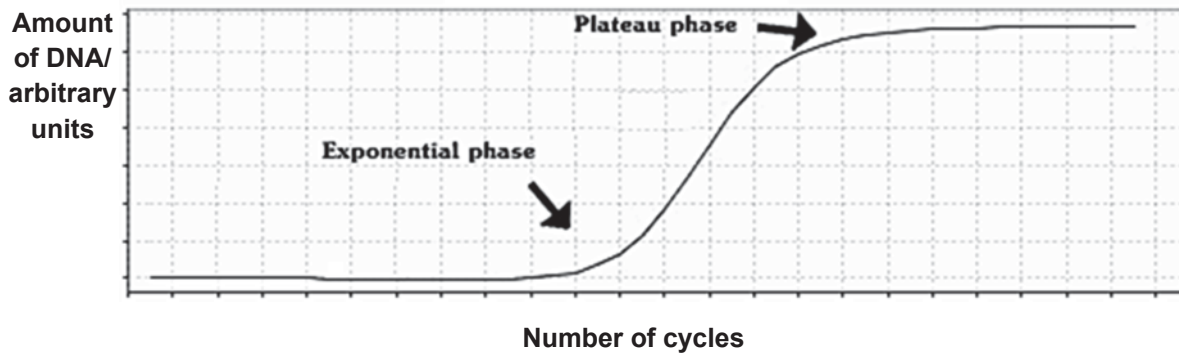
- 17 A plant researcher tried to investigate a cross between two heterozygous Snapdragon plants that produced red flowers. She predicted three possible phenotypic outcomes, namely plants with white flowers, pink flowers and red flowers, with a phenotypic ratio of 4:3:9 respectively. When the cross was performed, she found 50 plants with white flowers only, 41 plants with pink flowers, and 85 plants with red flowers. A chi-squared test was performed, and the chi-squared value was calculated to be 4.74

Degree of freedom	Probability, P				
	0.10	0.05	0.02	0.01	0.001
1	2.71	3.84	5.41	6.64	10.83
2	4.61	5.99	7.82	9.21	13.82
3	6.25	7.82	9.84	11.35	16.27
4	7.78	9.49	11.67	13.28	18.47

Which of the following statements is correct?

- A** The degree of freedom is 3.
- B** The calculated chi-squared value is greater than the critical chi-squared value.
- C** There is a high probability that the difference between the observed and expected values is due to chance.
- D** The probability that the difference between observed and expected values is due to chance is less than 5%.
- 18 Recent advances in the field of stem cell research have shown that induced pluripotent stem cells (iPS cells) can be artificially derived from adult somatic cells. iPS cells are mostly similar to natural pluripotent cells. This implies that iPS cells can
- A** theoretically differentiate into all cell types.
- B** theoretically differentiate into any of the three germ layers.
- C** theoretically differentiate into gametes.
- D** theoretically capable of transdifferentiation.

- 19 During the process of polymerase chain reaction (PCR), the amount of DNA synthesised can be traced using fluorescent probes and the measurements are shown in the following plot. The process initially goes through an exponential phase followed by a plateau phase eventually.



Which of the following statements is **true**?

- A During the exponential phase, the number of DNA molecules synthesized after 15 cycles is  $15^2$ .
  - B During the exponential phase, the temperature is always maintained at the optimum temperature of  $72^\circ\text{C}$  hence there is rapid amplification.
  - C During the plateau phase, the reaction mixture is being depleted of ribonucleotides.
  - D During the plateau phase, *Taq* polymerase may be denatured.**
- 20 The dashed lines in the template sequence represent a long sequence of bases to be amplified.

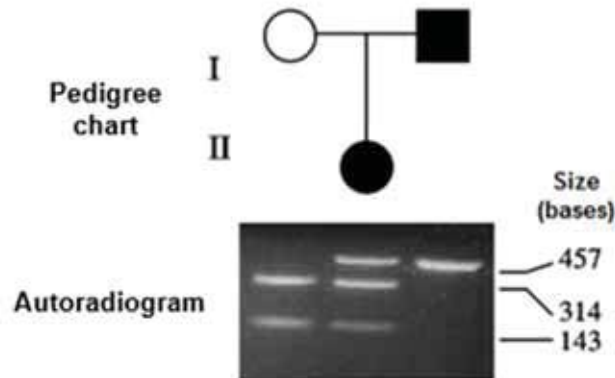
**Template**

5' ATTCGGACTTG ----- GTCCAGCTAGAGG 3'  
 3' TAAGCCTGAAC ----- CAGGTCGATCTCC 5'

Which of the following sets of primers can be used in the PCR for the amplification of the following DNA sequence?

- A 5' GTCCAGC 3' & 5' CCTGAAC 3'
- B 5' ATTCGGA 3' & 5' CCTCTAG 3'**
- C 5' GGAAGTTG 3' & 5' GCTGGAC 3'
- D 5' AUUCGGA 3' & 5' GAUCUCC 3'

- 21 A family with a history of a genetic disease is studied using restriction digestion of the DNA samples containing the gene responsible for the disease. The pedigree chart of the family is aligned with the autoradiogram obtained from Southern blotting. (Shaded symbols in the pedigree chart indicate individuals affected by disease.)



Based on the information given, which of the following can be deduced?

- A** The disease allele is dominant to the normal allele.
- B** The mutation creates a new restriction site in the affected gene.
- C** One of the parents in generation I is a carrier.
- D** The offspring in generation II is a carrier.
- 22 Which of the following statements correctly compares oxidative phosphorylation and non-cyclic photophosphorylation?
- A** Both types of phosphorylation produce ATP and oxygen as end products.
- B** Both types of phosphorylation produce ATP and the reduced form of a redox reagent.
- C** Oxidative phosphorylation is involved in the conversion of one form of chemical energy to another while non-cyclic photophosphorylation is involved in converting light energy to chemical energy.
- D** Water is an electron donor in non-cyclic photophosphorylation while it is an electron acceptor in oxidative phosphorylation.
- 23 What happens to most of the reduced NAD molecules in cell metabolism?
- A** They act as oxidising agents in glycolysis.
- B** They are oxidised in inner mitochondrial membrane for ATP formation.
- C** They are oxidised in the Calvin cycle.
- D** They combine with succinic acid as part of Krebs cycle.

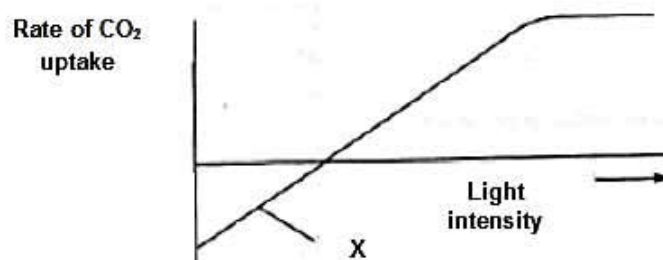
- 24 Rotene and oligomycin are two metabolic poisons which affect cellular respiration. The effects of rotene and oligomycin on aerobic respiration are summarised in the table.

	Ability to use glucose	Ability to use oxygen	ATP yield
<b>Rotene</b>	Yes	No	Decreases
<b>Oligomycin</b>	Yes	Yes	Decreases

Which of the following correctly identifies the specific functions of these two metabolic poisons?

	Rotene	Oligomycin
<b>A</b>	Electron transport inhibitor	Inhibits ATP synthase
<b>B</b>	Inhibits ATP synthase	Electron transport inhibitor
<b>C</b>	Dissipate proton gradient	Inhibits ATP synthase
<b>D</b>	Inhibits ATP synthase	Dissipate proton gradient

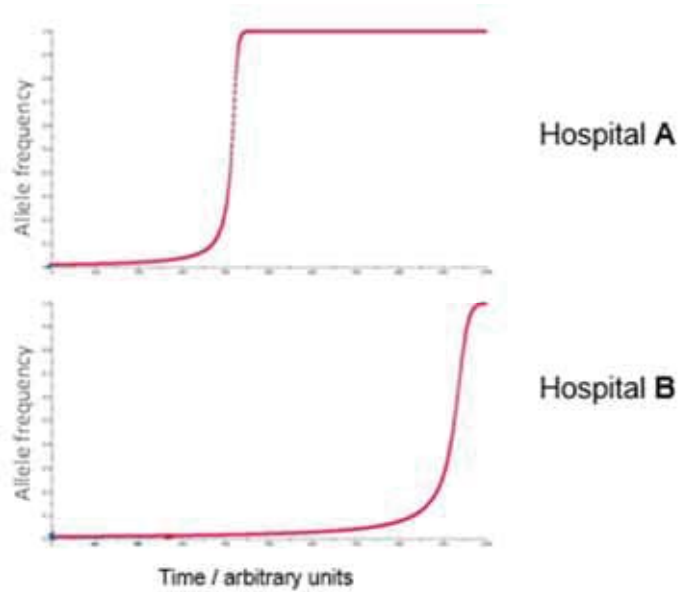
- 25 In the graph below, the rate of CO<sub>2</sub> uptake by plant cells is shown to vary with increasing light intensity.



Which of the following is true at point X?

- A** The plant is photosynthesizing.
- B** Rate of respiration equals rate of photosynthesis.
- C** CO<sub>2</sub> is a limiting factor.
- D** There is not enough light for photosynthesis to have commenced.

- 26 The two graphs below show the allele frequency of an antibiotic resistance gene *Neo* in the gene pool of *Streptococcus pneumoniae*, a bacteria that causes pneumonia.



Which of the following statements can be concluded from the graphs?

- A There is more genetic variation in the gene pool of *Streptococcus pneumoniae* in hospital **A** than hospital **B**.
  - B** Patients in hospital **A** were treated with antibiotic Neomycin more frequently than patients in hospital **B**.
  - C The rate of mutation in the genome of *Streptococcus pneumoniae* in hospital **B** occurs more slowly than that in hospital **A**.
  - D Patients in hospital **A** has a stronger immune system than patients in hospital **B**.
- 27 Which sequence of events correctly describes evolution?

- 1 Differential reproduction of the spiders occurs.
- 2 A new selection pressure occurs.
- 3 Allele frequencies within the spider population change.
- 4 Poorly adapted spiders have decreased survivorship.

- A** 2 4 1 3
- B 2 4 3 1
- C 4 1 3 2
- D 4 3 1 2

**28** The following statements are some findings of scientists in an attempt to investigate the evolutionary relationship between the anteater, armadillo and pangolin.

- I** Anteater, armadillo and pangolin feed primarily on insects such as ants.
- II** Anteater, armadillo and pangolin have long tongue and strong digging limbs.
- III** The tongues of the anteater and armadillo are connected to the hyoid bone while the tongue of pangolin is not.
- IV** There is a higher percentage similarity between the DNA sequences of Anteater and armadillo than with the pangolin.
- V** There is very low percentage similarity between the DNA sequences of anteater and pangolin as well as between the armadillo and pangolin.

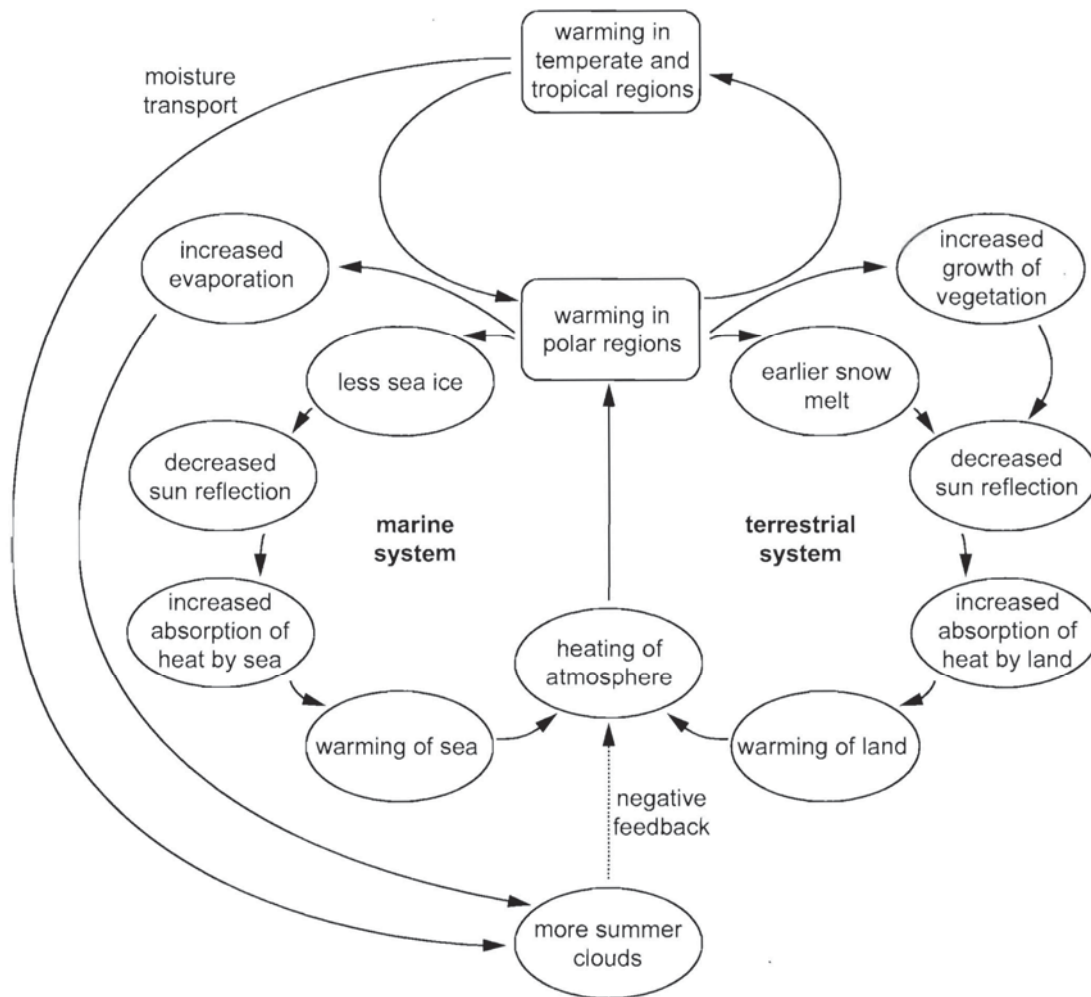
Which of the following conclusions can be drawn from the statements given above?

- A** The anteater and pangolin have experienced divergent evolution as shown by homologous structures between their hyoid bones and tongues.
- B** The anteater and pangolin have experienced convergent evolution as shown by homologous structures in their hyoid bones and tongues.
- C** The armadillo and pangolin have experienced divergent evolution as shown by the low similarity between their DNA sequences.
- D** The anteater and armadillo have experienced divergent evolution as shown by similarities in their DNA sequences and homologous anatomical structures.

**29** In order to initiate an adaptive immune response, antigenic peptide must be presented to antigen-specific T cells. Which one type of cell presents this antigen to T cells?

- A** Dendritic cell
- B** Epithelial cell
- C** Neutrophil
- D** Plasma cell

- 30 The diagram shows the effect of increasing temperatures on the ice and snow cover at the polar regions.



Which effect of higher temperatures in the polar regions could increase global warming?

- A Increased evaporation leads to more rainfall, which absorbs heat from the land and sea.
- B Melting of ice and snow results in less reflection of sunlight and more heat absorption by the Earth.**
- C Melting of sea ice causes more cloud formation, which increases absorption of heat in the atmosphere.
- D Earlier melting of snow allows vegetation cover to increase faster, reducing loss of heat from the surface of the Earth.

**End of Paper**

CANDIDATE NAME: \_\_\_\_\_

INDEX NUMBER \_\_\_\_\_

CG \_\_\_\_\_



SERANGOON JUNIOR COLLEGE  
JC2 PRELIMINARY EXAMINATION 2017

BIOLOGY  
Higher 2  
9744

## ANSWER SCHEME

### READ THESE INSTRUCTIONS FIRST

Write your name and index number in the spaces at the top of this page and on all the work you hand in.

Write in dark blue or black pen.

You may use a soft pencil for any diagrams, graphs or rough working.

Do not use staples, paper clips, highlighters, glue or correction fluid.

Answer **all** questions in all sections.

### INFORMATION FOR CANDIDATES

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

FOR EXAMINER'S USE	
Paper 1 (MCQ)	/30
Paper 2	
1	/15
2	/20
3	/15
4	/15
5	/18
6	/17
P2 Total	/100
Paper 3	/75
Paper 4	/55
TOTAL (100%)	

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This question paper consists of 19 printed pages.



Answer **all questions** in the spaces provided.

### Question 1

There have been many breakthroughs in stem cell research in recent years. It has been discovered that stem cells are involved in the replacement of worn-out cells and repair of damaged tissues. Further research is being conducted to better understand the mechanism involved in controlling the behaviour of stem cells in order to better manipulate them to treat various diseases and disorders.

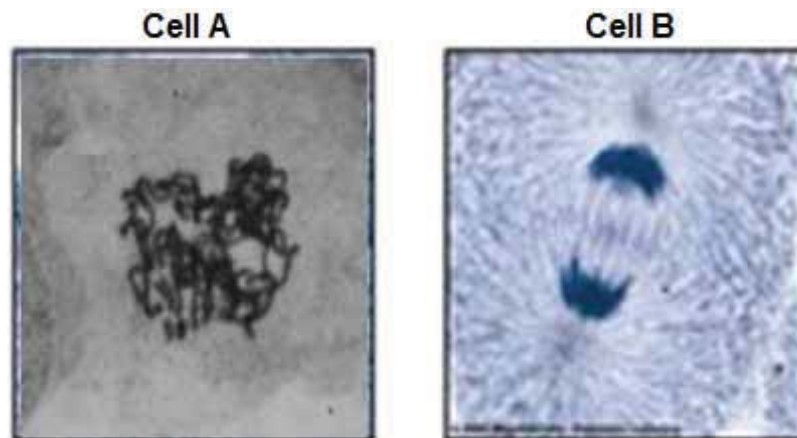
(a) State the type of stem cells involved in the replacement of worn-out cells and repair of damaged tissues, and describe the unique properties of this type of stem cells. [2]

- **Adult stem cells** [1]

*Any 2 properties [1]:*

- **Undifferentiated cells found in differentiated tissues**
- **Multipotent → Able to differentiate into a limited range of cell types**
- **Able to undergo mitotic cell division for self-renewal**

Stem cells undergo cell division to produce genetically identical daughter cells. **Fig. 1.1** shows two cells, each at a different stage of cell division.



**Fig. 1.1**

(b)(i) With reference to **Fig. 1.1**, state the stages of cell division in **Cell A** and **Cell B**. [1]

**Cell A: Prophase**

**Cell B: Anaphase**

(ii) The dysregulation of cell cycle can result in cancer. Outline the checkpoints that are present in normal cells to prevent this from occurring. [2]

Any 2

- **G<sub>1</sub> checkpoint:** assesses if the environmental conditions (presence of growth factors and nutrients, absence of DNA damage, adequate cell size) are favourable for cell division to proceed
- **G<sub>2</sub> checkpoint:** assesses if DNA replication is completed and cell size is adequate.
- **M checkpoint:** assesses if all chromosomes are attached to the mitotic spindle at their kinetochores and arrests the mitotic cell at metaphase if centromeres are not properly attached to kinetochore microtubules, hence preventing entry into anaphase.

Fig. 1.2 shows information about the movement of chromatids in a cell that has just started metaphase of mitosis.

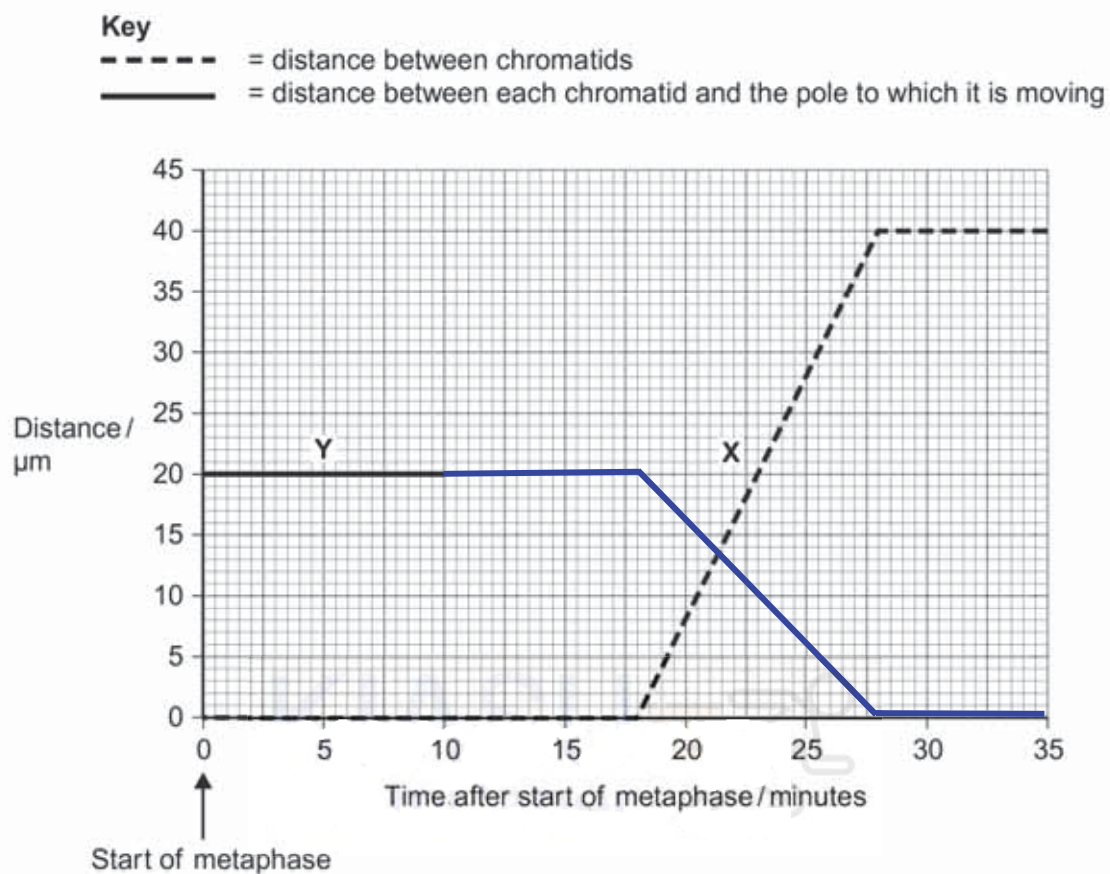


Fig. 1.2

(c)(i) With reference to Fig. 1.2, state the duration of metaphase in the cell. [1]

- 18 min

- (ii) Complete line Y on the graph. [1]
- Horizontal until 18 minutes, then decreases as straight line to 0  $\mu\text{m}$  at 28 minutes

- (iii) Account for your answer in (c)(ii). [3]

- Chromosomes align singly at the metaphase plate during metaphase of mitosis OR sister chromatids are attached to microtubules from opposite poles at metaphase
- Sister chromatids start to separate to become daughter chromosomes and migrate towards the opposite poles in anaphase, as shown at 18<sup>th</sup> min of line X when distance between chromatids starts to increase. Hence distance between chromatid and pole will start to decrease at 18<sup>th</sup> min.
- Distance between chromatids reach a plateau/maximum at 28<sup>th</sup> min, chromosomes arrived at opposite poles. Hence, distance between chromatid and pole will be minimum at 28<sup>th</sup> min.

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.

- (d)(i) Contrast between the structure of tubulin with that of DNA. [2]

Tubulin	DNA
• Tubulin is a polypeptide and hence made up of amino acid subunits	• DNA is made up of deoxyribonucleotides.
• Subunits by peptide bonds	• Subunits joined by phosphodiester bonds
• Globular	• Helical

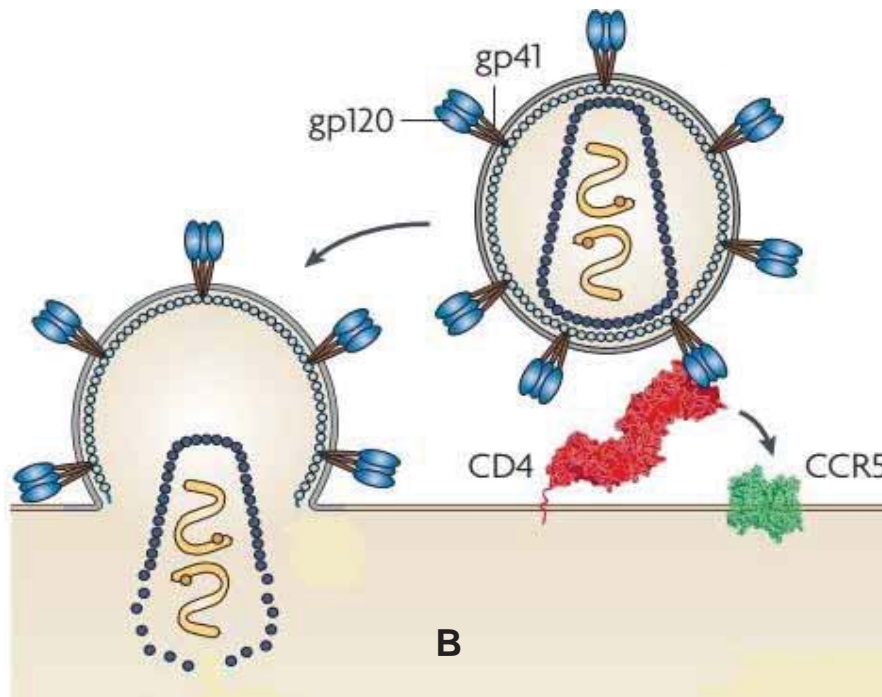
- (ii) Suggest how eribulin work to prevent tubulin polymerisation and explain its effect on the behaviour of chromosomes in mitosis. [3]

- Possible action of drug: bind to tubulin subunits to change its conformation that prevent joining of subsequent subunits
- Prevent kinetochore microtubules from attaching to the kinetochores at the centromeres of the chromosomes.
  - Cells cannot progress through metaphase, so that chromosomes cannot align singly at the metaphase plate OR sister chromatids could not separate/remain attached.

[Total: 15]

## Question 2

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. 2.1** shows the entry process of a HIV into a macrophage, which is a type of white blood cell.



**Fig. 2.1**

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

[2]

- Viruses with single stranded RNA as genome
- Has its own reverse transcriptase to produce DNA from its RNA genome

(ii) Compare the entry processes of the HIV and influenza virus into human host cells. [3]

- **Similarity:** Adsorption/ attachment of both viruses are by binding to specific cell surface receptors; AVP
- **Differences (any 2) :** 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 ; while haemagglutinin on the influenza viral membrane binds to sialic acid-containing receptors on the host cell membrane ;
  - The whole influenza virus enters the host cell by receptor-mediated endocytosis; while only the HIV capsid enters via membrane fusion where its 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.

(iii) Upon completion of the entry process, describe how the HIV genome is inherited by macrophage daughter cells. [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 which exist as a provirus.
- The provirus genome is also replicated along with the host cell genome and all daughter cells inherit the HIV genome

(iv) 'HIV-positive patients usually develop weak immunity.'

Explain what is meant by this statement. [4]

- Host cells of HIV viruses are macrophages and CD4 helper T cells
- Provirus begins viral replication to make viral proteins. Immune system responds by destroying the infected helper T cells.
- CD4 helper T cells level will decrease the ability of the patient to fight infections
- Patients develop AIDS and become more susceptible to opportunistic infections.

Another example of an enveloped virus is the herpes simplex virus. Viral DNA enters the nucleus of the host cell via nuclear pores and *directs synthesis of viral RNA and DNA*. The virus is able to grow in non-dividing cells because its genome encodes enzymes such as viral DNA polymerase and thymidine kinase. Thymidine kinase is involved in synthesising deoxyribonucleotides required for DNA replication.

(b)(i) Describe the normal function of nuclear pores. [2]

- Allows the movement of molecules across nuclear membrane from the nucleus to cytoplasm and vice versa
- Named example of molecules moving out of nucleus: mature mRNA / newly formed ribosome / tRNA OR into nucleus: enzymes involved in transcription/replication / ATP/ histones / spliceosome proteins

(ii) State the process that is involved in the [1]

*Synthesis of viral RNA:* **transcription**

*Synthesis of viral DNA:* **replication**

(iii) Describe two ways in which the named processes in (ii) are different. [2]

Replication	Transcription
<ul style="list-style-type: none"><li>• Product is DNA molecule</li></ul>	<ul style="list-style-type: none"><li>• Product is mRNA</li></ul>
<ul style="list-style-type: none"><li>• Enzyme involved is DNA polymerase</li></ul>	<ul style="list-style-type: none"><li>• Enzyme involved is RNA polymerase</li></ul>
<ul style="list-style-type: none"><li>• Both strands of DNA as template</li></ul>	<ul style="list-style-type: none"><li>• Only one strand serves as template</li></ul>

AVP

Acyclovir is an anti-viral drug which is used to treat herpes. It prevents the complete replication of viral DNA by viral DNA polymerase. The structure of acyclovir is shown below in Fig. 2.2.

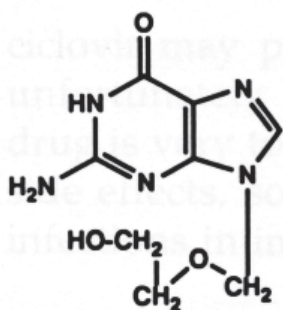


Fig. 2.2

When acyclovir enters the infected cell, it is first phosphorylated by thymidine kinase and subsequently becomes further phosphorylated by host cell kinases. When this phosphorylated acyclovir is incorporated into the newly-synthesised DNA strand, it prevents further elongation of the DNA strand.

(c)(i) With reference to Fig. 2.2, suggest how the incorporation of the phosphorylated acyclovir prevents further elongation of the DNA strand. [2]

- **Missing 3'OH group**
- **DNA polymerase cannot recognise/bind to 3' end of DNA strand and cannot catalyse formation of phosphodiester bonds between DNA strand and incoming deoxyribonucleotide**

(ii) Some strains of herpes simplex virus are now resistant to acyclovir. Suggest how the virus has gained resistance to this anti-viral drug. [1]

- **Mutation that affects the phosphorylation of acyclovir by thymidine kinase or other host cell kinases**

[Total: 20]

### Question 3

In a particular variety of tomato plant, the allele for red fruit colour (**A**) is dominant over the allele for orange fruit (**a**) and the allele for green base when ripe (**B**) is dominant over the allele for no green base when ripe (**b**).

Two students, Faiz and Jacob crossed plant with red fruit and green base when ripe with pure bred plant with orange fruit and no green bases when ripe. The phenotypes of 50 offspring of each of Faiz's and Jacob's crosses were recorded and are shown in **Table 3.1**.

**Table 3.1**

	Phenotypes of offspring of test crosses			
	Red fruit with green base	Red fruit with no green base	Orange fruit with green base	Orange fruit with no green base
Faiz's cross	23	4	3	20
Jacob's cross	3	21	23	3

degrees of freedom	probability, $p$				
	0.10	0.05	0.02	0.01	0.001
1	2.71	3.84	5.41	6.64	10.83
2	4.61	5.99	7.82	9.21	13.82
3	6.25	7.82	9.84	11.35	16.27
4	7.78	9.49	11.67	13.28	18.47

**(a)(i)** With the aid of the table of probabilities as shown above, carry out a  $\chi^2$  test on the results of Faiz's cross and provide a brief explanation for the results obtained. [6]

**Null Hypothesis:** There is **no significant difference** between the **observed occurrence** of tomato plant phenotypes and the **expected occurrence ratio of 1:1:1:1**. [1]

**Derivation of  $\chi^2_{\text{calculated}}$  values:** [1]

Category/Class	Observed Frequency (O)	Expected Frequency (E)	(O-E)	$\frac{(O - E)^2}{E}$
Red fruit with	23	$\frac{1}{4} \times 50 = 12.5$	10.5	8.82

green base				
Red fruit with no green base	4	$\frac{1}{4} \times 50 = 12.5$	-8.5	5.78
Orange with green base	3	$\frac{1}{4} \times 50 = 12.5$	-9.5	7.22
Orange with no green base	20	$\frac{1}{4} \times 100 = 25$	-5	4.50

**Calculation of  $\chi^2_{\text{calculated}}$  value**

$$\chi^2_{\text{calculated}} = 8.82 + 5.78 + 7.22 + 4.50 = \mathbf{26.32 \text{ (3 s.f)}}$$

[1]

Degree of freedom = 4 - 1 = 3

From the  $\chi^2$  table,

At 5 % level of significance and  $\nu = 1$ ,  $\chi^2_{\text{critical}} = \mathbf{7.82}$

Conclusion: Since  $\chi^2_{\text{calculated}} > \chi^2_{\text{critical}} / \mathbf{26.32 > 7.82}$ , the **calculated  $\chi^2$  value** is higher than the **critical value at  $p = 0.05$** , the **difference between the observed and expected results is statistically significant.** [1]

The difference between the observed and expected is not due to **chance alone**. Hence,  **$H_0$  is rejected/not accepted** and the pattern of inheritance in the survey does not follow Mendelian's pattern of inheritance.

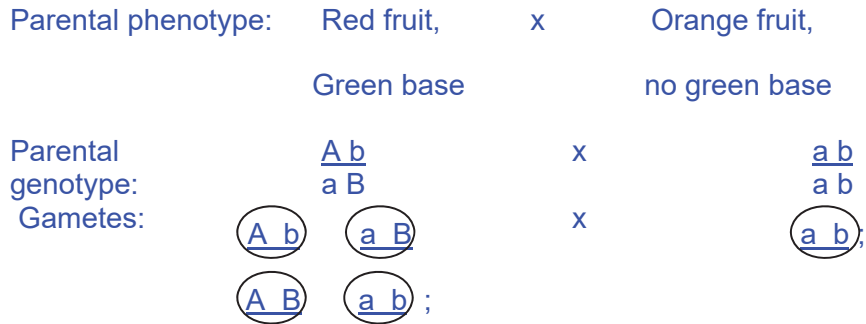
**Explanation: A and B gene loci are linked/found on the same chromosome.** [1] **The genes do not undergo independent assortment and are usually inherited together.** Hence, there is a higher proportion of the offspring having the parental phenotypes. [1]



- (ii) State the probability that the results of Faiz's cross depart significantly by chance from the expected ratio. [1]

$p < 0.001$

- (b) Draw a genetic diagram to explain the results of Jacob's cross. [5]



F<sub>2</sub> genotypes

	$\frac{A b}{a b}$	$\frac{a B}{a b}$	$\frac{A B}{a b}$	$\frac{a b}{a b}$
$\frac{a b}{a b}$	$\frac{A b}{a b}$	$\frac{a B}{a b}$	$\frac{A B}{a b}$	$\frac{a b}{a b}$

F<sub>1</sub> phenotypes

Red fruit with no green base	Orange fruit with green base	Red fruit with green base	Orange fruit with no green base
21	23	3	3

F<sub>1</sub> phenotypic ratio

1m each

(c) Explain the difference in results between Faiz's and Jacob's cross when the parental genotypes are the same. [3]

- Parental genotypes of red with green base tomato plant used are the same however the combination of the alleles of the two gene loci on the same chromosome are different

- Show or explain the genotypes of both parent tomato plant in two crosses

- AB x ab for Faiz's cross;

a b      a b

- Ab x ab for Jacob's cross

a B      a b

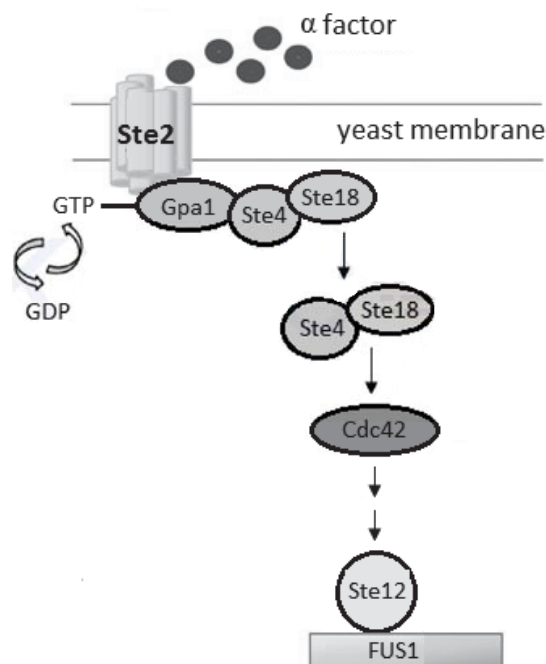
- Since the genes are linked, they are usually inherited together and hence would result in an overrepresentation of parental phenotypes among offsprings.

[Total: 15]

#### Question 4

Yeast haploid cells secrete  $\alpha$  factor to signal mating, and respond by growing a mating projection towards a potential mate. Upon contact of the two partner cells, these fuse to form a diploid zygote.

Fig. 4.1 shows the  $\alpha$  factor signaling pathway mediated by yeast G-protein coupled receptor, Ste2. The activation of the pathway induces the expression of *FUS1* gene which is required for yeast mating.



**Fig. 4.1**

**(a)(i)** With reference to **Fig. 4.1**, describe how  $\alpha$  factor triggers the activation of Cdc42. [4]

- $\alpha$  factor binds to Ste2 receptor at the binding site and triggers a conformational change in Ste2
- allowing Ste2 to bind Gpa1(-Ste4-Ste18 complex) and causes GTP to displace GDP
- activation of the (Gpa1-)Ste4-Ste18 complex
- Ste4-Ste18 dimer to dissociate from the complex to activate Cdc42

**(ii)** Briefly explain why  $\alpha$  factor cannot enter the yeast cell directly. [2]

- $\alpha$  factor is polar / charged or too large to pass through transient gaps
- cannot pass through the hydrophobic core of the phospholipid bilayer and requires a membrane receptor

**(iii)** Explain the possible role of Ste12 in the expression of FUS1 gene. [2]

- **General transcription factor that binds to the promoter of FUS1 gene**
- **Allows for the assembly of the transcription initiation complex to initiate transcription**

**(iv)** It has been observed that the binding of a protein Y to a region upstream of the promoter results in the mating projection to be produced at a rate higher than normal in the yeast cells.

Provide a reason for this observation. [3]

- **Region upstream of promoter is an enhancer**
- **Protein Y acts as an activator to bind to region upstream of promoter to increase the rate of transcription by**
- **Recruit histone acetylases to loosen chromatin cycle and increase the rate of assembly of the transcription initiation complex.**

**Fig. 4.2** shows the Ste2 receptor on another yeast cell membrane.

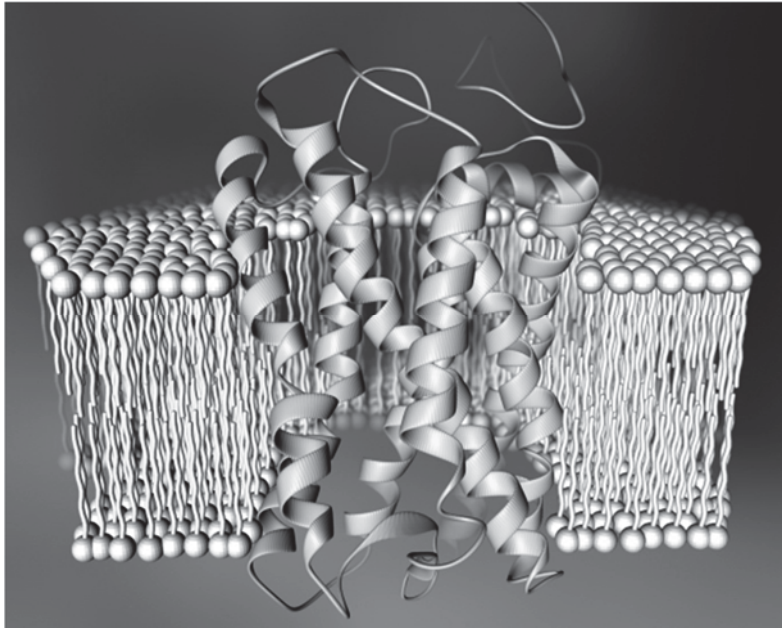


Fig. 4.2

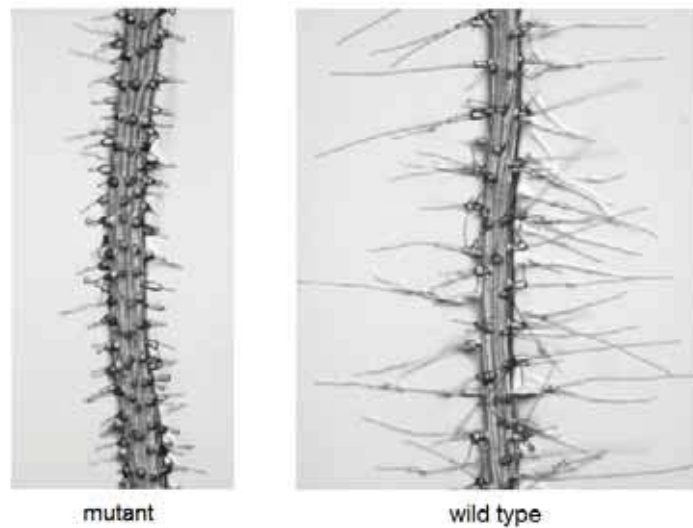
- (b)(i) Describe the structure of Ste2 receptor. [2]
- A single polypeptide chain consisting of (seven)  $\alpha$  helix segments that folds upon itself
  - Such that the  $\alpha$  helices are gathered together forming a cylindrical / globular structure
- (ii) Explain how Ste2 receptor remains embedded in the yeast cell membrane. [2]
- Hydrophobic non polar R groups of amino acid residues that make up Ste2 receptor forms hydrophobic interactions with the hydrophobic fatty acid tails of the phospholipid.
  - Polar or charged R groups of amino acid residues that make up Ste2 receptor forms favorable interactions with the hydrophilic phosphate head of the phospholipid.

[Total: 15]

### Question 5

*Arabidopsis thaliana* is a small flowering plant native to Asia. A mutation in the gene coding for NADP oxidase results in plants with short root hairs. NADP oxidase is an enzyme that converts NADPH to NADP<sup>+</sup>.

**Fig. 5.1** shows the root hairs in the two variant of *A. thaliana*.



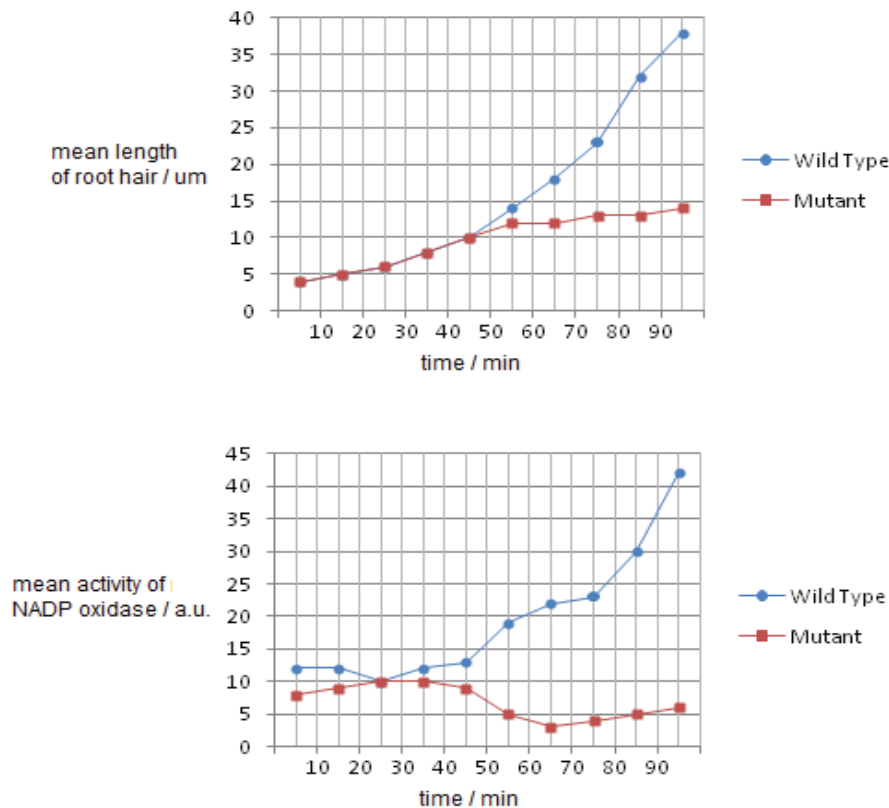
**Fig. 5.1**

(a) Explain the role of NADPH in photosynthesis.

[2]

- Provides reducing power/H<sup>+</sup> to reduce
- Phosphoglyceric acid (PGA)/glycerate-3-phosphate (GP) to glyceraldehyde-3-phosphate (GALP)/phosphoglyceraldehyde (PGAL)/triose phosphate (TP)

In a separate experiment, activity of NADP oxidase in the tips of the root hair cells in wild type and mutant *A. thaliana* were measured at intervals. Changes in mean length of the root hair cell was also measured to track the rate of growth, which is known to be an energy-requiring process. Both sets of results are shown in **Fig. 5.2**.

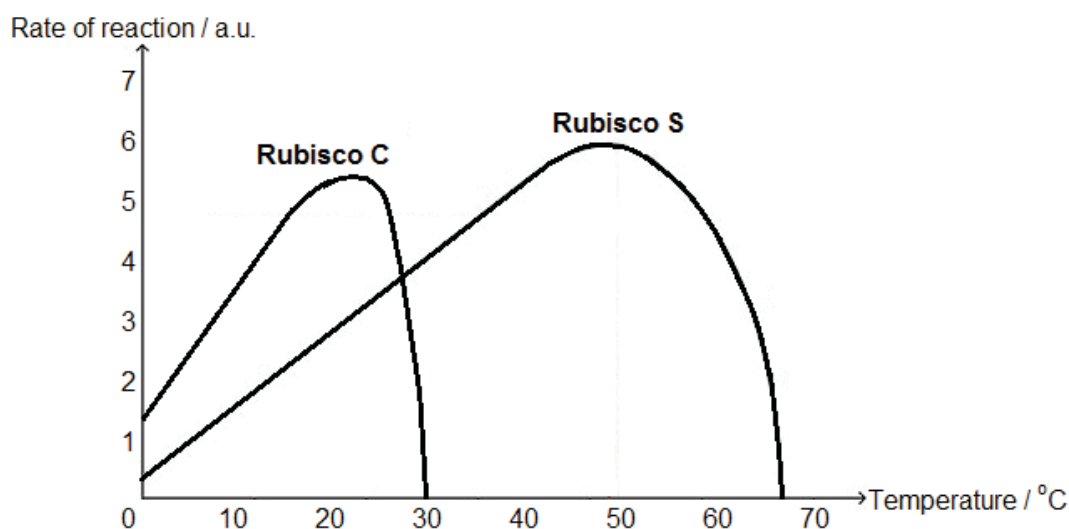


**Fig. 5.2**

(b) Using your knowledge of photosynthesis, provide an explanation for the difference in the growth of root hairs in the two types of *A. thaliana*. [4]

- Mean length of root hair for wild type *A. thaliana* increases from 4 um to 38um when mean activity of NADP increases from 13au to 42au
- Mean length of root hair for mutant *A. thaliana* increases from 4um to 14um when mean activity of NADP decreases from 9au to 6au after a small initial increase
- NADP oxidase catalyses the conversion of NADPH to NADP<sup>+</sup> for light reaction of photosynthesis where ATP is synthesised; (OR less reduction of PGA to GALP in Calvin cycle)
- Low NADP oxidase activity results in less carbohydrates produced and hence less growth

Rubisco is an enzyme required in the light-independent stage of photosynthesis. **Fig. 5.3** shows the effect of increasing temperature on the activity of two variations of Rubisco, **Rubisco C** and **Rubisco S**.



**Fig. 5.3**

(c) With reference to **Fig. 5.3**, compare the effect of temperature on the two enzymes. [3]

- Both Rubisco C and Rubisco S has an increased rate of reaction as temperature increases up to optimum temperature OR both Rubisco C and Rubisco S are denatured at temperatures higher than optimum.
- Rubisco C has a lower optimum temperature of 20°C as compared to Rubisco S at 50 °C where rate of reaction is at a maximum
- Rubisco C reaches a lower maximum rate of reaction of 5.5 a.u. at a faster rate as compared to Rubisco S which reaches a maximum rate of reaction of 6 a.u at a slower rate.

(d) It is known that **Rubisco C** is obtained from a species of coniferous tree found in Canada, while **Rubisco S** is obtained from a species of cactus found in the Sahara Desert.

(i) Explain how different alleles give rise to different Rubisco structure. [3]

- Different alleles have different DNA nucleotide sequence that results in a different mRNA/codon sequence after transcription
- Thus will result in different amino acid sequence / primary structure after translation
- Different R group interactions between amino acids affects folding of the polypeptide chain, giving rise to different 3D conformation in the tertiary structure

(ii) It has been predicted that **Rubisco S** will be found in more plant species in view of climate change. Explain how Darwin's theory of evolution supports this observation. [4]

- **Variation**: different types of Rubisco molecules that has different tolerance to high temperature
- **Selection pressure**: **rising global temperatures** that selects for plants with Rubisco S that are able to photosynthesise efficiently at high temperatures
- Plants with Rubisco S are able to **survive and reproduce** and **pass down** the favorable Rubisco S alleles to the next generation
- With time, **allele frequency** of the plant population gene pool will change with a **higher frequency** of the **Rubisco S** allele that confers a selective advantage, lower frequency of Rubisco C allele that confers selective disadvantage. Hence, more plants with Rubisco S.

(iii) Suggest two other ways plants can adapt to the changing climate. [2]

- Development of **more and longer roots** in order to access water deeper into the ground.
- **Reduction in the number and surface area of leaves** so as to lower transpiration rates.
- **Reduction in the number of stomata per leaf** to reduce transpiration rates.
- **Sunken stomata** which are concealed by **finger-like projections**.

[Total: 18]

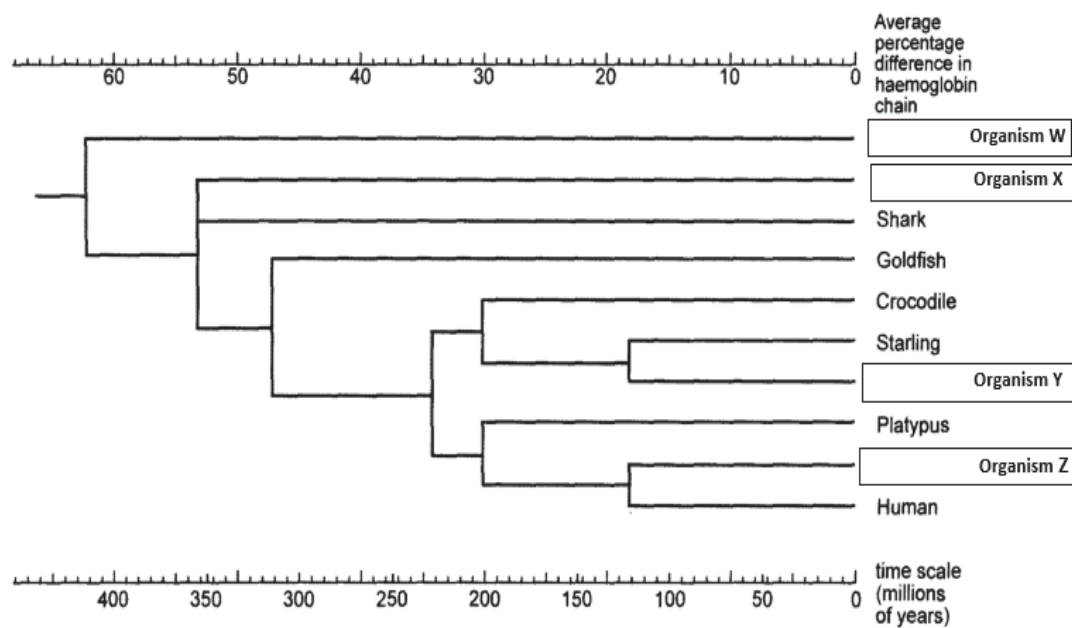


**Question 6**

The table below shows the amino acid differences in the cytochrome b protein between various vertebrates.

	Human	Elephant	Platypus	Ostrich	Starling	Crocodile	Lungfish	Coelacanth	Goldfish	Shark
Human		26	40	43	41	47	83	70	68	71
Elephant			45	45	48	50	84	72	63	74
Platypus				54	52	51	89	74	70	76
Ostrich					26	36	91	75	68	73
Starling						47	91	77	67	70
Crocodile							85	78	70	77
Lungfish								90	94	86
Coelacanth									83	78
Goldfish										88
Shark										

**Fig 6.1** shows the phylogenetic tree based on differences between the cytochrome b proteins.



**Fig. 6.1**

(a) Using information from the table and **Fig. 6.1**, identify organisms **W** to **Z**. [2]

Organism **W**: **lungfish**

Organism **X**: **coelacanth**

Organism **Y**: **ostrich**

Organism **Z**: **elephant**

(b) Distinguish between classification and phylogeny. [2]

- **Classification refers to grouping organisms based on similar characteristics while phylogeny involves grouping organisms based on evolutionary relationship.**
- **Similar characteristics in classification may be analogous and not homologous whereas in phylogeny, similarity is due to inheritance from common ancestry.**

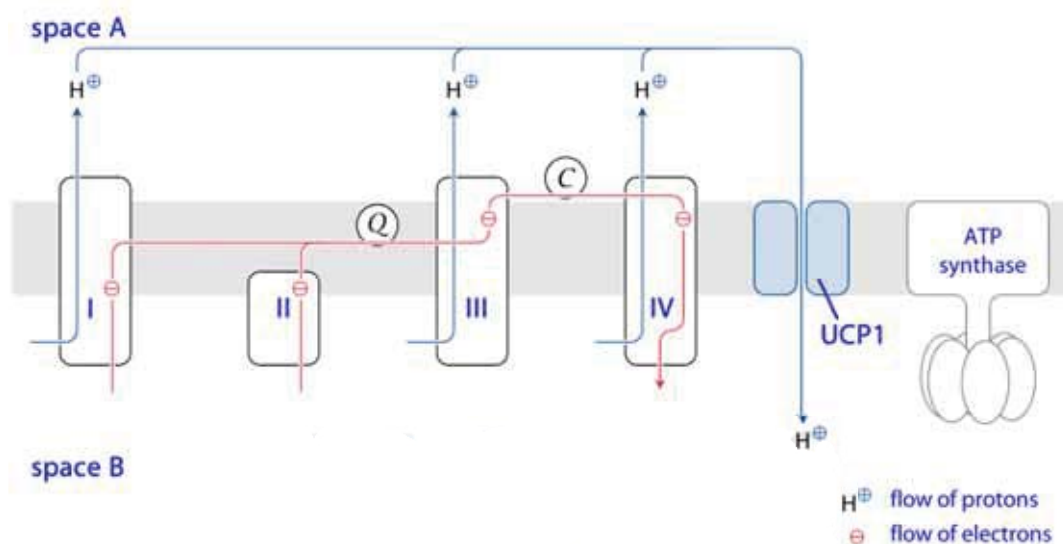
(c) Explain how differences in amino acid sequences in the cytochrome b chain allow the establishment of the phylogenetic tree. [2]

- **Percentage of amino acid difference indicates relatedness where few difference indicates recent common ancestor**
- **Provides quantitative data to construct phylogenetic tree**

(d) Suggest why homology still features prominently in evolutionary studies despite the advantages that molecular evidence can confer. [1]

- **Less expensive as it does not rely on machines**
- **DNA/protein samples might be limited or unavailable**

Brown adipocytes were one of the cells isolated for the above investigation. **Fig. 6.2** shows the schematic representation of a series of protein complexes found on the inner membrane of organelle X present in brown adipocytes.



**Fig. 6.2**

(e)(i) State the identity of organelle X. [1]

**Mitochondrion**

(ii) Outline how ATP is usually synthesised in the inner membrane of organelle X. [4]

- NADH and FADH<sub>2</sub> carry hydrogen in the form of protons and electrons where proton remain in the matrix and electrons are passed along the progressively lower energy electron carriers in the electron transport chain.
- Energy released is used to pump H<sup>+</sup> from the matrix to the intermembrane space via active transport which sets up a concentration gradient (high H<sup>+</sup> conc in intermembrane space, low conc in the matrix)
- H<sup>+</sup> diffuse down the concentration gradient from the intermembrane space to matrix via the stalked particle
- Provides a proton motive force that drives the synthesis of ATP by ATP synthase by phosphorylation of ADP and inorganic phosphate (chemiosmosis).

(f) Brown adipocytes contain a unique protein, UCP1, which is not found in organelle X in any other cell type.

Evaluate the impact of UCP1 on ATP synthesis and suggest the physiological significance of brown adipose tissue. [3]

- As UCP1 allows protons to leak back into the matrix without passing through the ATP synthase,
- Loss of H<sup>+</sup> concentration gradient, no ATP will be synthesized
- The energy released from the spontaneous flow of protons through UCP1 is lost as heat, which helps to keep the organisms warm.

(g) In other cell types, NADH and FADH<sub>2</sub> are used to drive ATP synthesis by ATP synthase. Using relevant information from **Fig. 6.2**, suggest and explain why more ATP is produced from NADH. [2]

- NADH donates electrons to complex I while FADH<sub>2</sub> donates to complex II. The energy released from transfer of electrons through the complexes is used to pump protons across the inner membrane.
- NADH allows for more chances to pumps more protons across the gradient, which powers the ATP synthase and gives us 3 ATP per molecule of NADH, while FADH<sub>2</sub> produces 2 ATP during the ETC because it gives up its electron to complex II, bypassing complex I.

[Total: 17]

END OF PAPER



SERANGOON JUNIOR COLLEGE  
JC2 PRELIMINARY EXAMINATION 2017

BIOLOGY 9744  
Higher 2

ANSWER SCHEME

PAPER 3

### Section A

Answer all the questions in this section.

#### Question 1

#### Repeat droughts may cause permanent damage to forests

By: [Alex Whiting](#)

ROME, Aug 9 (Thomson Reuters Foundation) - **Trees and their environment need as long as two years to recover from drought in some places, and if a second dry spell hits before then, it may cause permanent damage to the landscape,** researchers said on Wednesday.

With **climate change expected to bring more frequent and intense droughts,** the implications for areas that do not have time to bounce back fully could be severe, the researchers said in a paper to be published in *Nature* journal this week. "That could have a double whammy effect," said co-author William Anderegg, assistant professor of biology at the University of Utah. "A second drought could be harder on an ecosystem and have the potential to push it off a cliff."

In practice, **that means affected areas could eventually turn from lush forest to a land of grass and shrubs.** Boreal forests in northern parts of Europe, Russia and Canada can take up to two years to recover from drought, partly because they do not have a wide variety of plants, Anderegg told the Thomson Reuters Foundation. Forests in the tropics of South America and Southeast Asia have also taken the same amount of time to rebound.

**"That's worrisome because those regions store the largest chunks of carbon in ecosystems across the globe," Anderegg said.** Forests help tackle climate change by sucking carbon out of the air, reducing levels of planet-warming carbon dioxide, the main greenhouse gas. **But when trees die, most of the carbon they have absorbed is released back into the atmosphere.**

The Amazon rainforest suffered a double drought in the first decade of this century when dry spells, both of a once-in-a-100-years severity, hit the region. "Satellites showed that forests hadn't recovered from the 2005 drought by the time the 2010 drought struck," Anderegg said.

#### Adapted from a Reuters Article

(a) Discuss how anthropogenic climate change has affected the growth of forests in the world. [4]

- **Reduction in water content causes stomata to close which limits gaseous exchange thus affecting the availability of CO<sub>2</sub> for photosynthesis. (Negative Impact on Growth)**
- **Amount of RUBP carboxylase also drops significantly in low water content, leading to a decrease in CO<sub>2</sub> fixation in the Calvin cycle. (Negative Impact on Growth)**
- **Turgor pressure of cells and hence plant cell expansion is also slowed down, leading to retardation of plant growth. (Negative Impact on Growth)**

- **Increasing temperatures up to the optimal can increase plant growth and yield and rates of photosynthesis and respiration as a result of increased enzymatic activity. (Positive Impact on Growth)**
- **However, beyond the optimum, enzymes are denatured and plant death will result. (Negative Impact on Growth)**

**Any 4**

(b) The article talks about how forests may not be able to recover if a second drought soon after a first. Suggest how forests may recover from a drought and explain why a second drought soon after a first impedes its ability to recover. [4]

- **Forests recover from a drought by increasing rates of growth**
- **Forests recover from a drought by reproducing to increase their numbers**
- **All these take a significant length of time/ time for plants to grow; time for new plants to be formed and then grow**
- **A second drought will return the forest to a state before its recovery impeding its recovery/ reverse growth and reproductive gains**

(c) The article talks about forests storing carbon as shown by the following quote "*That's worrisome because those regions store the largest chunks of carbon in ecosystems across the globe,*" *Anderegg said.* Define the role of forests in storing carbon and explain briefly how they are able to do this. [4]

- **They act as carbon sinks**
- **A carbon sink refers to a natural or artificial reservoir that accumulates and stores some carbon-containing chemical compound for an indefinite period.**
- **The process by which carbon sinks remove carbon dioxide from the atmosphere is known as carbon sequestration.**
- **This is done by the removal of carbon dioxide from the air during photosynthesis**
- **and the storage of this carbon dioxide in the form of biomolecules like carbohydrates, proteins and lipids**

**Any 4**

Climate change does not just affect our forests, it also affects our farms. The following article elaborates on this.

*Aug. 4 (UPI) -- Scientists from Lancaster University suggest major changes in agricultural practices are needed to offset increases in nutrient losses due to climate change. The study, shows that phosphorus losses will continue to increase due to climate change unless major changes in agricultural practices are made.*

*"Although farmers are already doing what they can to prevent these losses, the currently adopted measures are not likely to be enough to offset the increase expected under climate change. This paper should alert policy makers and government to the help and support that farmers will need to achieve the scale of agricultural change that may be necessary to keep up with the increase in pollution due to climate change."*

*Although phosphorus and nitrogen are essential to crop and animal growth, too much of it can cause algae blooms in rivers and lakes.*

(d) Phosphorus and nitrogen are essential nutrients for plant growth and are typically found in large concentrations within the fertilizers used on farms. Suggest how climate change can lead to phosphorus and nitrogen from fertilizers ending up in rivers and water bodies. [2]

- **Heavy rainfall**
- **Washes the fertilizer off the land and into nearby rivers and water bodies.**

(e) The article talks about nitrogen and phosphorus causing algal blooms in rivers. Using a specific example of a plant or animal, discuss the possible impact of such blooms on the natural ecosystem in these rivers. [4]

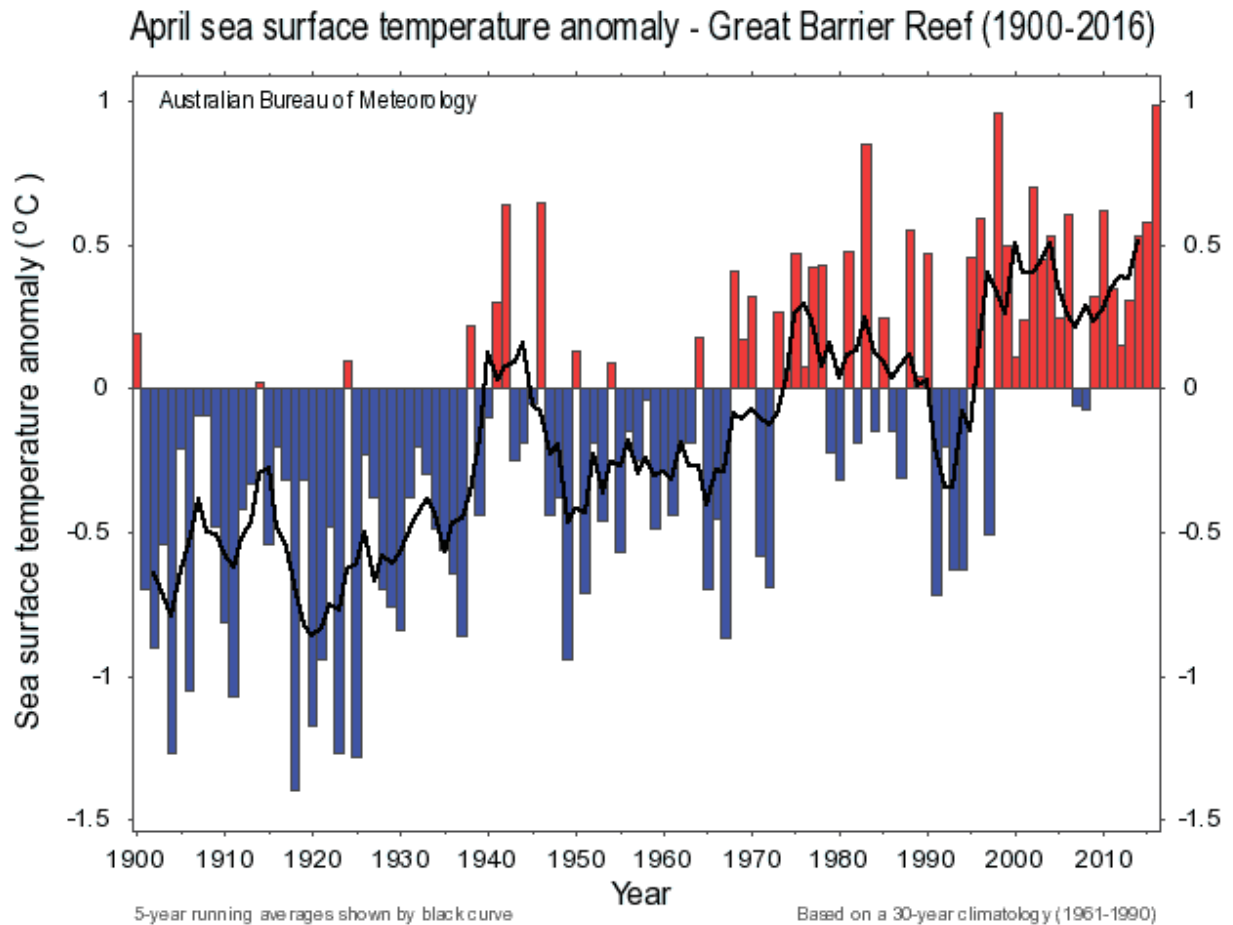
- **Specific named typed of organism [eg. Fish, plankton, aquatic insects]**
- **Death or migration of the organism**
- **With explanation of how algal bloom lead to death or migration eg. Lack of oxygen in the water**
- **Elaboration of impact of loss of organism on the ecosystem**

(f) The article suggests that farmers need to change their agricultural practices to limit the impact of phosphorus used in fertilizer on nearby aquatic bodies. Describe two changes they could make and suggest why it may be difficult for them to make such changes. [3]

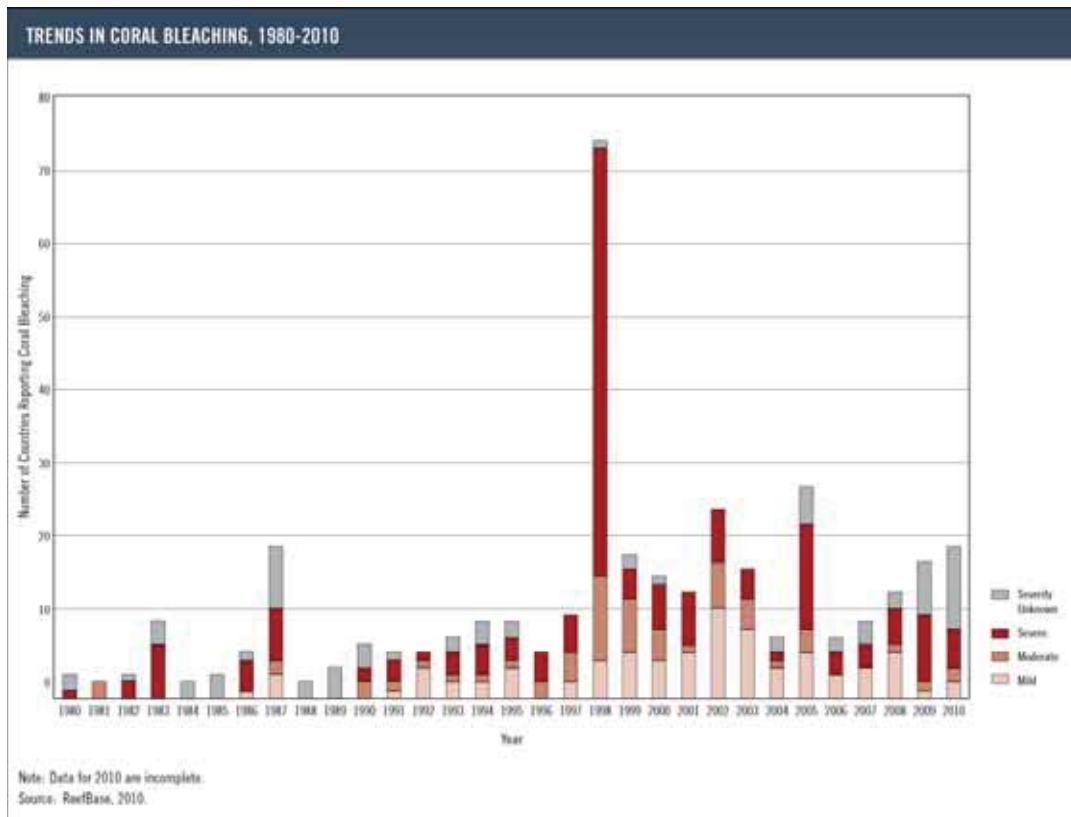
- **Use of plant strains that don't require as much fertilizer/crop rotation to minimize dependence on fertilizer/farming away from water bodies/AVP [any one properly described]**
- **Lank of funding/Lack of skill/Enculturation/AVP [Any one properly linked to an earlier change]**

Another aquatic ecosystem affected by climate change are coral reefs. The following graph, **Figure 1**, shows how sea water temperatures have varied over the last century or so at the Great Barrier Reef. 0°C is considered the average sea water temperature.

Rising sea level temperatures have been suggested as a reason for more frequent coral bleaching events. **Figure 2** shows the trend for coral bleaching events globally since 1980.



**Figure 1**



**Figure 2**

(g) With reference to **Figures 1 & 2**, discuss if the data provides evidence that the more frequent bleaching events seen in coral reef ecosystems over the last 15 years are due to rising water temperatures. [6]

- **Yes there is evidence.**
- **Most/More years above the average as compared with past 100 years. (Quote data)**
- **Peaks of high temperatures getting higher compared with past 100 years. (Quote data)**
- **Above average more frequent in the last 20 years compared with past 100 years. (Quote data)**
- **There have also been higher numbers of countries reporting coral bleaching events in the last 15 years. (Quote Data)**
- **There have also been higher number of countries reporting moderate to severe events in the last 15 years. (Quote Data)**
- **Higher sea temperatures can thus be directly correlated to coral bleaching events.**

(h) Other than warming water temperatures, describe two other ways anthropogenic climate change has impacted coral reefs. [2]

- **Rising sea levels**
- **Acidification of oceans**

**[Total: 29 marks]**

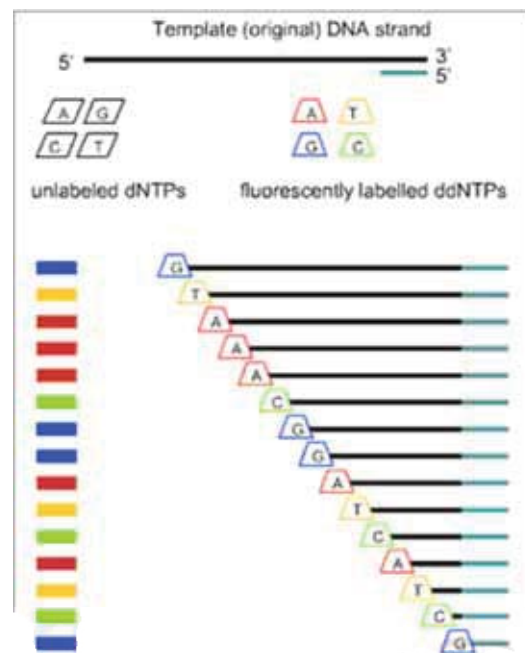


## Question 2

A method that has been used to sequence DNA is called Sanger Sequencing, named after the scientist that invented the process. Sequencing DNA essentially allows the sequence of bases on a DNA strand to be read. During Sanger sequencing, DNA polymerases copy single-stranded DNA templates by adding nucleotides to a growing chain (**extension product**). Chain elongation occurs at the 3' end of a primer, an oligonucleotide that anneals to the template. The extension product grows by the formation of a phosphodiester bridge between the 3'-hydroxyl group on the primer and the 5'-phosphate group of the incoming deoxynucleotide.

DNA polymerases can also incorporate analogues of nucleotide bases. The dideoxy method of DNA sequencing developed by Sanger et al. 1977 takes advantage of this characteristic by using 2',3'-dideoxynucleotides (ddNTPs) as substrates. When dideoxynucleotides are incorporated at the 3' end of the growing chain, chain elongation is terminated selectively at A, C, G, or T. This leads to the production of a DNA fragment with the ddNTP at its end.

The ddNTPs are usually labelled using fluorescent tags. Many fragments of the original DNA strand terminated in the manner shown in **Figure 3** are created using this processes. When all these fragments are separated on a gel using a form of gel electrophoresis, the sequence of bases can be read by reading the labelled ddNTPs from the shortest to the largest fragment.



**Figure 3**

(a) Suggest how the labelling of the ddNTPs allows for the identification of the base that normally occupies that position. [2]

- **ddNTPs will only occupy a position at the very end of the strand.**
- **Different ddNTPs with different bases are labelled with a different fluorescent tag.**
- **Normal nucleotides (non ddNTPs) are unlabelled and would not show up under fluorescent treatment.**

(b) Describe how the extension product (line 4) is created and explain the role of the template DNA strand to this process. [5]

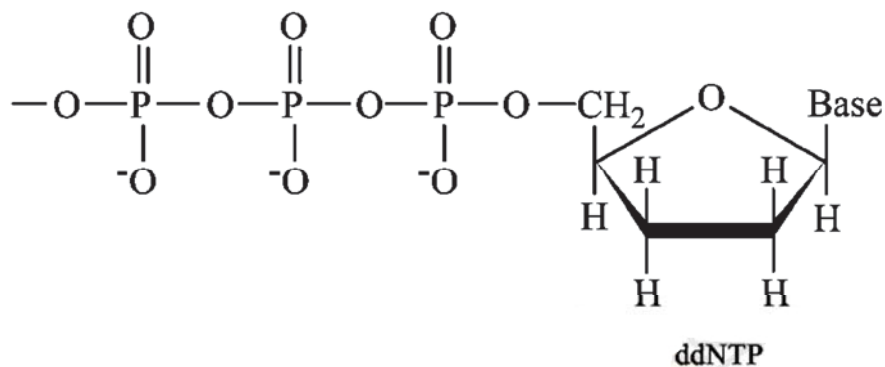
- **DNA polymerase extends the 3' end of the growing/daughter strand**
- **By adding deoxyribonucleotides via complementary base pairing with the bases on the template strand**
- **A:T, C:G**
- **Via hydrogen bonding**
- **Extension product (line 4) was created when DNA polymerase reads a 'T' on template strand, it adds an adenine ddNTP to the daughter strand**
- **Template strand thus carries the code for the sequence on the growing strand**
- **Template strand also ensures that the product is stable due to the hydrogen bonding with the template strand**

Any 5

(c) Explain how, when separated using gel electrophoresis, the strands produced by Sanger Sequencing end up in different positions on the gel. [3]

- **Strands are of different lengths**
- **Gel electrophoresis separates strands based in their lengths. Shorter fragments will move longer distances on the gel while longer fragments will move shorter distances**
- **Due to the resistance introduced by the gel that the fragments need to overcome (idea)**

The following **Figure 4** shows an example of dideoxynucleotide (ddNTP).



**Figure 4**

(d) Explain why dideoxynucleotides, such as the one shown in Figure 4, leads to the formation of fragmented DNA. [3]

- **No 3'-OH group**
- **DNA polymerase requires a 3'-OH group to extend a DNA strand.**
- **DNA polymerase cannot attach and extend growing strand, hence producing fragments of DNA.**

- (e) Methods such as Sanger Sequencing has enabled the sequencing of the human genome. This has opened up the possibility of detecting specific alleles in human genomes. Discuss the possible advantages of this and its possible ethical ramifications. [4]

#### **Possible Advantages**

- **Improved diagnosis of disease**
  - **Earlier detection of genetic predispositions to disease**
  - **Drug design**
  - **Gene therapy and control systems for drugs**
  - **Identify potential suspects whose DNA may match evidence left at crime scenes**
  - **Exonerate persons wrongly accused of crimes**
  - **Identify crime and catastrophe victims**
  - **Establish paternity and other family relationships**
  - **Match organ donors with recipients in transplant programs**
  - **Study evolution through mutations in lineages**
- Any 2 with brief elaboration

#### **Possible Ethical Ramifications**

- **Genetic testing - psychological impact and stigmatization**
  - **Genetic Testing - fairness in the use of genetic information and privacy and confidentiality**
  - **Reproductive issues - Prenatal testing / Reducing the number of deformities**
  - **Preimplantation testing - Gender selection**
  - **Commercialization - questions of the ownership of tissue and tissue derived products, patents, copyrights, and accessibility of data and materials**
  - **Conceptual and philosophical implications - free will vs genetic determinism / Do people's genes make them behave in a particular way? Can people always control their behavior?**
- Any 2 with brief elaboration

Human genomes include the DNA found in the mitochondria. Similar to the nuclear genome, the mitochondrial genome is made up of double-stranded DNA, and it encodes genes. However, the mitochondrial genome differs from the nuclear genome in several ways

- (f) Suggest two ways in which mitochondrial DNA may differ from the nuclear genome. [2]

- **The mitochondrial genome is circular, whereas the nuclear genome is linear.**
- **The mitochondrial genome is smaller/ less DNA base pairs, whereas the nuclear genome is larger/more DNA base pairs.**
- **The mitochondrial genome contains less genes.**

- (g) With reference to specific genes, explain the role of mitochondrial DNA. [2]

- **It carries genes that code for proteins used in the mitochondria.**
- **Examples include tRNA gene, rRNAs genes, genes of oxidative phosphorylation carrier proteins (any two logical genes stated)**

[Total: 21 marks]

## Section B (25 Marks)

Answer **only one** question

Write your answers in the space provided.

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

Your answers must be in continuous prose where appropriate.

Your answers must be set out in sections (a), (b), etc as indicated in the question.

### Question 3

- (a) All organisms need to replicate and transcribe/translate their DNA in the process of growth and development. Even viruses need to do so. Using the example of a dengue virus, compare and contrast the replication and protein synthesis process in a virus with the similar processes in a typical eukaryotic cell. [13]

#### Similarities

- Both use polymerases in replication and transcription
- Both use ribosomes in protein synthesis
- Both use amino acids as raw material for protein synthesis
- Both produce polypeptides in protein synthesis
- Enzymes used in protein synthesis include peptidyl transferase and aminoacyl t-RNA synthetase for both.

#### Differences

Criteria	Dengue Virus	Eukaryote
Polymerase for Replication	RNA Dependent RNA polymerase	DNA polymerase
Transcription	Not required as genome is positive RNA	Carried out by DNA Dependent RNA Polymerase
Replication	Two rounds of replication required to achieve product (positive strand RNA)	A single round of semi-conservative replication is sufficient
Template for Replication/Product of Replication	RNA/RNA	DNA/DNA
Raw material for replication	RNA nucleotides	DNA nucleotides
Source of replication and protein synthesis machinery	Host Cell	Own Cell
Unwinding of template	No unwinding as template for both Replication and Transcription is single stranded	Unwinding/separation of DNA template for replication carried out by helicase while for transcription carried out by RNA polymerase.
Post transcriptional modification	Absent	Present

Max 12

QWC – Criterion-based comparison (1 mark)

(b) Describe how viruses such as the influenza virus is able to create genetic variation and explain how this makes it difficult for us to eradicate harmful viruses with modern medicine. [12]

- **Via antigenic drift**
- **Antigenic drift is due to spontaneous mutations (e.g. point mutation) of the viral gene encoding haemagglutinin glycoprotein (antigen)**
- **Such mutations result in the production of new hemagglutinin proteins that are of a different conformation.**
- **Antibodies made against the original influenza virus can no longer recognize and bind to the new haemagglutinins antigens, thus the virus can evade host immune defenses.**
- **Mutations in influenza occur frequently because the viral RNA polymerase has no proofreading mechanism, providing a strong source of mutations.**

**Max 4**

- **Via antigenic shift**
- **Antigenic shift occurs when 2 different strains of influenza virus infect a single host cell at the same time**
- **When two different strains of influenza infect the same cell simultaneously, their protein capsids and lipid envelopes are removed, exposing their RNA genome.**
- **The RNA genome segments reassort and are randomly incorporated during assembly of new virus, thus forming a novel subtype of virus that has a mixture of glycoprotein antigens of 2 original strains.**
- **For example, H3N2 and H5N1 can form H5N2.**
- **Appropriate diagram**

**Max 4**

- **Many medicines target the glycoproteins on the influenza virus envelopes.**
- **To prevent adsorption and penetration.**
- **By physically blocking the receptor sites on the host cell**
- **Or interfering with the glycoproteins in the virus**
- **When the glycoproteins change due to antigenic shift and drift, the medicines will no longer work**
- **As they are specific to the glycoproteins they were designed to target.**

**Max 4**

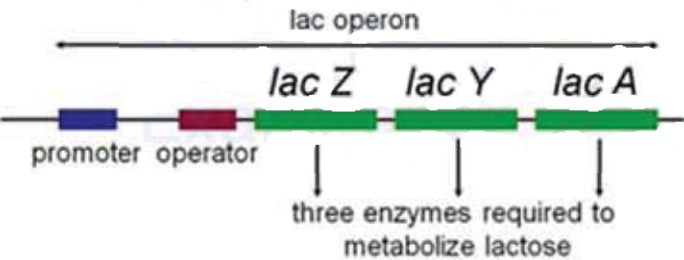
#### Question 4

- (a) All organisms need to control the expression of their DNA in the process of growth and development. Even bacteria need to do so. Using the example of *E. coli*, compare and contrast the control of gene expression in a bacteria with the similar processes in a typical eukaryotic cell. [13]

#### Similarity

- Expression of gene involves transcription and translation for both.

#### Differences

Control of Gene Expression		
Feature	<i>E. coli</i> Gene Expression	Eukaryotic Gene Expression
Genome Level	<ul style="list-style-type: none"> <li>• Chromatin modification e.g. histone acetylation <b>cannot occur</b> as prokaryotic DNA is <b>not associated with histones</b>.</li> </ul>	<ul style="list-style-type: none"> <li>• Chromatin modification e.g. histone acetylation can occur, resulting in conversion between <b>euchromatin and heterochromatin</b>.               <ul style="list-style-type: none"> <li>○ Eukaryotic DNA is associated with histones (and other proteins).</li> </ul> </li> </ul>
	<ul style="list-style-type: none"> <li>• <b>DNA sequences</b> (promoters and operators) serve as <b>on/off switches</b>.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Degree of condensation</b> of chromatin serves as <b>major on/off switches</b> for gene expression.</li> </ul>
	<ul style="list-style-type: none"> <li>• Gene amplification does not occur</li> </ul>	<ul style="list-style-type: none"> <li>• Gene amplification occurs to <b>increase number of copies</b> of the gene of interest.</li> </ul>
Transcription Level	<ul style="list-style-type: none"> <li>• Related genes are organised in an <b>operon</b>, under the <b>control of a single promoter</b>.</li> <li>• These genes are transcribed together to give rise to a <b>polycistronic mRNA</b>.</li> </ul> 	<ul style="list-style-type: none"> <li>• Genes are not organised into operons.</li> <li>• Each gene has its own promoter and gives rise to a <b>monocistronic mRNA</b>.</li> </ul>

	<ul style="list-style-type: none"> <li>• <b>Few control elements</b>, which are usually located <b>close</b> to the promoter and genes under its control. <ul style="list-style-type: none"> <li>○ E.g. operator is located close to the promoter of genes.</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• <b>Many control elements</b> that can be located <b>proximally or distally</b> upstream/ downstream of a gene. <ul style="list-style-type: none"> <li>○ E.g. proximal and distal enhancers and silencers.</li> </ul> </li> </ul>
	<ul style="list-style-type: none"> <li>• Only <b>one</b> RNA polymerase involved. All RNAs are synthesised by the same RNA polymerase.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Five</b> different RNA polymerases present. <ul style="list-style-type: none"> <li>○ Three main types of RNA (mRNA, tRNA and rRNA), synthesised by <b>three different RNA polymerase</b>.</li> </ul> </li> </ul>
	<ul style="list-style-type: none"> <li>• General transcription factors <b>not required</b> as RNA polymerase can directly recognize and bind to <b>pribnow box</b> of prokaryotic promoters. <ul style="list-style-type: none"> <li>○ Occurs with the aid of <b>sigma factors</b> which <b>reduces the affinity</b> of RNA polymerase for <b>non-specific DNA sequences</b>, and <b>increases</b> its affinity for the <b>promoter</b>.</li> <li>○ Prokaryotic RNA polymerase associates with the sigma factor to form RNA polymerase <b>holoenzyme</b>.</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• General transcription factors required. <ul style="list-style-type: none"> <li>○ General transcription factors and RNA polymerase II assemble at the <b>TATA box</b> of eukaryotic promoters to form the <b>transcription initiation complex</b>.</li> </ul> </li> </ul>
<b>Post-transcriptional Level</b>	<ul style="list-style-type: none"> <li>• Post-transcriptional modifications do not occur. Primary transcripts are the actual mRNA.</li> </ul>	<ul style="list-style-type: none"> <li>• Primary transcripts (pre-mRNA) undergo processing to produce mature mRNA; <ul style="list-style-type: none"> <li>○ Addition of 5' cap</li> <li>○ RNA splicing</li> <li>○ Addition of 3' poly (A) tail</li> </ul> </li> </ul>
	<ul style="list-style-type: none"> <li>• <b>Lower stability</b> of mRNA transcript <ul style="list-style-type: none"> <li>○ Degradation occurs within <b>seconds to minutes</b> of transcription.</li> <li>○ Allows prokaryotes to respond rapidly to environmental changes.</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• <b>Higher stability</b> of mRNA transcript <ul style="list-style-type: none"> <li>○ Degradation after <b>minutes or days</b> following transcription</li> <li>○ Degradation controlled by <b>length of poly (A) tail</b> → the</li> </ul> </li> </ul>

		longer the poly(A) tail, the later the mRNA is degraded.
<b>Translational Level</b>	<ul style="list-style-type: none"> <li>• Translation is often coupled to transcription. Both processes occur <b>simultaneously</b>.</li> </ul>	<ul style="list-style-type: none"> <li>• Translation is <b>not coupled</b> to transcription.</li> <li>• mRNA must move from the nucleoplasm, across the nuclear envelope, via a nuclear pore to the cytoplasm for translation to occur.</li> <li>• RNA transcript is not free to associate with ribosomal subunits prior to completion of transcription.</li> </ul>
	<ul style="list-style-type: none"> <li>• Control at this level is unlikely, due to simultaneous transcription and translation.</li> </ul>	<ul style="list-style-type: none"> <li>• Control can occur at pre-translational level, when <b>regulatory proteins bind at the 5' UTR or the 3' poly (A) tail of mature mRNA</b>. This prevents binding with the <b>small ribosomal subunit</b> and the assembly of the translation initiation complex.</li> </ul>
	<ul style="list-style-type: none"> <li>• mRNAs are <b>polycistronic</b> and have <b>multiple start codons</b>, allowing for the direct synthesis of <b>several different polypeptides</b>.</li> </ul>	<ul style="list-style-type: none"> <li>• mRNAs are <b>monocistronic</b> and have only <b>one</b> start codon, allowing for the synthesis of only <b>one kind of polypeptide</b>.</li> </ul>
<b>Post-translational Level</b>	<ul style="list-style-type: none"> <li>• Post-translational modification does not occur</li> </ul>	<ul style="list-style-type: none"> <li>• Post-translational modification can occur in the form of; <ul style="list-style-type: none"> <li>○ Chemical modification (glycosylation and phosphorylation)</li> <li>○ Proteolytic Cleavage and Activation</li> <li>○ Degradation (ubiquitination)</li> </ul> </li> </ul>

Max 12



**QWC – Criterion-based comparison (1 mark)**

(b) Describe how bacterial cells are able to create genetic variation and explain how this makes it difficult for us to eradicate harmful bacteria with modern medicine. [12]

- Transformation
- Transformation is the process by which a naked, foreign DNA molecule is taken up from the surrounding external environment
- and integrated into the bacterium's genome, via homologous recombination
- thus resulting in the change of recipient bacterial cell's genotype.
- The resultant transformant cell will now express genes from this new segment it has received, and pass them on to all subsequent daughter cells by binary fission.

Max 3

- Transduction
- Transduction is the process by which DNA is transferred from one bacterial cell (donor) to another (recipient) by bacteriophages,
- and is later integrated into the bacterium's genome of the recipient cell via homologous recombination.
- During the assembly stage, phage genome is randomly packaged within the phage capsid to form the mature phage particles. But occasionally, a small piece of the host cell's degraded DNA is packaged within the phage capsid in place of the phage genome.
- This 'defective' phage can attach to another recipient bacterium and inject the piece of bacterial DNA acquired from the first cell

Max 3

- Conjugation
- Conjugation is the process by which bacterial cells make direct contact with each other and DNA is directly transferred from one donor cell to the another recipient cell,
- The DNA donor uses appendages called sex pili for the transfer
- Sex pili attach the F<sup>+</sup> donor to the F<sup>-</sup> recipient cell.
- The sex pili facilitate the direct DNA transfer between donor and recipient cells by forming a temporary cytoplasmic bridge (also known as conjugation tube or mating bridge)
- the F plasmid replicates within the donor cell, and only one strand of the plasmid is transferred to the recipient through the conjugation tube joining the cells.

Max 3

- Modern medicine uses antibiotics to target bacteria.
- Antibiotics do not work on bacteria carrying genes for resistance to that antibiotic.
- Such antibiotic resistance genes can be transferred between bacterial cells via transformation, transduction or conjugation.
- This increases the number of resistant bacterial cells making it difficult to eradicate the bacteria.

Max 3

CANDIDATE NAME \_\_\_\_\_

CG \_\_\_\_\_



**SERANGOON JUNIOR COLLEGE**  
**JC2 PRELIM PRACTICAL EXAMINATION 2017**

**H2 BIOLOGY ANSWER SCHEME**

**9744**

**2.5 hours**

**Question 1**

You are provided with a quantity of vitamin C solution and a dye called DCPIP.

You are also provided with three test-tubes containing respectively lemon juice, orange juice, grapefruit juice, and labelled as such. These juices contain natural vitamin C and the dye DCPIP can be used to determine the concentration of this vitamin in the juices.

**Apparatus:** 6 test-tubes and a test-tube rack  
4 plastic teat pipettes  
Plastic ruler

**Material:** 30 cm<sup>3</sup> DCPIP solution, labelled '**DCPIP**'  
50 cm<sup>3</sup> vitamin C solution, labelled '**Vitamin C solution**'  
50 cm<sup>3</sup> lemon juice  
50 cm<sup>3</sup> orange juice  
50 cm<sup>3</sup> grapefruit juice

Proceed as follows:

1. Into a clean test-tube, transfer a quantity of the dye DCPIP to a depth of 0.5 cm. Take note its colour.
2. Fill a teat pipette with vitamin C solution. Add one drop of vitamin C solution to the DCPIP solution in the test-tube and shake gently. Continue to add the drops, counting the number of drops which are needed to bring about a colour change. Shake gently after each drop, refilling the pipette if necessary.
3. Record the initial colour of DCPIP (from step 1) and the first colour change after vitamin C is added as well as the number of drops counted to bring about this colour change in a suitable table.
4. After the first colour change, continue adding drops of vitamin C and counting the drops until the DCPIP solution becomes colourless/or consistent pale yellow. (Ignore any coloured granules that might form.). Record the number of drops counted in the same table from step 3.
5. Repeat steps 1 to 4 adequately to obtain enough data for analysis, cleaning all apparatus before use.

6. Place the DCPIP solution into each of three clean test-tubes to a depth of 0.5 cm. (The amount of DCPIP solution must be exactly the same in each of the tubes). Label the tubes A, B and C.
7. Fill a clean test pipette with lemon juice and drop by drop add this to the contents of tube **A**, shaking the tube gently after each drop. Count the number of drops needed to turn the DCPIP solution colourless. Repeat this step adequately to obtain enough data for analysis.
8. Repeat the step 7 with orange juice and grapefruit juice, using a clean pipette each time to add the juice to the DCPIP solution in tubes **B** and **C** respectively.
9. Record the results for the three juices in an appropriate table. [5]

<b>Solution</b>	<b>Replicates</b>	<b>Initial colour of DCPIP</b>	<b>First colour change</b>	<b>Drops required to achieve 1<sup>st</sup> colour change</b>	<b>Second Colour Change</b>	<b>Drops required to achieve 2<sup>nd</sup> colour change</b>
Vitamin C	1	Blue	Brown/Yellow	1	Pale Yellow	2-5
	2					
	Average					
Lemon Juice						
Grapefruit juice						
Orange juice						

Table with headings – 1

Results for Vitamin C with at least one replicate – 1

Results for Juices with at least one replicate – 1

Trend for Juices with at least one replicate – 1 (Lowest drops (Orange) to highest drops (Lemon))

Calculation of average for each solution - 1

10. What conclusions can you draw from your results? [3]

- Relate trend to relative concentrations of Vitamin C
- Higher concentrations of Vitamin C = lower pH = more H<sup>+</sup>
- DCPIP reduced faster = faster colour change

11. Comment on the main source(s) of error and the limitations of the measurements or experimental procedure. [3]

*identifies three of:*

- *uneven mixing of the juice with DCPIP solution;*
- *measuring DCPIP to a depth of 0.5 cm in test-tube;*
- *inconsistent drop size and release of drop from teat pipette;*
- *use of ruler to mark out a depth of 0.5 cm on test-tube;*
- *visual comparison of colour change (when DCPIP decolourises);*
- *varying drop size of vitamin C (or juice) due to way and the speed at which it is released from teat pipette;*
- *viscosity of fruit juice affecting size of the drop being formed before it is released from the teat pipette;*

12. What improvements could you make to the experimental procedures to overcome these sources of error? [3]

*Shake before using juice*

*Use colourimeter to determine colour change*

*use a syringe / graduated pipette to transfer a known volume of DCPIP;*

*use a burette to dispense equal sized drops / regulate the release the fruit juice;*

[Total: 15 marks]

## Question 2

You are provided with a leaf labelled Q. In this question you will be required to investigate the number of stomata present on the lower surface of leaf Q.

Proceed as follows:

1. Use nail varnish to cover a small area of the lower epidermis of leaf Q. Apply a thin layer over an area about 1cm<sup>2</sup>. Avoid any large veins that may be present.
2. Repeat this process for three different areas of the lower epidermis.
3. Allow the nail varnish about 20 minutes to dry. (Proceed to question 3 while the leaf is drying)
4. After 20 minutes, use a razor blade or a fine scalpel to lift one edge of the layer of nail varnish. Use forceps to then gently peel a layer of nail varnish off the leaf.
5. Transfer this layer of nail varnish to a slide and cover with a cover slip.
6. Examine the slide **using a microscope and count the number of stomata** you can observe in the field of view.
7. State which objective you chose to make this stomatal count and explain your choice. [1]

**X10. Idea that number of stomata is countable, not too many, not too few, just nice.**

8. Repeat the counting of stomata in the field of view for a second piece of peeled nail varnish. Calculate the mean number of stomata per field of view in the space below. [2]

**Working - 1**

**Precision 3sf - 1**

9. Make a high power drawing of three adjacent stomata from either of your stomatal peels. [3]

**Accurate Drawing of three adjacent stomata – 1**

**Accurate drawing of epidermal cells between stomata (Shape hexagonal plus cell wall as double line) - 1**

**Label of guard cell and stoma – 1**

10. Using the eyepiece graticule in your microscope and the provided stage micrometer, find the actual length, in  $\mu\text{m}$ , of one of the guard cells that you have drawn. [3]

**State objective used -1**

**Number of eyepiece graticule x calibrated value for that objective – 1**

**Final answer to 3sf in micrometer - 1**

**(40x – 30/35 eyepiece graticule – calibrated value = 0.0025, answer = 75.0 $\mu\text{m}$ )**

<b>Objective Lens</b>	<b>X4</b>	<b>X10</b>	<b>X40</b>	<b>X60</b>
<b>Diameter of Field of View (mm)</b>	5	2	0.5	0.333
<b>Length of one eyepiece division (mm)</b>	0.025	0.01	0.0025	0.00167

A researcher obtained leaves from two different plants, Plant A and Plant B. From the same forest. He found that the number of stomata on the underside of the both leaves differed. He ensured that he calculated the number of stomata based on per unit area and ensured he had sufficient replicates. However, the numbers still did not match. The mean density of stomata per  $1\text{cm}^2$  for leaves A and B were as follows:

Leaf A – 234

Leaf B – 297

11. While the researcher expected a difference in the number of stomata, he could not be sure if the difference seen was significant. Suggest a statistical test he could use to confirm if the difference was significant. [1]

**T-Test**

12. When carried out this test, the probability value he obtained was less than 0.05. Comment on what these results show and suggest an explanation for the pattern seen in Leaves A and B collected by the researcher. [4]

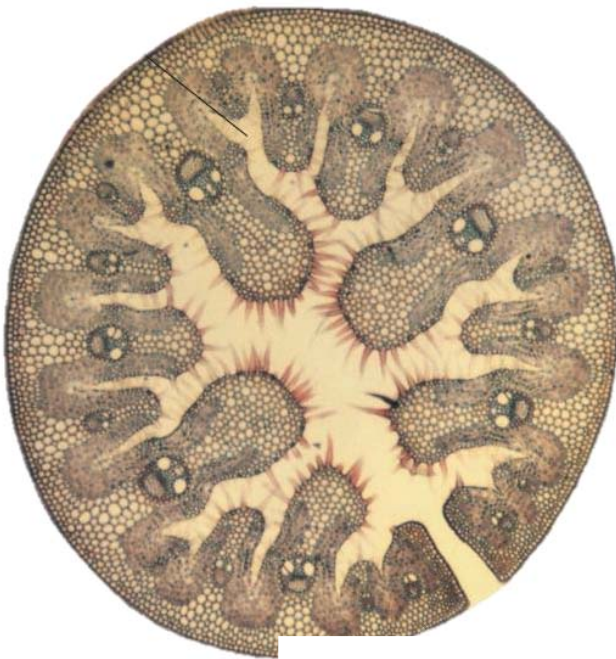
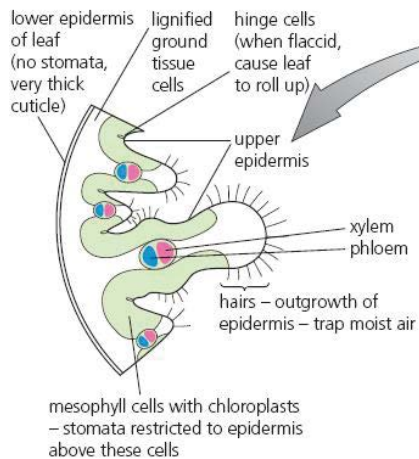
- Shows that the probability that the difference in density of stomata between the two leaves being due to chance is less than 5%.
- The difference is therefore statistically significant.
- Leaf A may be found in sunny areas while leaf B is in the shade/ Leaf A plant may be growing in low water content soil while leaf B plant is growing in more waterlogged soil/difference in carbon dioxide levels/AVP
- Ref to idea that higher stomatal density lead to increased water loss **or** increased carbon dioxide uptake and vice versa.

[Total: 14 marks]

### Question 3

Slide S1 is a transverse section from a leaf of the *Amophillia* plant.

(a) In the space below, draw a labelled plan drawing of this leaf. [6]



**Title – 1 (Plan drawing of *Amophillia* Leaf (T.S.) X40/X100)**

**Accurate size and shape of regions - 1**

**Mesophyll Labelled – 1**

**Vascular Bundle (with xylem and phloem) Labelled – 1**

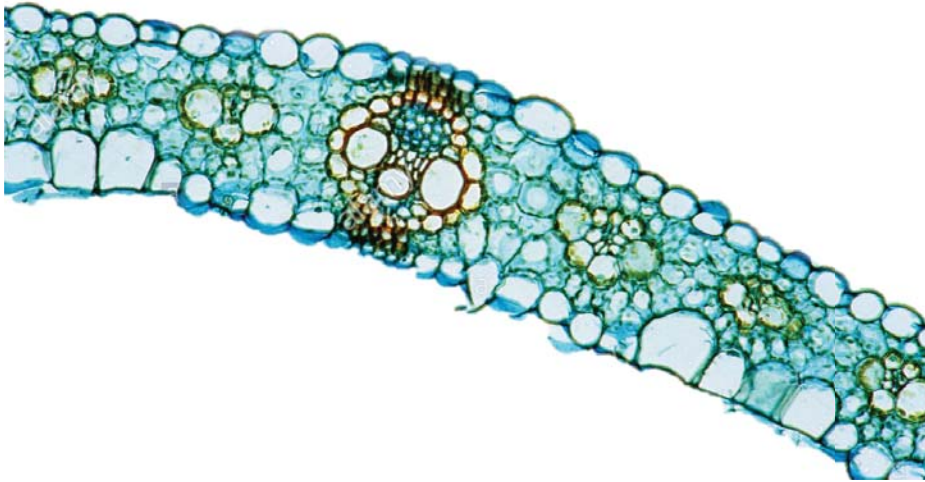
**Outer epidermal layer represented with a double line – 1**

**Calculation of magnification of drawing - 1**

- (b) Using the provided **slide graticule**, measure the diameter of the leaf. You may assume that the slide graticule can be used as you would use a ruler. [1]

**Diameter of leaf: 1.90 to 1.95 mm**

The following **Figure 2.1** is a microscope image of a section of a typical monocot leaf.



From *alamy.com*

**Figure 2.1**

- (c) Given that the magnification of Figure 2.1 is 50X, calculate the actual width of the specimen. [2]

**Working - 1**

**Precision 3sf - 1**

- (d) Identify three differences between a typical monocot leaf and the *Amophillia* leaf. [3]

**In *Amophillia***

- **Presence of epidermal hairs**
- **Folding/Rolling of leaf**
- **Sunken/hidden stomata (idea)**
- **No obvious guard cells**
- **AVP**

**Any 3**

- (e) Suggest reasons for the differences you have identified. [2]

- **Plant found in hot and dry regions**
- **Lacking in water**
- **Avoid excessive loss of water from evapo-transpiration**

**Any 2**

[Total: 14 marks]

#### Question 4

The Tasmanian Tiger or Thylacine (*Thylacinus cynocephalus*), the world's largest carnivorous marsupial, was once common throughout Australia and Papua New Guinea. The Thylacine resembled a large, short-haired dog with a stiff tail which smoothly extended from the body in a way similar to that of a kangaroo. The female Thylacine had a pouch with four teats, but unlike many other marsupials, the pouch opened to the rear of its body.

An example of convergent evolution, the Thylacine showed many similarities to the members of the Canidae (dog) family of the Northern Hemisphere: sharp teeth, powerful jaws, raised heels and the same general body form.

Due to human activities, the Thylacine was hunted to extinction by early 1930. A Thylacine specimen with soft tissue remaining is found in the Australian Museum in Sydney.

Imagine that you are a researcher in the Australian Rare Fauna Research Association. Recently it was reported that locals near Mount Carstensz in Western New Guinea had sighted creatures that resemble Thylacines. Some members of the Association believe that the creatures sighted may be descendents of the Thylacine, while other members believe that the creatures may be a new species. If the former were true, then the Thylacine is not extinct and conservation effects may revive the species.

Plan an investigation to investigate if these creatures found in Western New Guinea are descendents of the Thylacine that was thought to be extinct.

Your planning must be based on the assumption that you have been provided with the following equipment and materials.

- Tissue sample from the museum specimen and from the Western New Guinea creatures under investigation
- Pestle and mortar
- DNA extraction buffer solution
- Microcentrifuge tubes
- Centrifuge
- Restriction enzymes
- Agarose or polyacrylamide gel plate
- Suitable source of electric current
- Radioactive probe
- Nitrocellulose membrane
- Autoradiography equipment

Your plan should have a clear and helpful structure to include:

- An explanation of the theory to support your practical procedure
- A description of the method used including scientific reasoning behind the method
- The type of data generated by the experiment
- How the results will be analysed including how the origin of the organism can be determined

[Total: 14]



1. Theoretical consideration or rationale of the plan to justify the practical procedure;
2. Method of DNA extraction including homogenization and use of buffers;
3. Preparation of samples for electrophoresis, including use of centrifuge
4. Selection of restriction enzyme and reasons for the selection;
5. Amplification of DNA fragments using PCR including detail of PCR;
6. Separation of fragments by gel electrophoresis and the principles behind the separation;
7. Transfer of DNA onto nitrocellulose membrane;
8. Hybridization with radioactive labeled DNA probe;
9. Autoradiography method;
10. Method of band visualization, e.g. exposure to X-ray or chemiluminescent substrate;
11. Significance of matching bands;
12. The correct use of technical and scientific terms; valid safety concerns

1. Theoretical consideration or rationale of the plan to justify the practical procedure;
  - **Homologous regions of DNA** in the Thylacine and the creatures found in Western New Guinea have **variable number of tandem repeats (VNTR)**.
  - Restriction digestion of homologous regions produces **restriction fragments of different lengths**, the animals exhibit **Restriction Fragment Length Polymorphism (RFLP)**.
  - **Comparison of the DNA banding patterns** from Thylacine with the creatures found in Western New Guinea **will determine if they are the same species**.
2. Method of DNA extraction including homogenization and use of buffers;
  - Add DNA extraction buffer to tissue sample before homogenization
  - **Mechanically break tissue samples** during homogenization using mortar and pestle, to release DNA
3. **Centrifuge homogenized samples in a microcentrifuge tube to separate DNA from the rest of the cellular debris.**
  - Use a micropipette to transfer the **supernatant** which contains the DNA into another clean microcentrifuge tube.
  - Add ice-cold ethanol to **precipitate the DNA out of solution**.
4. **The same restriction enzyme, for example, EcoRI, which is used to cleave DNA from the Thylacine and the creatures found in Western New Guinea at different restriction sites, producing differentiating number and length of restriction fragments between the species.**

5. The purified DNAs from both specimens are subjected to Polymerase Chain Reaction (PCR) in a thermocycler to amplify a particular region of the DNA using Taq polymerase.
  - One PCR cycle consists of 3 steps:
    - Denaturation of DNA molecules at (95°C), to break the hydrogen bonds so that the double-stranded DNA separates into single strands.
    - Annealing of Primer to DNA strands at (55°C) to complementary sequences flanking the target sequence to be amplified
    - Extension step at (72°C) for Taq polymerase to catalyse the synthesis of a complementary strand of DNA for each of the single strands of the target DNA.
  
6. Separation of fragments by gel electrophoresis and the principles behind the separation;
  - Mix DNA fragments after restriction digestion with loading dye.
  - Load the DNA samples from the 2 animals into separate wells of the agarose gel to perform gel electrophoresis at 100 V for 30 min.
  - Load DNA ladder onto a separate well as a reference for identification of band sizes.
  - Gel electrophoresis separate DNA fragments based on size - Smaller size fragments migrate more rapidly/at a faster rate than large fragments to the positive end.
  - DNA is negatively-charged and will migrate to the positive electrode.
  
7. Transfer of DNA onto nitrocellulose membrane;
  - Restriction fragments in the agarose gel are transferred to a piece of nitrocellulose membrane by capillary action during Southern Blotting.
  - The gel is immersed in alkaline solution (sodium hydroxide) to denature double-stranded DNA into single strands.
  
8. Hybridization with radioactive labeled DNA probe;
  - The nitrocellulose membrane is then incubated with a radioactively labelled, single-stranded DNA probe which is complementary and thus hybridised to the target VNTR sequences, via formation of hydrogen bonds. Excess probe was washed off.
  
9. Autoradiography method;
  - Detect the position of the hybridised probes by autoradiography. A sheet of X-ray film is placed over the nitrocellulose membrane.

**10. Method of band visualization, e.g. exposure to X-ray or chemiluminescent substrate;**

- **Bands of radioactivity will be detected on the X-ray film where the radioactively labelled probe hybridised to complementary VNTR sequences.**

**11. Significance of matching bands;**

- **Compare DNA banding patterns** from Thylacine with the creatures found in Western New Guinea.
- **Identify bands that** creatures found in Western New Guinea **have in common/match** with the Thylacine.
- Presence of a **high number of common or matching bands at the chosen RFLP loci** indicates a **high possibility** that creatures found in Western New Guinea are **evolutionarily related** to and are likely to be the **descendants of the Thylacine. If the banding patterns are different, then they are not the same species.**

**12. Valid safety concerns**

- **Sodium hydroxide can be an irritant, avoid direct contact with hands and wear gloves.**
- **Risk of electrocution, do not switch on/off power socket and power pack of gel electrophoresis with wet hands to avoid being electrocuted.** Ensure hands are dry when using appliances to prevent electrocution.
- **Probes are radioactive, wear protective gear and gloves when handling probes during southern blotting and autoradiography and work behind a radioactive shield.**
- **Ethidium bromide is a carcinogen.** Handle gel using protective gloves.

**End of Paper**

