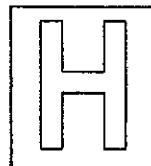


1



NATIONAL JUNIOR COLLEGE, SINGAPORE
Senior High 2
Preliminary Examination
Higher 2

CANDIDATE
NAME

BIOLOGY
CLASS

2bi2_

REGISTRATION
NUMBER

BIOLOGY

9744/01

Paper 1 Multiple Choice

19 September 2019

Additional Materials: Multiple Choice Answer Sheet

1 hour

READ THESE INSTRUCTIONS FIRST

Write in soft pencil.

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

Write your name, Biology class and registration number above and on the Answer Sheet in the spaces provided.

There are **thirty** questions on this paper. Answer all questions. For each question there are four possible answers A, B, C and D.

Choose the one you consider correct and record your choice in soft pencil on the separate Answer Sheet.

Read the instructions on the Answer Sheet very carefully.

Each correct answer will score one mark. A mark will not be deducted for a wrong answer. Any rough working should be done in this booklet.

The use of an approved scientific calculator is expected, where appropriate.

For Examiner's Use	
Paper 1	
Total	/ 30

This document consists of 13 printed pages.

1 Which structures are found in *Tuberculosis mycobacterium*?

- 1 70S ribosomes
 - 2 80S ribosomes
 - 3 Linear DNA (chromosomes)
 - 4 Circular DNA
- A 1 and 2
B 1 and 4
C 2 and 3
D 1, 2 and 3

2 Tests were performed on samples from a mixture of biological molecules. When iodine in potassium iodide solution was added to a sample, the mixture turned black. When the biuret test was carried out on another sample, the mixture turned purple.

Which biological molecules were in the mixture?

- A amylase and starch
B cellulose and starch
C phospholipid and cellulose
D starch and phospholipid

3 Collagen is a macromolecule with three polypeptides lying closely side by side in the form of a triple helix.

Every third amino acid in each polypeptide has the shortest possible R-group or side chain (— H) to allow close packing of the polypeptides.

Which is the amino acid?

- A glucose
B glycerol
C glycine
D guanine

- 4 Which row shows two pairs of nucleotides formed during transcription?

	first base pair transcribed		second base pair transcribed	
	bases	number of hydrogen bonds	bases	number of hydrogen bonds
A	AU	2	CG	2
B	AU	2	CG	3
C	AU	2	TU	2
D	AU	3	CG	2

- 5 Sickle cell anaemia is caused by a mutation in an allele of the gene that codes for the β -globin polypeptide of haemoglobin.

The diagram shows the sequence of bases in a small section of the coding strand of DNA for both the HbA (normal) and HbS (sickle-cell) β -globin alleles.

HbA: CTGACTCCTGAGGAGAAAGTCT

HbS: CTGACTCCTGTGGAGAAAGTCT

How will the mutation in the HbS allele result in the production of an altered version of the β -globin polypeptide?

- A A tRNA molecule with the anticodon CAC will hydrogen bond to the altered codon on mRNA.
- B All the amino acids coded for after the mutation will differ from those in the HbA protein.
- C mRNA transcribed from the HbS allele will contain the codon CAC instead of the codon CTC.
- D The ribosome will be unable to continue translation of the HbS mRNA after the altered codon.

- 6 Which of the following about transcription is correct?

- A DNA is synthesized in the 5' \rightarrow 3' direction.
- B RNA is synthesized in the 3' \rightarrow 5' direction.
- C The template strand is read in a 3' \rightarrow 5' direction.
- D The template strand is read in a 5' \rightarrow 3' direction.

- 7 In *E. coli*, the production of enzymes for tryptophan synthesis is carefully controlled according to the organism's metabolic needs. A mutation in the gene encoding the tryptophan repressor has occurred, such that the repressor can bind DNA without the co-repressor.

What effect on enzyme production can be expected under this condition?

- A constitutive, high-level enzyme production
 - B high-level enzyme production in the absence of tryptophan, no activity in the presence of tryptophan
 - C no enzyme production in the absence of tryptophan, high-level activity in the presence of tryptophan
 - D no enzyme production under any conditions
- 8 Which of the following statements about RNA Splicing of a single pre-mRNA are correct?
- 1 Alternative splicing controls the amount of gene products formed by having different promoters.
 - 2 Alternative splicing controls the type of gene products formed by having different exons.
 - 3 Different gene products can be formed at the same time within the same cells.
 - 4 Different gene products can be formed at different stages of an organism's life cycle.
 - 5 Different mRNA transcripts can be produced at the same time.
- A 2 and 4 only
 - B 3 and 5 only
 - C 1, 2 and 4 only
 - D 1, 3 and 5 only
- 9 Which of the following about genetics of bacteria is correct?
- A A bacteria cell can transfer DNA during conjugation when it has been infected by a bacteriophage.
 - B Binary fission allows for chromosomal and non-chromosomal DNA to be transferred.
 - C Inducible systems in bacteria are regulated by positive control.
 - D RNA polymerase binds to the operator for transcription to begin.

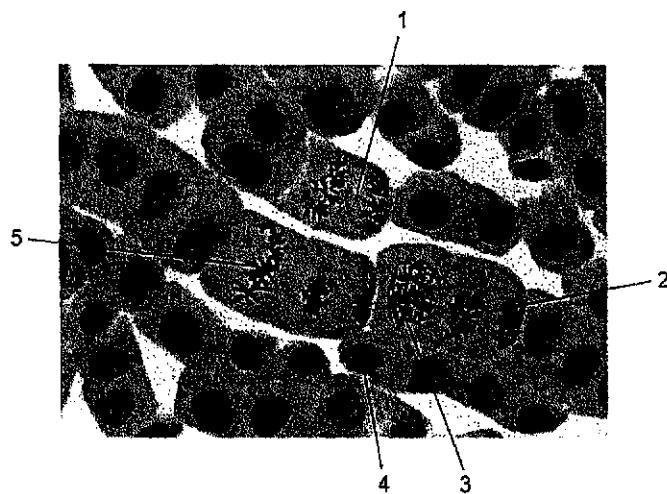
5

- 10 The cell cycle includes mitosis.

Which are features of nuclear division?

- 1 forms cell of equal size to parent cell
 - 2 forms genetically identical cells
 - 3 semi-conservation replication of DNA
- A 1, 2 and 3
B 1 and 2 only
C 1 and 3 only
D 2 only

- 11 The photomicrograph shows cells in the different stages of mitosis.



In which order do these stages occur?

- A $3 \rightarrow 5 \rightarrow 2 \rightarrow 1 \rightarrow 4$
B $3 \rightarrow 5 \rightarrow 1 \rightarrow 2 \rightarrow 4$
C $4 \rightarrow 3 \rightarrow 5 \rightarrow 1 \rightarrow 2$
D $4 \rightarrow 5 \rightarrow 1 \rightarrow 2 \rightarrow 3$

- 12** Exposure to which of the following increases the risk of developing cancerous growth?

	UV light	viruses	carbon monoxide	X-rays	Key
A	✓	✓	X	✓	✓ Increases risk
B	✓	X	✓	✓	X Does not increase risk
C	X	✓	✓	X	
D	✓	X	✓	X	

- 13** Stem cells have active telomerase that prevents chromosome shortening with every DNA replication.

Which of the following describes the role of telomerase?

- A It acts as a buffer to prevent erosion of subtelomeric (segments of DNA between telomeric caps and chromatin) genes.
- B It extends the parental DNA strand at the 3' end.
- C It lengthens the daughter DNA strand at the end of replication.
- D It prevents the end replication problem from occurring.

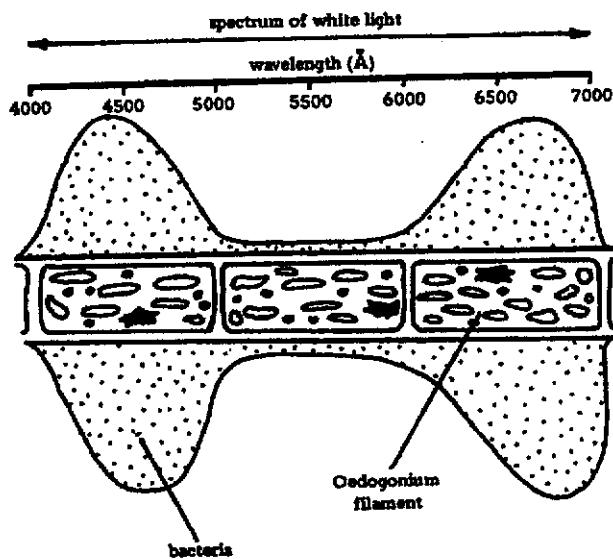
- 14** A single species evolves into several species which occupy different habitats.

What best describes this evolutionary process?

- A adaptive radiation
- B divergent evolution
- C directional selection
- D mutation

- 15 Which of the following increases variation within a gene pool?
- A chromosome inversion
 - B gene mutation
 - C reassortment of chromosome in meiosis
 - D random fusion of gametes
- 16 Which of the following processes could still occur in a chloroplast in the presence of an inhibitor that prevents H⁺ from passing through ATP synthase complexes?
- 1 sugar synthesis
 - 2 photolysis of water
 - 3 transfer of electrons down the electron transport chain
 - 4 oxidation of NADPH
- A 1 and 2
 - B 1 and 4
 - C 2 and 3
 - D 3 and 4

- 17 The diagram represents a filament of the green alga *Oedogonium* sealed in an airtight chamber together with oxygen sensitive bacteria. The filament was illuminated by a micro-spectrum of white light along the length of three cells. The motile oxygen-sensitive bacteria distributed themselves along the cells as shown.

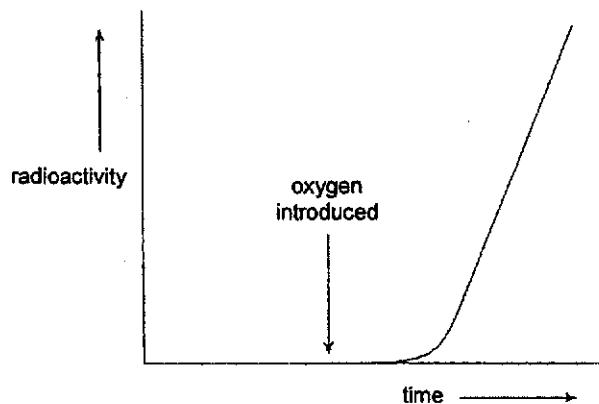


Which of the following best explains the distribution of the bacteria?

- A The bacteria were distributed according to the absorption spectrum of the chlorophyll.
- B The central cell had been killed by the blue light and therefore could not attract bacteria.
- C The distribution of chlorophyll in the cells was uneven and this influenced the bacteria.
- D The two end cells were dead and the bacteria were decomposing them.

- 18** In an investigation, a culture medium containing glucose labelled with radioactive carbon atoms was placed in a flask. A sample of animal cells was added to this medium. The conditions in the flask at the start were anaerobic. Oxygen was later bubbled through the medium. Samples of gas produced by the cells were tested for radioactivity at regular intervals.

The graph shows the results.



What is the best explanation for the appearance of radioactivity in the gas produced by the cells after oxygen was introduced?

- A carbon fixation of RuBP
 - B decarboxylation of pyruvate
 - C oxidation of reduced NAD
 - D phosphorylation of ADP
- 19** Which of the following statement about anaerobic respiration is correct?
- A Animals are unable to use lactate for the production of ATP.
 - B From one molecule of glucose, ethanol and lactate production yield the same amounts of ATP.
 - C Plant cells do not have mitochondria for ATP production due to the presence of the chloroplast.
 - D Yeast is able to respire ethanol for the production of ATP.

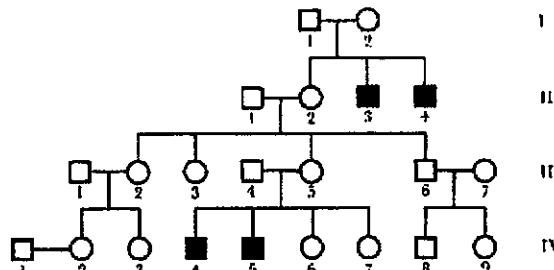
10

- 20 In birds, sex is determined by a ZW chromosome scheme. Males are ZZ and females are ZW. A lethal recessive allele is sometimes present on the Z chromosome in pigeons.

What would be the sex ratio in the offspring of a cross between a male heterozygote and a normal female?

- A 1:1 male to female
- B 1:2 male to female
- C 2:1 male to female
- D 3:1 male to female

- 21 Haemophilia is a rare genetic disorder in which the blood does not clot normally. The mutation that causes haemophilia is located on the X-chromosome. The pedigree below shows the inheritance of haemophilia in a family. Squares represent males while circles represent females. Dark symbols represent affected individuals.



What is the probability that a son born to IV-2 will be a haemophiliac?

- A 0.5
- B 0.25
- C 0.125
- D 0.0625

- 22** An experiment was carried out to investigate the release of glucose by liver cell cultures under various conditions.

culture	conditions	observations
1	no addition of glucagon or caffeine	minimal glucose release
2	addition of glucagon, followed by its removal	release of glucose into the media, but rapid reduction in release after glucagon was removed
3	addition of caffeine	minimal glucose release
4	addition of both glucagon and caffeine, followed by the removal of glucagon	release of glucose into the media, but with slow reduction in release after glucagon was removed

What could be deduced about the action of caffeine from the observations?

- A caffeine binds to the active site of adenylyl cyclase
 - B caffeine inhibits the action of phosphodiesterase
 - C caffeine prevents the breakdown of glucagon
 - D caffeine stimulates the synthesis of adenylyl cyclase
- 23** A drug designed to inhibit the response of cells to a lipid soluble signalling molecule would almost certainly result in which of the following?
- A a decrease in G-protein activity
 - B a decrease in transcriptional activity of certain genes
 - C an increase in receptor tyrosine kinase activity
 - D lower cytoplasmic levels of cAMP
- 24** Which of the following cell types of the innate immune system does not perform phagocytosis?
- A neutrophils
 - B monocytes
 - C macrophages
 - D all of the above

25 All of the following are true of antigen EXCEPT which one of the following?

- A They are protein in nature.
- B They can elicit an immune response.
- C They contain epitopes.
- D They will react with antibodies.

26 Which of the following is a potential risk of vaccination?

- 1 severe side effects
 - 2 attenuated virus used for vaccination may pose the risk of virulence in the patient
 - 3 decreased herd immunity of the population
- A 1 only
 - B 1 and 2
 - C 1 and 3
 - D None of the above

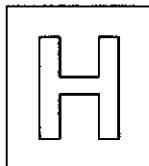
27 Cell-wall biosynthesis is inhibited by antibiotics by inhibiting the biosynthesis of which of the following?

- A cellulose
- B liposaccharide
- C peptidoglycan
- D protein

28 Which of the following is not a characteristic of the dengue virus?

- A Immunity to one serotype of the virus will lead to immunity to the other serotypes.
- B It is spread by both *Aedes aegyptii* and *Aedes albopictus*.
- C Source reduction is an important method to reduce the spread of the disease.
- D The spread of dengue has spread as far as Europe due to climate change.

- 29** Which of these is not an expected effect of climate change?
- A** expanding glacier
 - B** extreme weather
 - C** flooding in coastal cities
 - D** sea level rising
- 30** Which of the following will be the least likely to be a direct result of the rise in water level in oceans and seas due to melted glaciers?
- A** desertification
 - B** destruction of infrastructure
 - C** destruction of human settlements
 - D** endangering of species



NATIONAL JUNIOR COLLEGE, SINGAPORE
 Senior High 2
 Preliminary Examinations
 Higher 2

CANDIDATE
NAME

BIOLOGY
CLASS

2bi2_____

REGISTRATION
NUMBER

Biology

9744/02

Paper 2

29 August 2019

2 hours

Candidates answer on the Question Booklet.

No Additional Materials are required.

READ THESE INSTRUCTIONS FIRST

Write your name and Biology class on all the work you hand in.

Write in dark blue or black pen on both sides of the paper.

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 parts of the question in the spaces provided on the Question Booklet.

The use of an approved scientific calculator is expected, where appropriate.

You may lose marks if you do not show your workings or if you do not use appropriate units.

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

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

For Examiner's Use	
1	/12
2	/12
3	/14
4	/14
5	/12
6	/10
7	/12
8	/14
Total	/100

This document consists of 24 printed pages.

Answer all the questions.

- 1 Fig. 1.1 below shows a section of a cell surface membrane.

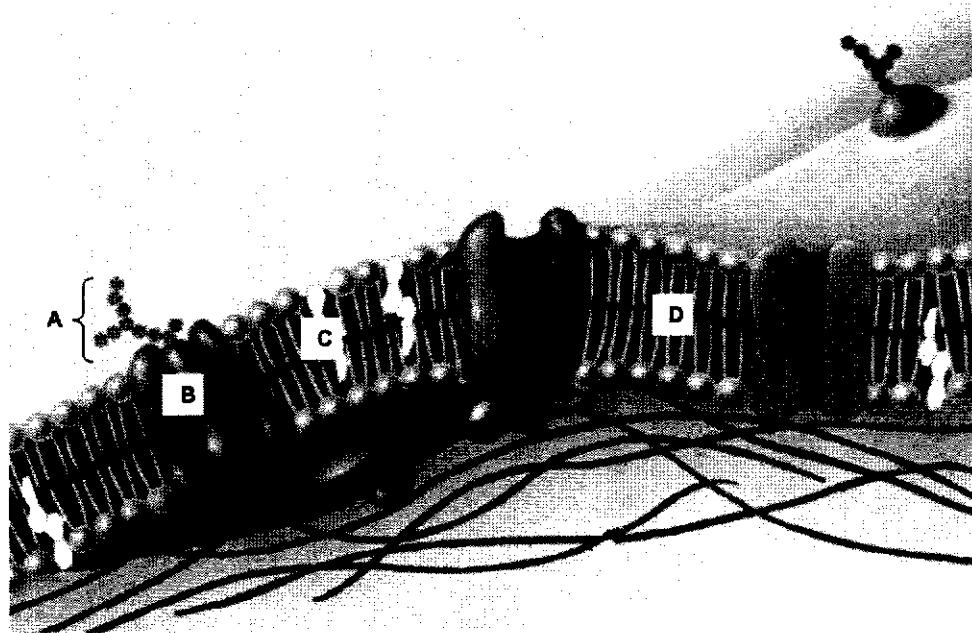


Fig. 1.1

- (a) (i) Name the structures labelled C and D.

D: _____ [2]

- (ii) Describe three possible functions of the structure labelled A.

[3]

- (b) Listed below is the amino acid sequence that makes up the transmembrane segment of structure B.

*... Ile – Thr – Leu – Ile – Tyr – Phe – Gly – Val – Met – Ala –
Gly – Val – Ile – Gly – Thr – Ile – Leu – Leu – Ile – Ser – ...*

Suggest why such an amino acid sequence would enable the protein to span the membrane.

[3]

[3]

- (c) For hydrophilic molecules to enter a cell, they require the help of either carrier proteins or channel proteins.

State which kind of membrane proteins can transport molecule at a faster rate and give reasons to support your answer.

[4]

[4]

[Total: 12]

- 2 (a) Many amino acids are needed to form the structure of a typical haemoglobin molecule shown in Fig. 2.1.

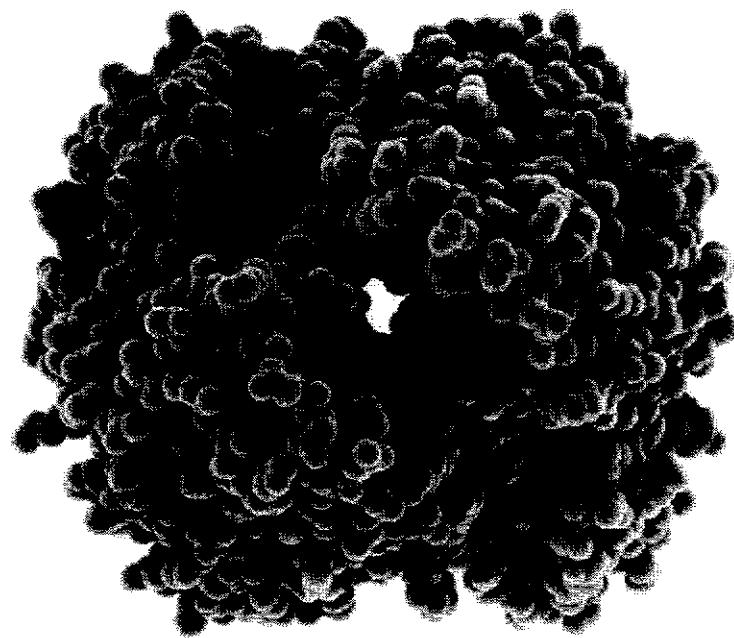


Fig. 2.1

Briefly describe how the structure of haemoglobin is adapted for its function.

[3]

- (b) Fig. 2.2 shows a three-dimensional model of an important signal molecule XDF in glucose regulation. It is used to send signals to the cells of the Islets of Langerhans to make insulin. XDF is a globular protein made up of 212 amino acids.



Fig. 2.2

- (i) Explain what determines the three-dimensional shape of the XDF.

[3]

[3]

- (ii) Suggest the consequence of the shape of XDF to its function, when subjected to high pH treatment.

[2]

[2]

- (c) An experiment was carried out to monitor the changes in blood glucose level and blood insulin level in a healthy individual over a 12-hour period. Table 2.1 below shows the results of the experiment.

Table 2.1

time	meal taken	average blood insulin level / units per 100 ml	average blood glucose level / units per 100 ml
0900	Yes	10	100
1000	No	65	170
1100	No	10	110
1200	No	10	100
1300	No	10	100
1400	Yes	10	100
1500	No	70	165
1600	No	10	110
1700	No	10	100
1800	Yes	10	100
1900	No	70	180
2000	No	10	110
2100	No	10	100

With reference to Table 2.1, outline how blood insulin level is regulated in the body.

[4]

[Total: 12]

- 3 Fig. 3.1 shows the eukaryotic chromatin in two states, **A** and **B**.

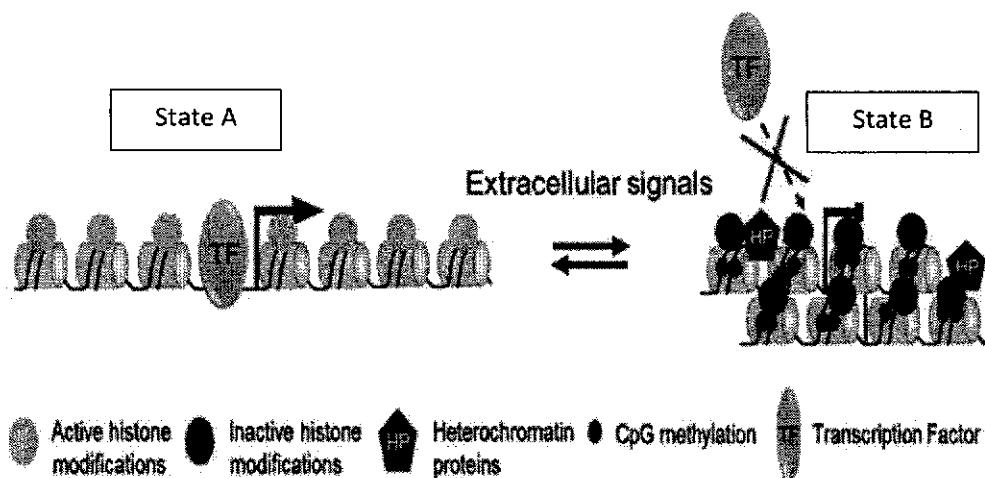


Fig. 3.1

- (a) (i) State the type of chromatin in state **A** and in state **B**.

A:

B:

[2]

- (ii) Using your knowledge of histone modification, describe the process that may give rise to chromatin in state **A**.

[4]

- (b) Describe how the difference in the structure of DNA in state A and B affects gene expression.

[2]

[2]

- (c) Eukaryotic DNA has non-coding regions that do not get transcribed and some that may be transcribed but not translated. One such example is the promoter sequence.

Apart from the non-coding DNA involved in regulation of transcription, state and describe the roles of three other types of non-coding DNA.

[6]

[6]

[Total: 14]

- 4 (a) The electron micrographs below shows the organelles of a eukaryotic cell.

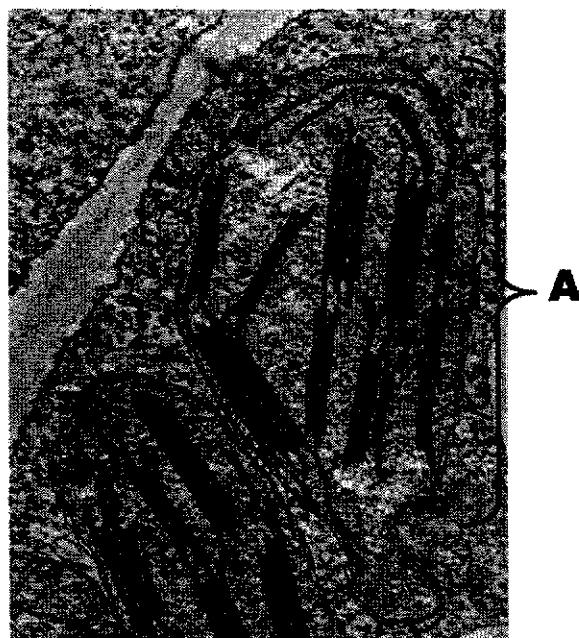


Fig. 4.1



Fig. 4.2

- (i) Name the structures labelled A (Fig. 4.1) and B (Fig. 4.2).

A:

B:

[2]

- (ii) State two similarities and two differences in the structural features of A and B.

[4]

[4]

- (b) Fig. 4.3 is a scaled up image of Fig. 4.2 depicting a single organelle W. The labelled arrows X and Y both represent a structural feature of W.

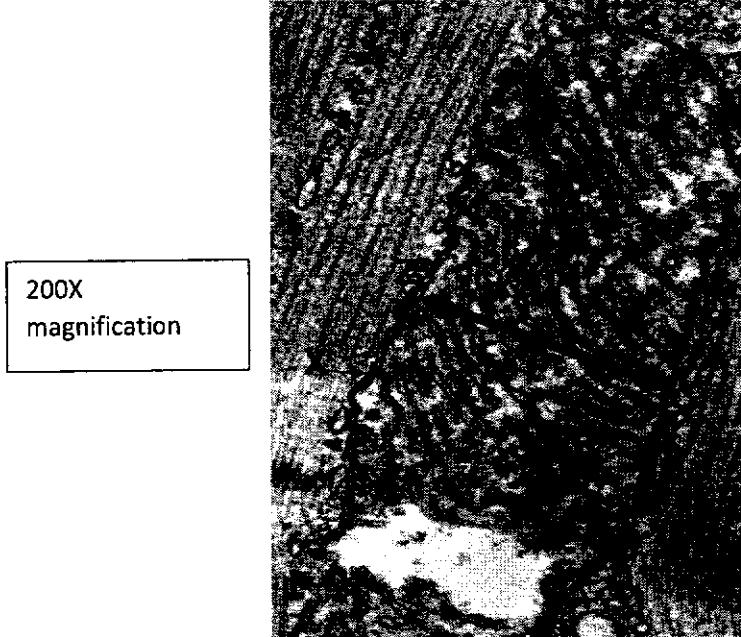


Fig. 4.3

The table below shows the protein composition of various areas in organelle W in Fig. 4.3.

Table 4.1

Labels	Protein composition (%)
X	6
Y	21
Region between X & Y	6
Inside W	67
Total	100

Using the information in Table 4.1 above, account for the

- (i) abundance of protein inside organelle W.

[2]

- (ii) greater amount of protein in Y compared to X.

[3]

12

- (iii) Calculate the **actual** width of the organelle at the position marked by line **Z** in Fig. 4.3.

You should show your working and use appropriate units.

[3]

[Total: 14]

- 5 Growth and development in organisms is controlled by a number of mechanisms that operate at the cellular level. The control elements involved in these mechanisms include hormones, the second messenger molecule cyclic AMP and regulatory genes. In eukaryotes the most important regulatory genes contain homeobox sequences and are called homeotic genes.

The regulatory genes of the *lac* operon in prokaryotes are studied to help us to understand how regulatory genes and their products interact to switch structural genes on and off.

- (a) Use your understanding of the biochemical identity and interactions of these control elements to complete Table 5.1 by putting a tick (✓) or a cross (✗) in each box.

Table 5.1

control element	made of protein	binds with a protein	codes for a protein
insulin		✓	
cyclic AMP			✗
<i>lac I</i> gene		✓	
<i>lac O</i> gene	✗		
Homeotic gene product		✗	

[5]

- (b) RNA polymerase and DNA polymerase are both enzymes. RNA polymerase is involved in the action of some control elements, whereas DNA polymerase is not.

Describe and explain the difference between the **functions** of these two enzymes.

[4]

- (c) The control of the expression of the *lac* operon genes, which allow uptake and digestion of lactose in the bacterium *Escherichia coli*, is well known.

Fig. 5.1 shows the arrangement of the elements of the *lac* operon.

```

graph LR
    R1[regulator gene] --- P1[promoter]
    R2[regulator gene] --- O[operator]
    P1 --- O
    O --- GZ[structural gene Z]
    O --- GY[structural gene Y]

```

The diagram illustrates the lac operon structure. It features two regulator genes positioned above a single promoter. The promoter is located upstream of an operator, which in turn controls the expression of two structural genes, labeled Z and Y.

Fig. 5.1

Describe how genes Z and Y are switched on in bacteria that are moved to a nutrient medium that contains lactose.

[3]

[3]

[Total: 12]

- 6 (a) Fig. 6.1 represents part of a DNA molecule.

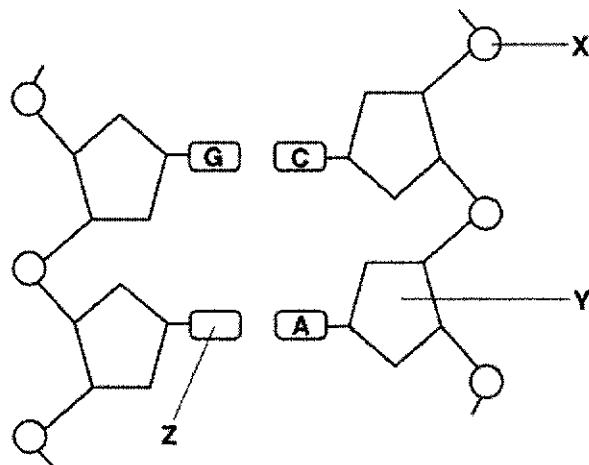


Fig. 6.1

- (i) State the precise name of each of the parts of the DNA molecule labelled **X**, **Y**, and **Z**.

X: _____

Y: _____

Z: _____ [3]

- (ii) Describe how the DNA molecule replicates.

[5]

- (b) Explain why the mRNA molecule is shorter than a DNA molecule.

[2]

[Total: 10]

7 Vaccination can protect against the infectious disease tuberculosis (TB).

(a) Define the terms:

(i) vaccination

[2]

(ii) infectious disease.

[2]

(b) TB is an important disease worldwide. Table 7.1 shows recent information about TB cases reported during one year in six different countries.

Table 7.1

country	region	number of cases	number of cases per 100 000 population
Germany	Europe	4000	5
India	Asia	2 300 000	185
Japan	Asia	27 000	21
South Africa	Africa	490 000	981
Swaziland	Africa	15 000	1287
United Kingdom	Europe	7900	13

With reference to Table 7.1, explain the advantage of calculating the number of cases of TB per 100 000 population rather than stating the number of cases alone.

[2]

- (c) Describe how a person may become infected with TB.

[3]

- (d) Suggest why TB is more likely to be fatal in people who have HIV/AIDS than in those who do not have HIV/AIDS.

[3]

[Total: 12]

- 8 (a) Sometimes a gene has more than two alleles, termed *multiple alleles*. The ABO blood group system in humans is controlled by a gene with three alleles, I^A, I^B, and I^O. Alleles I^A and I^B are codominant and I^O is recessive to both.

Explain what is meant by *codominance*.

[3]

- (b) In humans, a gene that codes for the production of a protein, called factor VII, is located on the X chromosome. The dominant allele for this gene produces factor VIII, but the recessive allele does not produce factor VIII.

A person who is unable to make factor VIII has haemophilia in which the blood fails to clot properly.

Explain why a man with haemophilia cannot pass haemophilia to his son but may pass haemophilia to his grandson.

[3]

- (c) A gene for feather colour in chickens is carried on an autosome. This gene has two alleles, black (C^B) and splashed-white (C^W). When a male chicken with black feathers is mated with a female chicken with splashed-white feathers, all the offspring have blue feathers. This also occurs when a male chicken with splashed-white feathers is crossed with a female with black feathers.

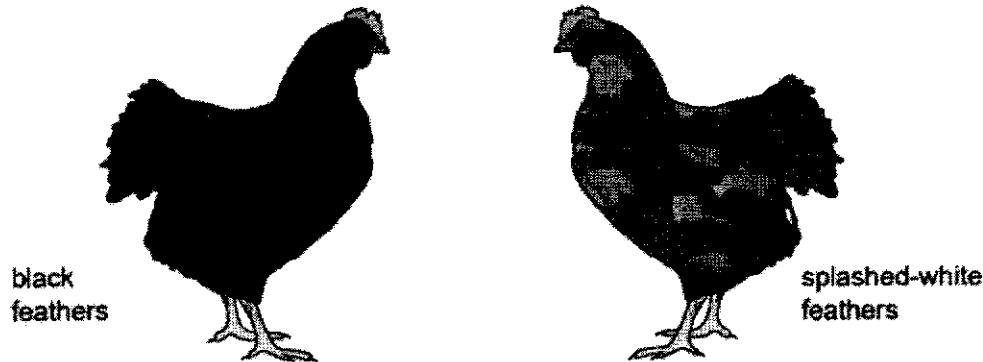


Fig. 8.1

Another gene may cause stripes on feathers (barred feathers). This gene is carried on the X chromosome. The allele for barred feathers (X^A) is dominant to the allele for non-barred feathers (X^a).

In chickens the male is homogametic and has two X chromosomes while the female is heterogametic and has one X chromosome and one Y chromosome.

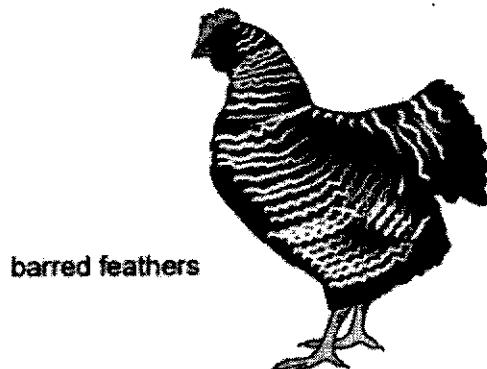


Fig. 8.2

- (i) A male chicken with black, non-barred feathers was crossed with a female chicken with splashed-white, barred feathers. All the offspring had blue feathers, but the males were barred and the females were non-barred.

Using the symbols given above draw a genetic diagram to show this cross.

[5]

- (ii) Explain how a farmer could use a breeding programme to find out the genotype of a male chicken with blue, barred feathers.

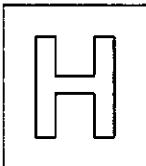
[3]

[Total: 14]

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NATIONAL JUNIOR COLLEGE, SINGAPORE
 Senior High 2
 Preliminary Examinations
 Higher 2

CANDIDATE
NAME

BIOLOGY
CLASS

2bi2 _____

REGISTRATION
NUMBER

Biology

9744/03

Paper 3

3 September 2019

Additional Materials: Answer Booklet

2 hours

READ THESE INSTRUCTIONS FIRST

Write your name and Biology class on all the work you hand in.

Write in dark blue or black pen on both sides of the paper.

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

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

Section A

Answer all parts of the question in the spaces provided on the Question Booklet.

Section B

Answer both parts of the question in the spaces provided on the Answer Booklet.

The use of an approved scientific calculator is expected, where appropriate.

You may lose marks if you do not show your workings or if you do not use appropriate units.

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

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

For Examiner's Use	
Section A	
1	/30
2	/8
3	/12
Section B	
4/5	/25
Total	/75

This document consists of 14 printed pages.

Section A

Answer all the questions in this section.

- 1 (a) Cyclin-dependent kinases (CDKs) are important cell-cycle regulators. To study them, the gene can be amplified using polymerase chain reaction (PCR), and the fragments ligated into cloning vectors.

PCR was used to amplify the CDK coding sequence using the following primers, which were designed to incorporate restriction sites:

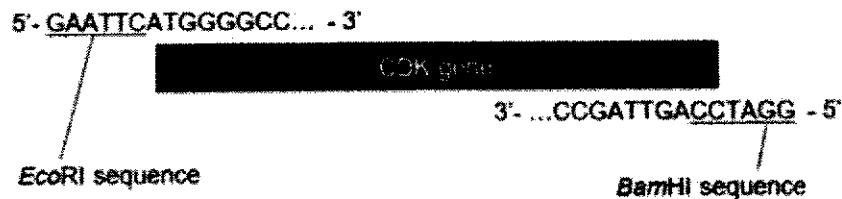


Fig. 1.1

- (i) Name **two** restriction sites that the CDK coding sequence should not contain.

[1]

- (ii) Suggest what additional feature the amplified gene must have to enable the expression of the eukaryotic gene.

[1]

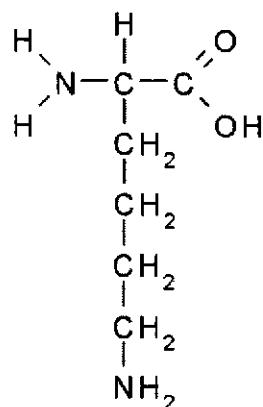
- (iii) Outline three differences between prokaryotic and eukaryotic control of gene expression.

[3]

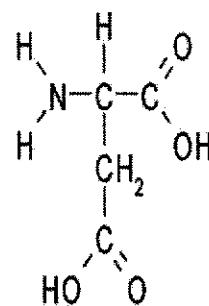
- (iv) Suggest the advantage of designing primers that incorporate the restriction site.
-
-
-

[2]

- (b) Two of the amino acids that may be found in CDKs are shown in Fig. 1.2.



Lysine



aspartate

Fig. 1.2

With reference to the amino acids in Fig. 1.2, explain the effect of extreme pH on enzyme activity of CDKs.

[3]

- (c) Enzymes can be found in cell cytoplasm as well as embedded on membranes of the cell.

- (i) Suggest one **important** structural difference between intrinsic membrane-bound enzymes and cytoplasmic enzymes.

[2]

- (ii) Explain the significance of the difference that you have stated in (i) to the intrinsic membrane-bound enzymes.

[2]

- (d) Fig. 1.3 shows the various stages of the cell cycle and how it is being regulated at the various check points.

Important control points of the cell cycle lie at the end of the G2 phase (G2/M transition), in mitosis (metaphase/anaphase transition) and in G1 phase (restriction point). Controls are shown in both solid and broken arrows.

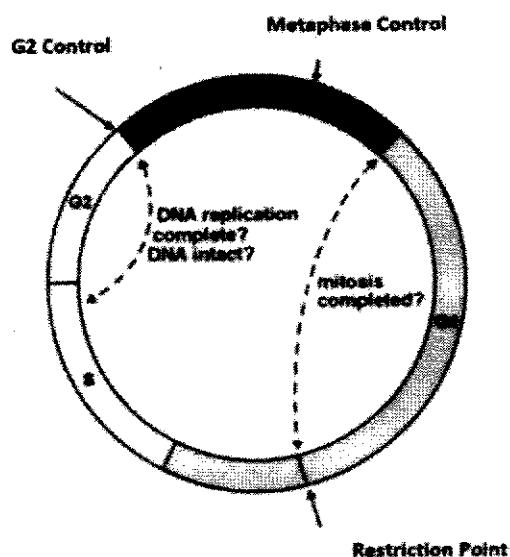


Fig. 1.3

Describe the function of metaphase control and how this may prevent the development of cancer.

[3]

- (e) Spindle fibres are polymers made from tubulin monomers. The removal of tubulin monomers causes spindle fibres to shorten. Scientists investigated the effect of the rate of tubulin removal on the speed of movement of chromatids during mitosis. The results are shown in Fig. 1.4.

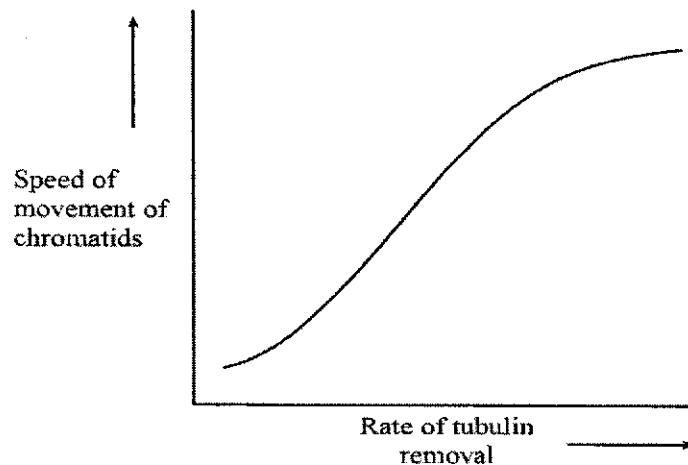


Fig. 1.4

- (i) Describe how these results support the role of spindle fibres in mitosis.

[2]

- (ii) Explain why drugs that stabilise microtubule structures are effective as anti-cancer therapeutic.
-
-
-

[2]

- (f) Leukemia is an uncontrolled proliferation of one type of white blood cell. One of the most common form is chronic myelogenous leukemia (CML). In most cases of CML, the leukemic cells share a chromosomal abnormality not found in any leukemic white blood cells, nor in any other cells of the patient's body.

This abnormality is shown in Fig. 1.5 where one chromosome 9 is longer than normal and one chromosome 22 is shorter than normal.

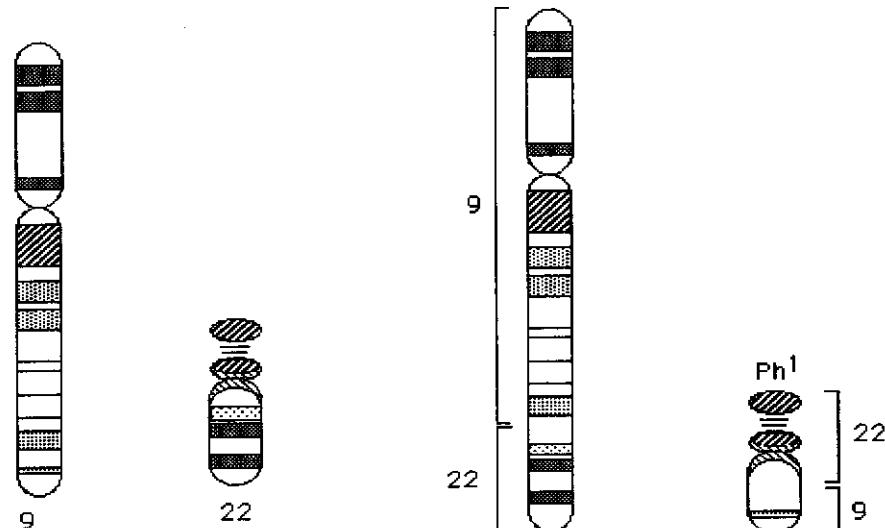


Fig. 1.5 (a) above showing the banding pattern on Normal Human chromosomes 9 and 22.

Fig. 1.5 (b) above showing the longer chromosome 9, and a shorter chromosome 22 which is known as the Philadelphia chromosome (Ph¹).

The gene that is affected in CML is the *bcr* gene on chromosome 22.

In a normal cell, *bcr* gene codes for the receptor tyrosine kinase, which receives growth factor required for cell division.

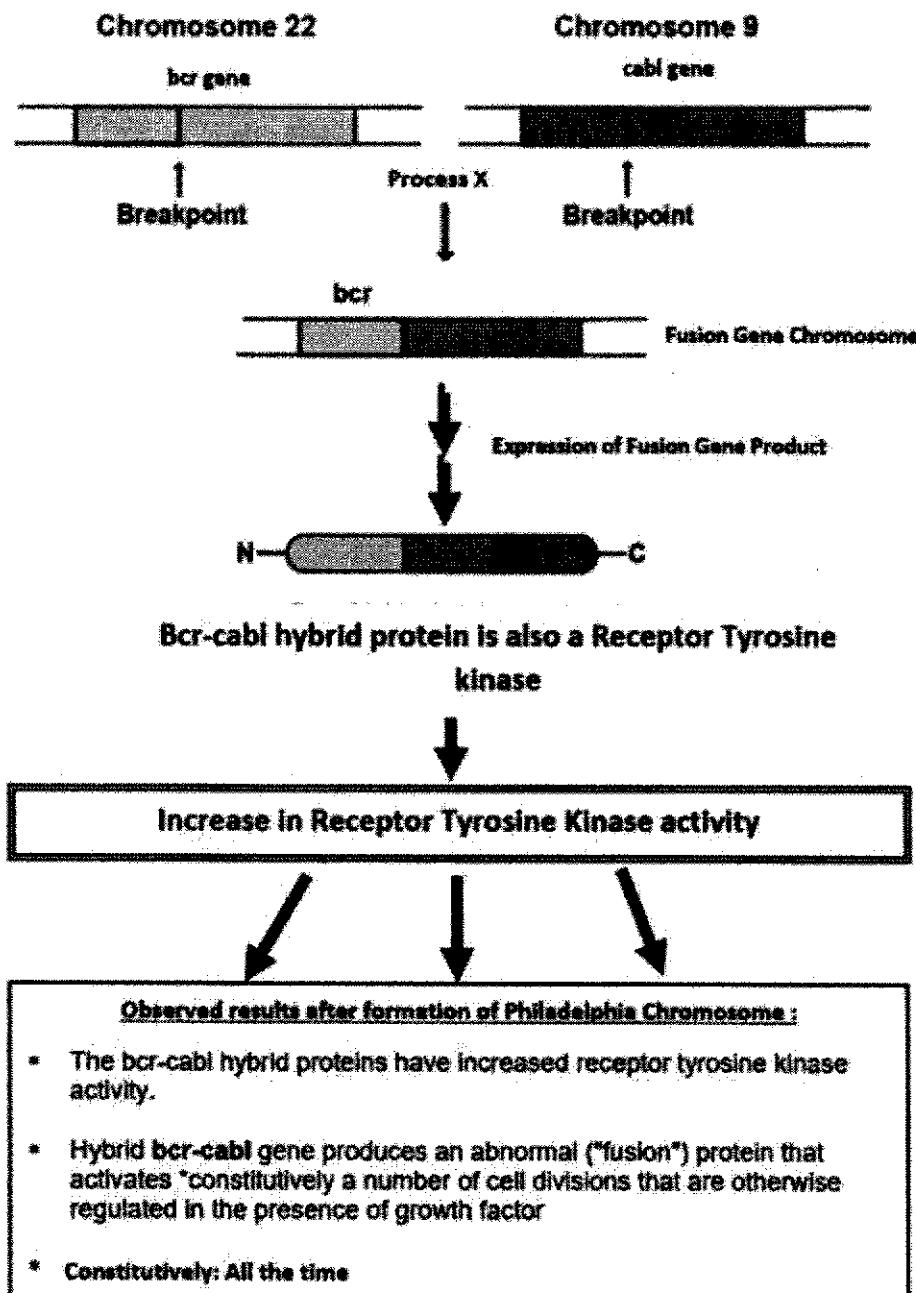


Fig. 1.6

- (i) Briefly describe the kind of mutation that occurs between chromosome 9 and 22 shown as process X in Fig. 1.6 that leads to the formation of Philadelphia chromosome (Ph^1).

[1]

- (ii) Based on the high tyrosine kinase activity of the Bcr-abl hybrid protein, explain whether *bcr* gene is considered a proto-oncogene or tumor suppressor gene.

[2]

- (iii) Describe three mechanisms by which oncogenes can arise.

[3]

- (iv) Mutation of *p53* gene was also commonly observed in these CML cells. This additional mutation causes the rate of mitosis in these cells to increase sharply.

Explain whether this is a dominant or recessive mutation.

[3]

[Total: 30]

- 2 The spruce bark beetle feeds on and breeds in spruce trees. If a large number of beetles are on a spruce tree, the tree will die. These dead trees will appear red when viewed from the air.

Alaska has experienced recent changes in the number of spruce bark beetles. It is thought that the number of beetles is affected by climate change.

Each year, the extent of damage to the woodland was estimated by measuring the size of the 'red area' from aerial photographs.

The drought index of the woodland was also determined. A high drought index indicates warm, dry conditions and low drought index indicates cool, moist conditions.

The graphs below show the changes in 'red area', mean summer temperature and drought index in Alaskan woodland, from 1930 to 2000.

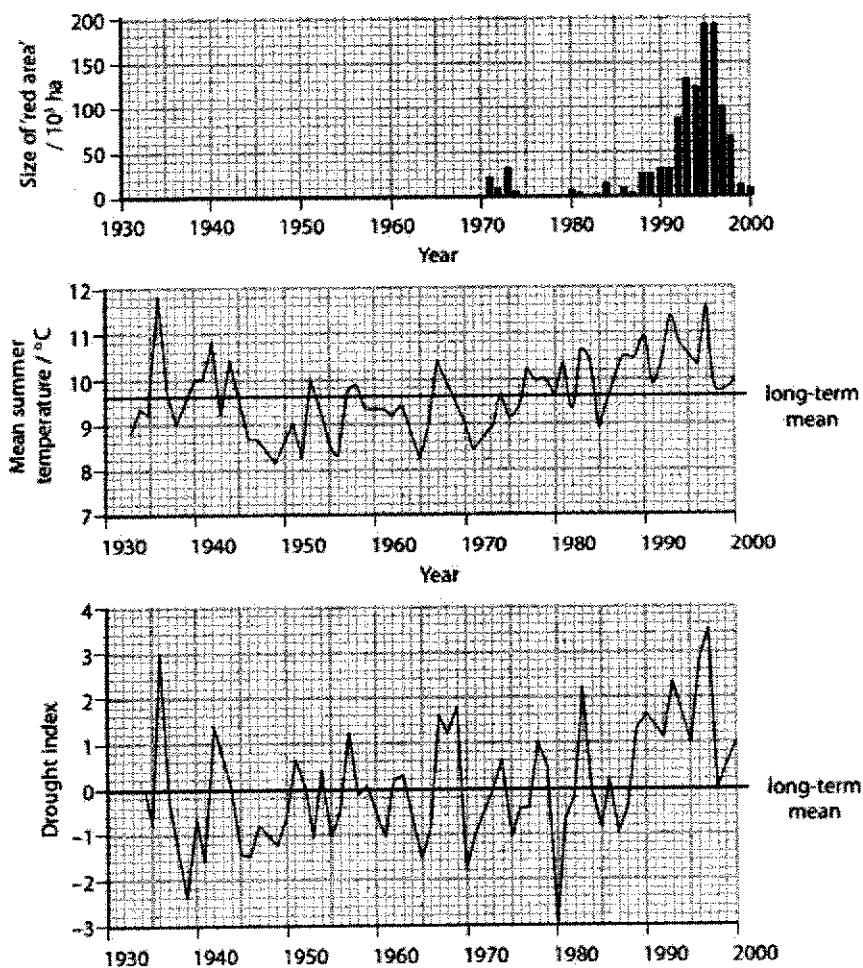


Fig. 2.1

- (a) Describe the changes in the size of the 'red area' from 1970 to 2000.

[2]

- (b) Suggest why there is no data for the size of the 'red area' before 1970.

[1]

- (c) Suggest why the number of spruce bark beetles is affected by temperature.

[2]

- (d) Using the information in the graphs, describe the evidence for climate change being responsible for the size of the 'red area'.

[3]

[Total: 8]

- 3 (a) The following boxes show the names of different stages that occur during meiosis.

Anaphase I	Metaphase II	Anaphase II
Telophase II	Prophase I	Metaphase I

State the stage(s) in which the following events occur:

Independent assortment

Formation of the spindle apparatus

Separation of sister chromatids

Formation of nuclear membranes

Chromosomes pulled to opposite poles

[5]

- (b) Meiosis is used in many organisms for the production of gametes.

Explain the importance of meiosis in the production of gametes.

[4]

[4]

- (c) Several days after fertilisation between gametes, the ball of cells becomes a blastocyst. The diagram below shows a section through a blastocyst.

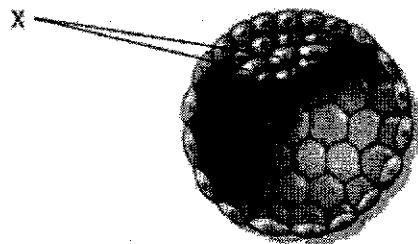


Fig. 3.1

- (i) Identify the type and state of potency of the cells labelled X.

[1]

- (ii) Explain what is meant by the state of potency identified in (c)(i).

[2]

[Total: 12]

Section B

Answer **one** question in this section.

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

Your answers must be in continuous prose, where appropriate.

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

- 4 (a) Explain the role of isolating mechanisms in evolution of new species. [15]

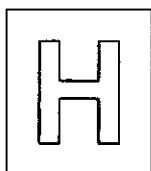
- (b) Explain, using **named** examples, how mutation can affect phenotype. [10]

[Total: 25]

- 5 (a) Describe how non-cyclic photophosphorylation produces ATP and [15] reduced NADP, and outline the steps of the Calvin cycle.

- (b) Outline the role of anaerobic respiration in both mammals and yeast [10] cells.

[Total: 25]



NATIONAL JUNIOR COLLEGE, SINGAPORE
Senior High 2
Practical Examination
Higher 2

CANDIDATE
NAME

BIOLOGY
CLASS

2bi2 _____

REGISTRATION
NUMBER

Biology

9744/04

Paper 4 Practical

22 August 2019

2 hours 30 min

Additional Materials: Answer Booklet

READ THESE INSTRUCTIONS FIRST

Write your name, Biology class and registration number on all the work you hand in.

Circle your practical shift and laboratory in the boxes provided.

Write in dark blue or black pen.

You may use an HB pencil for any diagrams or graphs.

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

Answer questions one and two in the spaces provided on the Question Paper.

Answer question three on the Answer Booklet provided.

The use of an approved scientific calculator is expected, where appropriate.

You may lose marks if you do not show your workings or if you do not use appropriate units.

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

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

Shift		
1	2	3
Laboratory		
BI23	BI24	CM44

For Examiner's Use	
1	20
2	20
3	15
Total	55

This document consists of 16 printed pages.

- 1 In humans, kidneys are the organs that remove waste products from the blood and produce urine. Small, useful molecules such as glucose are also removed from the blood in the kidney. Glucose must be reabsorbed into the blood so that very little is lost in the urine.

The concentration of glucose in urine can be estimated in order to check that the kidneys are working.

You will not be testing real urine. You will be testing solutions that represent urine and will be referred to as "mock urine".

You are required to test each of three samples of mock urine for the presence of glucose. These represent samples taken at different times from the same person.

You are provided with:

labelled	contents	hazard	Volume / cm ³
U1	mock urine	none	20
U2	mock urine	none	20
U3	mock urine	none	20
Benedict's	Benedict's solution	none	20
G	2% glucose solution	none	20
W	distilled water	none	20

If **Benedict's** comes into contact with your skin, wash off immediately under running water. It is recommended that you wear suitable eye protection.

Read step 1 to step 7 before proceeding.

Proceed as follows:

1. Set up a water-bath and heat to boiling for use in step 6.
2. Put 2 cm³ of **U1** into a test-tube.
3. Put 2cm³ of Benedict's solution into the same test-tube.
4. Shake the test-tube gently to mix contents.
5. Repeat step 2 and step 4 for **U2** and **U3**.
6. Put all three test-tubes into the water-bath you prepared in step 1 and immediately start timing.
7. Record in (a)(i) the time taken to the first colour change for each tube and record the final colour at 90s. After 90s, remove each of the test-tubes from the water-bath. If there has been no colour change during the 90s, record the time to the first colour change as "more than 90". You should still record the final colour at 90s.

- (a) (i) Record your results in an appropriate table.

[4]

- (ii) Use your results in (a)(i) to state which of **U1**, **U2**, and **U3**, do **not** contain glucose.

..... [1]

- (iii) State how you will use your results in (a)(i) to identify which of **U1**, **U2**, and **U3**, has the highest concentration of glucose.

..... [1]

- (iv) State which of **U1**, **U2**, and **U3** has the highest concentration of glucose.

..... [1]

If the sample with the highest concentration of glucose is more than 0.5%, then this may mean that a kidney is not working.

You are required to estimate the concentration of glucose in the sample stated in (a)(iv) by :

- Preparing 10 cm³ of a 0.5% glucose solution
- Carrying out a Benedict's test on the 0.5% glucose solution
- Using your results to estimate the concentration of glucose in the sample stated in (a)(iv).

- (v) You are provided with a 2% glucose solution, G.

Complete Table 1.1 to describe how G could be diluted to produce 10 cm³ of a 0.5% glucose solution.

Table 1.1

final percentage concentration of glucose	volume of 2% glucose solution / cm ³	volume of distilled water, W / cm ³
0.5		

[1]

- (vi) State one variable that must be standardized when carrying out the Benedict's test, to allow you to make a valid comparison between the results collected in (a)(i) and the result you will collect for the 0.5% glucose solution that you have prepared.

..... [1]

8. Prepare the 0.5% glucose solution as shown in Table 1.1 in the sample tube provided.
9. Repeat the Benedict's test with the 0.5% glucose solution and the sample stated in (a)(iv).
10. Record in (a)(vii) the time taken to the first colour change. After 90s, remove the test-tubes from the water-bath.
If there has been no colour change during the 90s record the time to the first colour change as 'more than 90'.

- (vii) Record your results in an appropriate table.

[2]

- (viii) Use your results from (a)(vii) to complete Table 1.2 by using **one tick (✓)** to show your estimate of the concentration of glucose in the sample stated in (a)(iv).

Table 1.2

percentage concentration of glucose in mock urine sample/%	estimate tick (✓)
below 0.5	
0.5	
above 0.5	

[1]

- (ix) This procedure enabled you to estimate the concentration of glucose in the mock urine sample.

Suggest how you would improve this procedure to find a more accurate estimate of this concentration.

.....

 [3]

- (b) (i) Fig. 1.1 is a photomicrograph of a stained transverse section through an animal organ. This organ is used to transport urine from the kidney to the bladder.

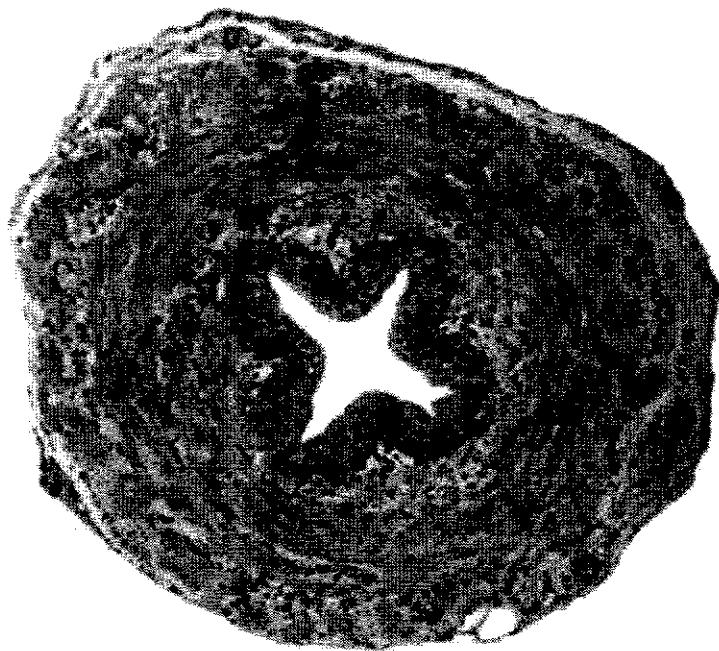


Fig. 1.1

You are not expected to be familiar with this specimen.

Use a sharp pencil for drawing.

Draw a large plan diagram of half of the organ in Fig. 1.1, shown by the shaded area in Fig. 1.2.

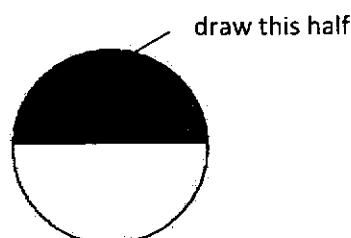


Fig. 1.2

You are expected to draw the correct shape and proportions of the different tissues.

[3]

- (ii) You are provided with a sample slide of a bladder wall showing the transitional epithelium. Fig. 1.3 shows the position of the transitional epithelium.

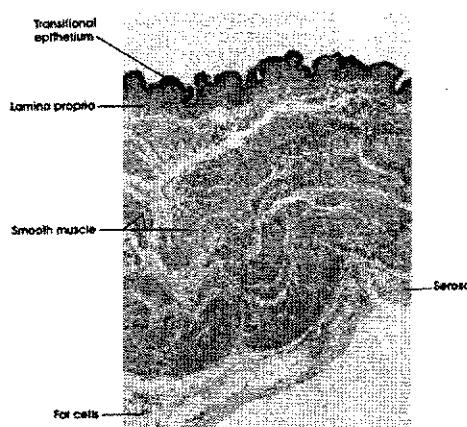


Fig. 1.3

Determine the thickness of the transitional epithelium at high power.

Show your working.

You are not expected to be familiar with this specimen.

[2]

[Total: 20]

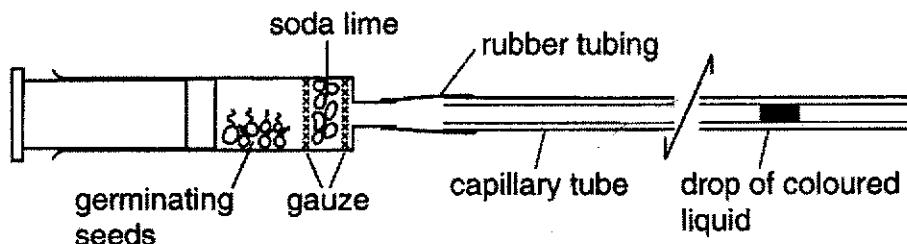
Question 2 starts on page 10

- 2 You are going to investigate the respiration rate in a particular seedling species and relate this to the age of the seedling.

In this experiment the respiration rate of seedlings can be monitored by the rate of absorption of oxygen, carbon dioxide is absorbed by soda lime within the apparatus.

Take care. Do not remove the soda lime from the syringe as it is corrosive and will burn your skin.

Diagram:



1. Remove the plunger from the syringe.
2. Take 3 two-day old seedling, carefully remove and discard its seed coat.
3. Introduce the two-day old seedlings into the syringe between the soda lime and the plunger.
4. Connect the capillary tube to the syringe via the rubber connecting tube.
5. Holding up the whole apparatus, dip the free end of the capillary tube into the vial of coloured water such that a small drop of coloured water is introduced into the end of the capillary tube. (There is no need to pull the plunger of syringe)
6. Wipe excess colored water from outer surface of the capillary tube. The size of the drop of coloured water in the capillary tube is not important as long as it can be seen clearly.
7. Place the respirometer horizontally on the separate piece of graph paper which you have been provided.
8. Leave the seedlings alone for 1 minutes for it to acclimatize to the environment.
9. Adjust the drop of coloured water to the start of the scale (by moving the strip of graph paper)
10. Start a stop watch.
11. Measure the movement of the drop of colored water for 3 minutes.
12. Immediately after you have recorded your results, detach the syringe from the capillary tube by pulling gently from the rubber connecting tube. Using an empty 5 cm³ syringe, pump air through the capillary tube to push out all the coloured water within the capillary tube onto a piece of filter or blotting paper (do not use water to flush the capillary tube).
13. Repeat step 1 to 12 with another set of seedlings of the same age-group.
14. Repeat the experiments with one-day old seedlings.

15. Use the space below to record in standard units of measurement, your results.

[5]

16. Weigh each of the set of seedlings that were used and record their masses.

[2]

12

17. Using your data from step 15 and 16, account for the trends observed.

.....
.....
.....
.....
.....
..... [4]

18. Predict the trend of results, should the experiment be carried out for three-day old seedlings and five-day old seedlings. Explain your predictions. (At five days, seedlings usually start to sprout small leaves)

Three-day old seedlings:

.....
.....
.....
..... [2]

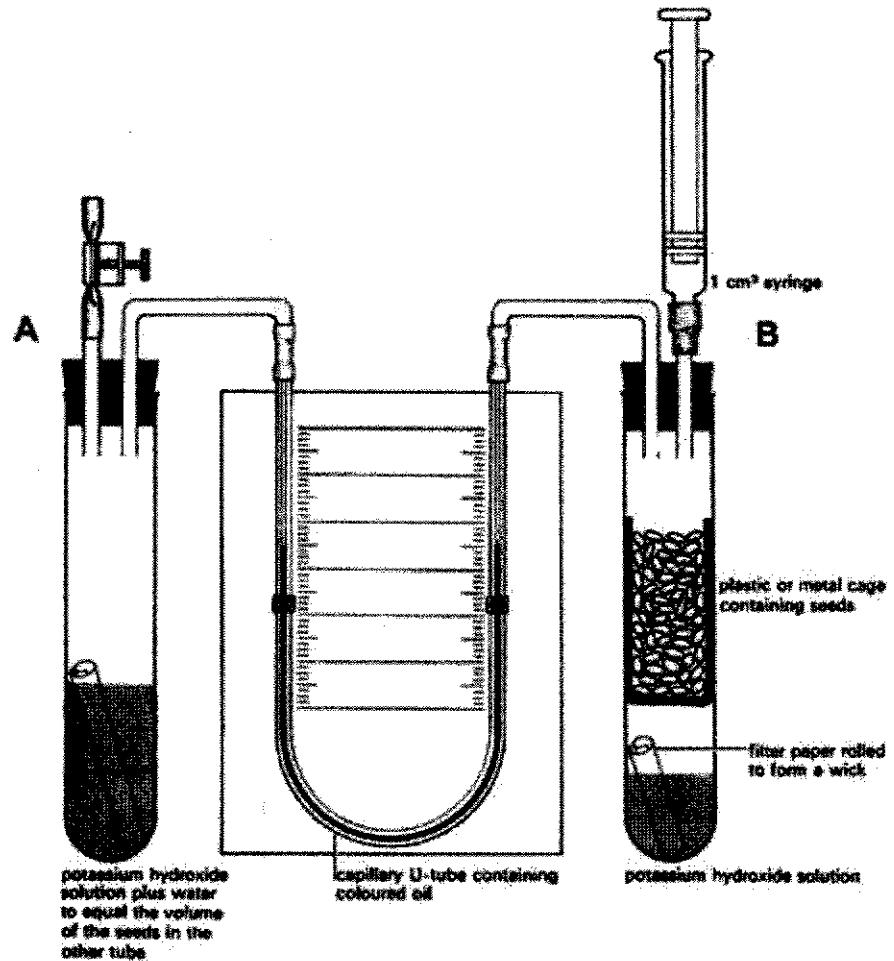
Five-day old seedlings:

.....
.....
.....
..... [2]

19. Identify one source of error in the procedure.

.....
..... [1]

20. A more complex apparatus for measuring respiration is shown below. Glass beads of same mass as the seedlings are added into a metal cage in setup A, before the start of the experiment.



Explain two ways in which this apparatus is significantly more accurate than the one used in this experiment.

.....

.....

.....

.....

.....

.....

[4]

[Total: 20]

Question 3 starts on page 15

- 3 The Java Rhino is one of the rarest animals in the world. It is estimated that only 60 of them are known to exist.

A few of them have been successfully bred in captivity and released in a wildlife sanctuary in Sumatra.

A baby Java Rhino has been found smuggled in one of the shipping containers by the customs officers. Plan an investigation to verify if the Java Rhino was one that was bred in captivity and released to the Sumatran wildlife sanctuary or poached from the wild.

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

- tissue samples (epidermal) from the Java Rhino provided by the customs and a known Java Rhino from the wildlife sanctuary in Sumatra.
- pestle and mortar
- DNA extraction buffer solution
- glass rods
- microcentrifuge tubes
- restriction enzyme
- agarose gel plate
- suitable source of electrical 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 the 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: 15]

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SCHEME OF ASSESSMENT

All candidates are required to enter for Papers 1, 2, 3 and 4.

Paper	Type of Paper	Duration	Mark Allocation (%)	Charts
1	Multiple Choice	1 h	15	30
2	Structured Questions	2 h	30	100
3	Long Structured and Free-response Questions	2 h	35	75
4	Practical	2 h 30 min	20	55

Mark Scheme**Paper 2**

Question	Marking Points	Comments
1 (a) (i)	C : cholesterol [1] D : phospholipid bilayer / membrane phospholipids / lipid bilayer [1]	
1 (a) (ii)	(Any 3 of the following) <ul style="list-style-type: none"> • Increases the hydrophilic characteristics of lipids and proteins [1] • Stabilises the conformation of many membrane proteins [1] • Contribute to cell-cell recognition / cell-cell communication [1] • Contribute to cell-cell adhesion [1] • Contribute to signal transduction [1] • Used as antigens in the body's immune responses [1] • Lubricates and protects the cell membrane from mechanical damage [1] • AVP 	
1 (b)	<ul style="list-style-type: none"> • The amino acids present in the sequence are predominantly non-polar [1] • with hydrophobic side chains / R groups [1]. • They are thus able to interact with the non-polar / hydrophobic fatty acid chains of the membrane phospholipids and span the membrane. [1] 	
1 (c)	<ul style="list-style-type: none"> • Via channel proteins [1] • Hydrophilic molecules do not need to bind to the channel proteins in order to enter a cell. [1] <ul style="list-style-type: none"> • Channel proteins do not need to undergo any conformation change to allow the entry of the hydrophilic molecules into a cell. [1] • Carrier proteins, on the other hand, require the hydrophilic molecules to bind to them before they undergo a conformational change that results in the transport of the hydrophilic molecules into a cell. [1] • Haemoglobin is a respiratory pigment in animal's blood and *carries vital O₂ to the tissues. (compulsory point) Ottwe 	

2	(a)	<ul style="list-style-type: none"> • It consists of four polypeptide chains, *each of which has a specific globular conformation that (compulsory Point) • holds its haem group in a strategic position for binding with O₂. [1] • The four globular chains are held tightly by various types of interactions which *ensures stability of the molecule and efficiency of the four haem groups in binding with O₂. [1] max 3 	
	(b)	<p>(i) • It is determined by primary structure of arrangement of its constituent amino acids i.e No., types, sequence.</p> <ul style="list-style-type: none"> • The intramolecular interactions between amino acid residues by H-bonds, ionic bonds hydrophobic interactions, etc, (mention at least 3) • result in the folding of the chain to form the most stable 3D configuration. • The shape also depends on the prevailing conditions of pH and temperature as changes in pH and temperature affects different intramolecular bonds/interactions and alters the 3-D shape. [any3, max 3] 	
		<p>(ii) • The specific shape/conformation of the protein allows it to fit/bind correctly to the receptor.</p> <ul style="list-style-type: none"> • When the shape of the protein is destroyed by factors such as high pH changes, XDF is unable to pass down signals to the interior of the cell. [2] 	

		destroyed ; Blood insulin level returns to normal ; [1]	[4]	
3	(a)	(i) Euchromatin [1 mark] Heterochromatin [1 mark]		Question was generally well done. Minority could not recall the names accurately.
		(ii) Acetyl groups added to histone tails (lysine); [1] neutralising the positive charge of lysine residues [1] Less interaction between negatively charged DNA [1] histones leading to a looser / less condensed structure. [1]	[4 marks]	Answers were impressive with appropriate keywords showing that the candidates have internalised the concept well. There were still some who were confused with the actual mechanism. Other answers of demethylation were also accepted.
	(b)	In state B, Transcription factors are unable to bind to the DNA due to the high level of condensation, [1] thus the formation of transcription initiation complex will be prevented / lower efficiency, decreasing level of expression. [1] OR In state A, Transcription factors are able to bind to the DNA due to the lower level of condensation, [1] thus TIC forms and transcription can proceed / rate is increased. [1] [2 marks]		Answers were well elaborated. Most are able to understand that access of transcription factors to the promoter is important for initiation.
	(c)	Introns, telomeres, centromeres, untranslated region (UTR) [1 mark each] Introns are spliced out after transcription; this allows for alternative splicing to produce variations of protein from one gene OR some introns may contain control elements that can regulate transcription. [1] Centromeres bind to kinetochores which provide an attachment site for microtubules for the segregation / separation / movement of chromosomes. [1]		Failure to obtain full marks was a result of inability to name at least 3 non-coding region. Most answers hovered around introns and telomeres only. Better

		<p>Telomeres: Prevent nucleases from degrading the ends of the linear DNA molecules. OR Prevent fusion with the ends of other broken chromosomes. OR Although a small amount of the non-coding and repetitive telomeric DNA fails to replicate (and is lost) each time the cell divides, a cell can divide many times before it starts losing essential genetic information (OWTTE) [1]</p> <p>Untranslated region on mRNA provides a binding site for regulatory proteins that regulate the level of translation.</p> <p>2 marks max each for naming region and explaining. [1]</p>	<p>candidates were able to identify the centromere or the genetic sequence coding for UTR on mRNA. Promotor will not be accepted as it was already mentioned in the question.</p>						
4	(a)	<p>(i) A: Chloroplast [1] B: Mitochondria</p> <p>(ii) Similarities :</p> <ul style="list-style-type: none"> - possess double membrane (outer and inner membrane) - possess DNA, ribosomes and enzymes in its matrix/ stroma - possess stalked particles (= ATPase) and electron carriers on their membrane. <p>[any 2; max 2 marks]</p> <p>Differences :</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: center;">Structure of A (Chloroplast)</th> <th style="text-align: center;">Structure of B (mitochondrion)</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">Inner membrane not folded</td> <td style="text-align: center;">Inner membrane folded to form cristae</td> </tr> <tr> <td style="text-align: center;">Presence of internal membrane system ie. grana and intergrana lamellae</td> <td style="text-align: center;">Absence of internal membrane system</td> </tr> </tbody> </table>	Structure of A (Chloroplast)	Structure of B (mitochondrion)	Inner membrane not folded	Inner membrane folded to form cristae	Presence of internal membrane system ie. grana and intergrana lamellae	Absence of internal membrane system	<p>Structures were well identified.</p> <p>It is important to note that chloroplast also has a double membrane, which includes the chloroplast envelope. Some candidates are still not giving appropriate one to one comparison or relevant examples. Misconceptions include thylakoid membrane as a continuation from the inner membrane. Marks were lost for failing to explain with clarity and precision. Yes and no answers will not be accepted.</p>
Structure of A (Chloroplast)	Structure of B (mitochondrion)								
Inner membrane not folded	Inner membrane folded to form cristae								
Presence of internal membrane system ie. grana and intergrana lamellae	Absence of internal membrane system								

	Presence of pigments,	Absence of pigments.							
	Stalked particles (ATPase) and electron carriers are present on thylakoid membrane	Stalk particles (ATPase) and electron carriers are present on the cristae of outer membrane							
	Presence of enzymes for dark reactions in the stroma	Presence of enzymes for Kreb's cycle in the matrix.							
	[Any 2; max 2 marks]								
(b)	(i)	<ul style="list-style-type: none"> • Protein composition inside W is high at 67% [1] • as there are abundant enzymes needed for oxidation of food/ respiration/ Kreb's cycle [1] 	Question was well answered.						
	(ii)	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%;">Y (inner membrane)</td> <td style="width: 50%;">X (outer membrane)</td> </tr> <tr> <td>21% or 15% more than X</td> <td>6% or 15% less than Y</td> </tr> <tr> <td>Because Y is the site of oxidative phosphorylation / ATP synthesis during respiration</td> <td>No ATP synthesis occurs on X</td> </tr> </table>	Y (inner membrane)	X (outer membrane)	21% or 15% more than X	6% or 15% less than Y	Because Y is the site of oxidative phosphorylation / ATP synthesis during respiration	No ATP synthesis occurs on X	Candidates had difficulty coming up with 3 differences. Specifics were not given and marks were not allocated for the point.
Y (inner membrane)	X (outer membrane)								
21% or 15% more than X	6% or 15% less than Y								
Because Y is the site of oxidative phosphorylation / ATP synthesis during respiration	No ATP synthesis occurs on X								

	Thus contains abundant enzyme ATPase (= stalked particles) needed for ATP synthesis	Thus does not contain ATPase (= stalk particles)	
	It also contain transport proteins/ intrinsic proteins to allow diffusion of gases, O ₂ and CO ₂ and	Only contains transport proteins/ intrinsic proteins to allow diffusion of gases, O ₂ and CO ₂ and	
	for entry of raw materials for Kreb's cycle (eg. Pyruvate, Coenzyme A, , ADP, phosphate ions etc.) and exit of water and ATP	for entry of raw materials for Kreb's cycle (eg. Pyruvate, Coenzyme A, , ADP, phosphate ions etc.) and exit of water and ATP	
	Any 3 comparison	[3]	
	(iii) Breath of organelle = 36 mm [1] Formula: Magnification (200x) = size of drawing / actual size ; [1] Actual size = 36 / 200 = 0.18 mm ; [1]	There are still a handful who do not know how to calculate the actual breath using the magnification given. Full marks will also not be awarded if the derivation of the answers was not shown. Some were still unclear how to convert mm to um.	Award one mark for each correct row.
5 (a)	control element	made of protein	binds with a protein
		codes for a protein	

	insulin	✓	(✗)	✗		multiple answers or hybrid ticks (a tick that has been crossed through, so it cannot be judged if it's a tick or a cross)
	cyclic AMP	✗	✓	(✗)		
	<i>lac I</i> gene	✗	(✗)	✓		
	<i>lac O</i> gene	(✗)	✓	✗		
	Homeotic gene product	✓	(✗)	✗		
(b)	RNA Polymerase					3 Must be clear statement 4 Credit: replicates/replicated 5 Must be clear statement 6 Credit: before, mitosis/meiosis/cytokinesis or Credit in S phase (of interphase)
		1. Makes mRNA / tRNA/ rRNA / RNA				
		2. Transcription A: transcribes/transcribed				
		3. One strand (DNA) used / short section used / one strand formed;				
		DNA Polymerase				
		4. DNA replication;				
		5. Semi-conservation / both strands used / whole length used / 2 strands formed;				
		6. Before nuclear/cell division				
		Comments: RNA pol not involved in protein synthesis.				
(c)		1. Lactose binds to repressor protein				1 Do not credit: regulator substance
		2. Changes, shape/structure (of protein)				2 Ignore ref. to active site
		3. Removes it from / stops it from binding to, operator				4 Do not credit: DNA polymerase
		4. RNA polymerase binds to promoter				5 Credit: lactose permease and β -galactosidase ignore gene, switched on/expressed
		5. Idea that (so that Z and Y) are transcribed / mRNA made				

6	(a)	(i)	X: phosphate Y: deoxyribose Z: thymine	Mark the first answer for each letter. If the answer is correct, and an additional answer given is incorrect or contradicts the correct answer, zero marks.	Do not credit PO4 or phosphate backbone/molecule	Do not credit deoxyribose, ignore (pentose) sugar	Do not credit incorrect spelling, ignore (nitrogenous) base / T	IGNORE anything after it becomes clear that a candidate is describing transcription 2 IGNORE straightens DO NOT CREDIT α -helix 3 IGNORE unzips 5 IGNORE in cytoplasm 6 IGNORE A to T / C to G (as given in Q) ACCEPT base pair rule 8 CREDIT formation of
				(ii)	1. Semi conservative (replication)	2. (double) helix, untwists / uncoils / unwinds / unravels;		

	<p>phosphodiester bond</p> <p>9 ACCEPT in context of H bonds forming</p> <p>10 DO NOT CREDIT half old and half new strand</p> <p>11 e.g. correct ref to , (DNA) helicase (in context of unwinding or unzipping) / (DNA) ligase (in context of joining Okazaki fragments or role in backbone formation) / leading or lagging strand / 3' / 5' / antiparallel / activation of free nucleotides / 3 H bonds between C and G / 2 H bonds between</p>	<p>e.g. mRNA only codes for 1 protein</p> <p>DO NOT CREDIT '1 DNA molecule contains <i>all</i> the genes'</p> <p>Note: 'mRNA only codes for 1 protein but DNA codes for</p>
(b)	<p>Idea that only copies one, gene / section / part (of DNA);</p> <p>Idea that DNA comprises many, genes / alleles</p>	

7	(a)	(i)	<p>1. (method to) stimulate/AW, an immune response; A gives immunological memory</p> <p>2. Giving/AW, antigens;</p> <p>3. (method to provide long-term) artificial active immunity;</p> <p>4. One relevant detail; e.g. no ability to cause disease, ref. to harmless / AW, form of pathogen used, (protection through) production of (specific) memory cells, (contains, pathogen/antigen) in an injection or an oral dose</p>	Max 2 many proteins' = 2 marks
		(ii)	<p>1. (disease) caused by, a pathogen/microorganism; A: two of bacteria, virus, fungi, protist, One relevant detail, e.g. Transmissible/communicable/passed from one organism to another/AW; A: spread to others if quantified</p> <p>2. Affecting the normal function of the body/causing ill health</p>	Max 2 R: vector (may not always be transmitted through vectors)
	(b)		<p>1. (number of cases per 100 000) shows, proportion/AW, of population affected; AW</p> <p>2. Idea that easier to visualize the severity of the problem;</p> <p>3. Useful/more reliable, qualified; e.g. for making comparisons between different countries</p> <p>4. (as) countries with larger populations will usually have more cases/higher number of cases may just mean larger population of country;</p> <p>5. Comparative data quote to support;</p>	Max 2 AW
	(c)		<p>1. Infected person, coughs/sneezes/breathes out / AW, droplets;</p> <p>2. Droplets containing bacteria/pathogen/<i>M. tuberculosis</i>;</p> <p>3. Airborne droplets, inhaled/inspired/breathed in (by uninfected person); A: infection/transmission</p> <p>4. Consumption of, meat containing pathogen/<i>M. tuberculosis/M. bovis</i></p>	Max 3
	(d)		<p>1. (HIV/AIDS) leads to) weak immune system/reduced immunity (to disease); 2. Details; e.g. reduced action of phagocytes / Th lymphocytes low in number / B-</p>	Max 2

		lymphocyte response!																	
8	(a)	3. (so TB pathogens) can multiply faster / are not destroyed before they cause disease; 4. Idea that important organs/systems, may already be suffering from consequences of HIV/AIDS (so more likely to stop functioning); 5. Ref. to inactive/dormant/latent, TB more likely to be active	Max 3																
	(b)	1. Both alleles, influence phenotype/are expressed; 2. ref. more than 2 phenotypes possible; 3. phenotype of heterozygote different from either homozygote;	Max 3																
	(c)	1. son receives Y chromosome from father; 2. Y chromosome does not carry hemophilia allele; 3. Father will pass hemophilia allele to daughter(s); 4. Daughter will be, a carrier/heterozygous/ $X^H X^h$; 5. Daughter may pass allele to, her son/his grandson; accept on diagram	Max 3																
	(i)	<table border="1"> <thead> <tr> <th>Parents' phenotype</th> <th>Male, black, non-barred feathers</th> <th>Female, splashed-white, barred feathers</th> </tr> </thead> <tbody> <tr> <td>Parents' genotype</td> <td>$C^B C^A X^a$</td> <td>$C^W C^W X^A Y$</td> </tr> <tr> <td>Gametes/cross</td> <td>$C^B X^a$</td> <td>$C^W X^A$ or $C^W Y$</td> </tr> <tr> <td>Offspring genotypes</td> <td>$C^B C^W X^A X^a$</td> <td>$C^B C^W X^a Y$</td> </tr> <tr> <td>Offspring phenotypes</td> <td>Male, blue, barred</td> <td></td> </tr> </tbody> </table>	Parents' phenotype	Male, black, non-barred feathers	Female, splashed-white, barred feathers	Parents' genotype	$C^B C^A X^a$	$C^W C^W X^A Y$	Gametes/cross	$C^B X^a$	$C^W X^A$ or $C^W Y$	Offspring genotypes	$C^B C^W X^A X^a$	$C^B C^W X^a Y$	Offspring phenotypes	Male, blue, barred		Accept other symbols but only with key If male XY and female XX then mark gametes and offspring genotypes to max 2 If other symbols used but no key, then mark to max 2	
Parents' phenotype	Male, black, non-barred feathers	Female, splashed-white, barred feathers																	
Parents' genotype	$C^B C^A X^a$	$C^W C^W X^A Y$																	
Gametes/cross	$C^B X^a$	$C^W X^A$ or $C^W Y$																	
Offspring genotypes	$C^B C^W X^A X^a$	$C^B C^W X^a Y$																	
Offspring phenotypes	Male, blue, barred																		
	(ii)	1. Blue colour is heterozygous / $C^B C^W$; 2. Test cross 3. With non-barred female; 4. If all offspring <u>barred</u> , must be $XAXA$ / homozygous; 5. If some offspring <u>non-barred</u> , must be $XAXa$ / heterozygous;	Max 3																

Paper 3

Question		Marking Points		Comments
1	(a)	(i)	EcoRI [1] BamHI [1]	
		(ii)	Promoter [1]	Many are able to identify that the promoter is needed for expression. Enhances or Silencers or operators will not be accepted.
		(iii)	<p>Prokaryotes</p> <p>Eukaryotes</p> <p><i>Genes that encode proteins serving the same metabolic pathway are usually <u>clustered</u>.</i></p> <p><i>Genes that encode proteins serving the same metabolic pathway are usually <u>dispersed</u> on different chromosomes.</i></p>	This question is about control in gene expression and not just organization. Answer about the arrangement of chromosome with histones will not be accepted unless it is explicitly stated

		<i>Transcribed as a polycistronic mRNA.</i>	<i>Transcribed as monocistronic mRNAs.</i>	that the compactness will regulate the expression. Other answers were not clear or comparisons were inappropriate.
		<i>Clusters of related genes regulated by a single promoter.</i>	<i>Co-expressed genes are individually transcribed under the control of its own promoter.</i>	
		<i>Absence of post transcriptional machinery, lacks of introns in mRNA and absence of mRNA splicing.</i>	<i>Alternative mRNA splicing could occur which leads to a variety of proteins being translated from the same DNA sequence.</i>	
		<i>RNA polymerase can bind directly to the promoter region and initiate transcription.</i>	<i>Requires binding of general transcription factors prior to the binding of RNA polymerase to initiate gene transcription.</i>	
			Any 3 comparison [3]	
	(iv)	The PCR product will have restriction sites [1] The CDK gene can be inserted into a vector/plasmid/genetic material digested with the same RE sites. [1]		Majority were not able to answer this question. Most answers hover around how primers were useful for the DNA polymerase process.
	(b)	pH changes cause R groups to lose their charges; when H ⁺ ions are lost or gained. [1]		Question was

		Thus there may be a disruption of intramolecular bonds between amino acids (or between amino acids and substrate); leading to a change in the 3D conformation of the enzyme / active site OR active site no longer complementary to substrate. [1] Therefore substrate can no longer bind to active site / binds less efficiently, lower / loss of enzyme activity. [1]	generally well done. However, using keywords like disrupting 3D structure or altering charges were not used.
(c)	(i)	Cytoplasmic enzymes would have hydrophilic (or polar) R groups hydrophilic amino acids on the exterior / surface of the enzyme;[1] membrane-bound enzymes would have some hydrophobic (or non-polar) R groups / amino acids on their exterior / surface. [1]	Candidates must be mindful in explaining the positions of the these amino acids. exterior or interior. Full keywords like amino acid R groups should be used.
	(ii)	Allows membrane bound enzymes to be embedded / remain in (within) the membrane; [1] hydrophobic amino acids would interact with hydrophobic fatty acid tails / hydrocarbon tails of phospholipids. [1]	Question was well answered.
(d)		<ol style="list-style-type: none"> M checkpoint serves to safeguard the integrity of the genome by ensuring that all chromosomes are attached to the spindle tubules in preparation for anaphase [1]. In the event of defective spindle assembly (e.g. presence of unattached kinetochores), the cell cycle will be arrested at metaphase / Transit into anaphase is prevented until all chromosomes are aligned properly at metaphase [1]. The possibility of aneuploidy (abnormal number of chromosomes in daughter cells) occurring is thus ruled out as this could lead to an increase in copy number proto-oncogenes leading to cancer cells formation / prevent over expression of proteins leading to excessive cell growth. [1]. Compulsory *Pt 3. Must be mentioned to explain how development of cancer is prevented. 	Some candidates were not able to identify the correct function of the M check point. Keyword Apoptosis must be stated, as it is important in order for the destruction of cancer cells.
(e)	(i)	As the rate of tubulin removal increases, the speed of movement of chromatids also increases in a constant rate until it reaches a plateau. Max speed of movement of chromatids. [1]	Many failed to identify the plateau

		This suggest that tubulin removal is required to allow separation of sister chromatids during mitosis [1]	This stage. One must clearly elucidate that removal of tubulin is important in separation of chromatids.
	(ii)	During cancer cell division, shortening of microtubules is required to separate the sister chromatids with the kinetochores tubules. OTTWE [1] Presence of taxol prevents shortening of the microtubules/affecting arrangement of chromosomes. [1] This cause the cell cycle to arrest at M phase checkpoint often leading to apoptosis. [2] (Any two)	Question was not well answered as it was not well understood.
	(f)	(i) Chromosomal mutation which involves translocation of c-abl gene of chromosome 9 to the bcr gene of chromosome 22 or vice versa	Most were able to answer this question. Vague answers like chromosomal aberration are not accepted.
		(ii) Proto-oncogene; [1] codes for protein involved in cell signaling; gains function on mutation leading to increased in cell division in Philadelphia chromosome (hybrid gene) [1]	Candidates who had the correct answer were not awarded full marks for using key word gain of function.
	(iii)	Any one of the following: Reject answers that states mentioned chromosomal/DNA translocation: Due to gene amplification of proto-oncogene; leading to production of extra copies of proto-oncogenes, which can lead to increasing copies of proto-oncogene proteins leading to onset of cancer. [1] Point mutations within a control element/promoter/enhancer which controls the proto-oncogene	A handful were still not able to identify the mechanisms behind how the oncogenes will

		may result in an increased expression of the proto-oncogene. [1] Point mutations in the proto-oncogene itself may give rise to a protein product that is more active or more resistant to degradation than the normal protein. E.g. RAS Oncogene. [1]	arise.
	(iv)	Recessive mutation for mutation of tumour suppressor genes [1] loss of function mutation as the normal, non-mutated gene can still synthesise tumour suppressor protein to inhibit cell cycle/to cause apoptosis should DNA damage becomes irreparable. [1] Both genes must be mutated/knocked out for tumour suppressor protein to be non-functional. [1]	Most were able to answer clearly using keywords.
3	(a)	<p>Independent assortment</p> <p>Formation of the spindle apparatus</p> <p>Separation of sister chromatids</p> <p>Formation of nuclear membranes</p> <p>Chromosomes pulled to opposite poles</p>	<p>Metaphase I and metaphase II</p> <p>Prophase I</p> <p>Anaphase II</p> <p>Telophase II</p> <p>Anaphase I</p>
	(b)	<ol style="list-style-type: none"> 1. Halves the chromosome number 2. To produce a haploid nucleus 3. So that at fertilization the (full complement / diploid number) of chromosomes is restored 4. Allows for genetic variation (in gametes) 5. Through independent assortment 6. Through crossing over 	
	(c)	<p>(i)</p> <ol style="list-style-type: none"> 1. Embryonic stem cells 2. Pluripotent <p>(ii)</p> <ol style="list-style-type: none"> 1. Able to differentiate into all cell types except for extra-embryonic tissues. 	

2	(a)	<ol style="list-style-type: none"> 1. Idea that area varies (from 1970 to 2000); 2. Description of a change in 1970s e.g. red areas disappear; 3. Description of a change in 1980s, e.g. red areas increase towards the end; 4. Description of a change in 1990s, e.g. red areas increase to 1995 5. Credit correct manipulation of figures 	<p>1. Do not piece this statement together</p> <p>2. Ignore ref.</p> <p>fluctuations in MP2, 3 and 4</p> <p>4 Accept increases and decreases in 1990s</p>
	(b)	<ol style="list-style-type: none"> 1. Idea that (there were no damaged trees/ there were no beetles/ survey had not started / photographic equipment not available / technology not available / no one realized what "red areas" were / no records kept 	<p>Ignore planes not invented</p>
	(c)	<ol style="list-style-type: none"> 1. Idea that temperature affects (enzyme activity / metabolic reaction) 2. Idea that (growth / reproduction / life cycle) of beetles affected 3. Credit appropriate comment about availability of food in relation to temperature; 4. Credit appropriate comment about numbers of (competitors/predators) 5. Beetles die if conditions very cold 6. Credit appropriate comment about availability of food in relation to lack of water (due to high temperatures) 	<p>1 Accept named metabolic reaction e.g. photosynthesis</p>
	(d)	<ol style="list-style-type: none"> 1. Idea that before 1970 the temperature was low / below mean and there was no 'red area' 2. Idea that before 1970 the drought index was low / below mean and there was no 'red area' 3. Idea that as temperature increases so does the 'red area' 4. Idea that as the drought index increases so does the red area 	<p>NB: If years are quoted they must be sensible</p> <p>A: converse for MP3, 4, 5 and 6</p>
4	(a)	<p>Explain the role of isolating mechanisms in evolution of new species.</p> <ol style="list-style-type: none"> 1. allopatric speciation ; 2. geographical isolation / spatial separation ; 3. e.g. of barrier ; 4. e.g. of organism ; 5. sympatric speciation ; 6. meiosis problems ; 7. polyploidy ; 	<p>Max 15</p> <p>Mp 6 – 11 (max 3)</p> <p>Mp 6 – 11 award additional marks for examples (max 3)</p> <p>1 mark awarded</p>

			for stating both examples of allopatric speciation and sympatric speciation
		<p>8. behavioural;</p> <p>9. temporal;</p> <p>10. ecological;</p> <p>11. structural isolation ;</p> <p>12. examples for mp 6 - 12</p> <p>13. (isolated) populations, prevented from interbreeding / can only breed amongst themselves ;</p> <p>14. no, gene flow / gene mixing, (between populations) ;</p> <p>15. different selection pressures operate ;</p> <p>16. natural selection ;</p> <p>17. change in allele frequencies ;</p> <p>18. resulting in different gene pool ;</p> <p>19. over time <u>differences</u> prevent interbreeding ;</p> <p>20. becoming reproductively isolated ;</p>	Max 6 for gene mutation
(b)		<p>Explain, using named examples, how mutation can affect phenotype.</p> <p>1. (gene) example ; (sickle cell / PKU)</p> <p>2. change in gene / DNA / base change ;</p> <p>3. different amino acid ;</p> <p>4. different polypeptide / different protein / non-functional protein ;</p> <p>5. AVP ; details</p> <p>6. AVP ; details</p> <p>7. (chromosome) example ; (Down's, Turner's syndromes) structural</p> <p>8. changes in chromosomes ;</p> <p>9. change in number of chromosomes ;</p> <p>10. change in sets of chromosomes / ref. polyploidy ;</p> <p>11. AVP ; details</p> <p>12. AVP ; details</p>	Max 4 for chromosomal mutation 1 mark awarded for stating genetic AND chromosomal mutation example

S	<p>(a) Describe how non-cyclic photophosphorylation produces ATP and reduced NADP, and outline the steps of the Calvin cycle.</p> <p>Max 15</p> <p>1 mark awarded for proper paragraphing</p> <p>1. photosystem I (PI) and photosystem II (PII) involved ; light harvesting clusters ; 2. light absorbed by accessory pigments ; 3. primary pigment is chlorophyll a ; 4. energy passed to, primary pigment / chlorophyll a ; 5. electrons, excited / raised to higher energy level ; 6. (electrons) taken up by electron acceptor ; 7. (electrons) pass down electron carrier chain (to produce ATP) ; 8. PII has (water splitting) enzyme ; 9. water split into protons, electrons and oxygen ; 10. An equation photolysis ; 11. electrons from PII pass to PI / electrons from water pass to PII ; 12. to replace those lost ; give either in relation to PI or PII 13. protons and electrons combine with NADP (to produce reduced NADP) ;</p> <p>Max 10</p> <p>21. RuBP combines with carbon dioxide + rubisco ;</p>

P4 - Practical Paper

Question	Answer	Marks
1(a)(i)	<ol style="list-style-type: none"> 1. Heading for independent variable – sample / mock urine / solution; 2. Time to first colour change / seconds; 3. Colours and time for U1 (0.3%), U2 (5.0%), and U3 (0.0%); 4. Records whole numbers only; 	4
1(a)(ii)	Interprets correct answer from results; - U3	1

1(a)(iii)	Mock urine sample with shortest time to first colour change;	1
1(a)(iv)	Interprets correct answer from results; - U2	1
1(a)(v)	Volume of 2% glucose is 2.5 cm^3 and total volume of glucose concentration is 10.0 cm^3	1
1(a)(vi)	Interprets correct answer from results;	1
1(a)(vii)	Time for 0.5% glucose; Time for mock urine sample stated in (a)(iv);	2
1(a)(viii)	Tick in correct place based on candidates results;	1
1(a)(ix)	<ol style="list-style-type: none"> 1. Use more concentrations; 2. Named concentrations in narrower range / described method of dilution of 2% glucose solution; 3. Plot graph and read off graph; 	3
1(b)(i)	<ol style="list-style-type: none"> 1. Minimum size + no shading + no cells; 2. At least 2 layers; 	3

3.	Half star shape of central lumen;											
1(b)(ii)	<p>1. Show working highlight eye piece graticule unit and micrometer length</p> <p>2. Correct answer</p>	2	<p>Age of seedlings (days) Rate of movement of coloured water (mm / 3min)</p> <table> <thead> <tr> <th></th> <th>Seedling 1</th> <th>Seedling 2</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>15 to 27 divisions on graph paper = 30 to 54 mm / 3min</td> <td></td> </tr> <tr> <td>2</td> <td>33 to 45 divisions on graph paper = 66 to 90 mm / 3min</td> <td></td> </tr> </tbody> </table> <p>1 mark for table with headings with appropriate units; i.e. recorded in rate i.e. /3min</p> <p>1 mark for all the 4 readings (regardless of precision),</p> <p>1 mark for correct precision (2 points of estimation, start and end point, thus precision is to the nearest division only)</p> <p>1 mark for converting divisions on graph paper to standard units: (either mm or cm);</p> <p>1 mark for trend. either higher or lower.</p>		Seedling 1	Seedling 2	1	15 to 27 divisions on graph paper = 30 to 54 mm / 3min		2	33 to 45 divisions on graph paper = 66 to 90 mm / 3min	
	Seedling 1	Seedling 2										
1	15 to 27 divisions on graph paper = 30 to 54 mm / 3min											
2	33 to 45 divisions on graph paper = 66 to 90 mm / 3min											

(16)	<p>1 mark in total for 4 set of masses 1 mark for correct precision: to 1 decimal places (according to the electronic weighing balance)</p>
(17)	<ul style="list-style-type: none"> • Two-day old seedlings show a higher rate of movement of ink than one-day old seedlings • Distance moved by coloured ink is directly proportional to volume of oxygen absorbed for respiration and thus is an indication of the rate of respiration, which is higher in two day old seedlings. • Two-day old seedlings also have a higher mass than one-day old seedlings • There are more respiring cells in two-day old seedlings than one-day old seedlings <p>OR</p> <ul style="list-style-type: none"> • Two-day old seedlings show a lower rate of movement of ink than one-day old seedlings, even though more respiring cells. • Because the stored sugar left in the seed is less at 2 days than at 1 day / Additional point given to: any observation of respiration rate per unit mass of each age group of seedlings...i.e respiration per unit mass may be higher in two day old than one day old • Two-day old seedlings also have a higher mass than one-day old seedlings • There are more respiring cells in two-day old seedlings than one-day old seedlings <p>1 mark each</p>

<p>(18)</p> <p>Three-day old seedlings</p> <ul style="list-style-type: none"> • Three-day old seedlings show a higher rate of respiration than both two-day old and one-day old seedlings • Cell division results in increase in number of respiring cells in the seedling (award once only for either three-day / five-day old seedlings) <p>Five-day old seedlings</p> <ul style="list-style-type: none"> • Five-day old seedlings show a higher rate of respiration than younger seedlings • However, rate of movement of ink may not be as great due to oxygen released as a result of photosynthesis by leaves <p>1 mark each</p>	<p>(19)</p> <p>Respiration rate of different seedlings of the same age may vary and only 2 seedlings from each age group were tested.</p> <p>External air temperature may fluctuate / handling of syringe causing internal air temperature to fluctuate / pressure changes inside and outside the syringe – affect the movement of the coloured water</p> <p>Length of drop of coloured water may affect the rate of movement</p> <p>Graph paper used as scale is not precise enough</p> <p>1 mark</p>
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- (20) • Movement of coloured liquid due to pressure / temperature changes can be eliminated
 • Both the tubes will experience the same temperature and pressure changes
- OR
- More seedlings of the same age respiring in the apparatus
 • Thus movement of the coloured fluid will be more significant than for just 3 seedling / reduces % error as compared to using on 1 seedling
- OR
- Calibrated scale is more precise than graph paper used thus allowing us to make more accurate measurements
 • Graph paper's precision is 2mm per division
- OR
- Tube B acts as a control tube
 • Tube B has an equal mass of glass beads that do not respire, and subject to the same experimental conditions, the effect of environmental conditions on both tubes would cancel each other out / movement of coloured water in U shaped tube only due to respiration of seedlings and no other factors
- OR
- Apparatus is a closed system
 • Therefore movement of fluid not affected by external air pressure changes

<p>OR</p> <ul style="list-style-type: none"> • The coloured fluid can be reset in this apparatus (by pushing down on the syringe in tube A whilst opening tap in tube B) • This eliminates the error from leftover ink within the capillary tube that cannot be removed <p style="text-align: center;">2 mark for each example with explanation</p>	<p>Mark Scheme for Planning</p> <p>[Rationale]: - principle of method</p> <p>Differences in DNA sequences / Short Tandem Repeats among different individuals results in differences in the number of restriction site locations within the DNA molecules. [1]</p> <p>This generates different number and length of the restriction fragments in the restriction enzyme digestion outcome. [1]</p> <p>Therefore, by running gel electrophoresis, we can perform comparison of the DNA banding patterns of the Java Rhino specimen confiscated with those of the wildlife sanctuary. [1]</p> <p>[Procedure]</p> <p>Step 1 – 10: extraction and purification of DNA</p>
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- | | |
|--|--|
| 1. | Remove some cells from the confiscated sample and take extra care when removing cells to prevent damage to the specimen. [1] |
| 2. | Homogenise and Lyse the cells with DNA extraction buffer. [1] |
| 3. | The microfuge tube containing the lysate is then subjected to centrifugation to remove the cell debris. [1] |
| 4. | Transfer the supernatant containing DNA to a new microfuge tube and add RNase buffer and protein precipitation buffer to precipitate out RNA and protein respectively. [1] |
| 5. | Centrifuge the tube to remove the RNA and protein precipitate. |
| 6. | Store the supernatant containing the dodo specimen's DNA in a microfuge tube. |
| 7. | To purify, transfer the confiscated specimen DNA into an affinity chromatography column. [DNA affinity chromatography will cause DNA molecules to stick to the column] |
| 8. | Add washing buffer into the column and centrifuge to remove impurities. |
| 9. | Subsequently, add elution buffer into the column and centrifuge to elute (means to cause detachment of the DNA from the column) out the purified DNA into a microfuge tube and store it. |
| 10. | DNA from a few of the Sumatra wildlife sanctuary are also obtained through the same process. [1] |
| Step 11- 17: Amplification of DNA using PCR | |
| 11. | The purified DNAs from both specimens are subjected to Polymerase Chain Reaction (PCR) to amplify a particular region of the DNA using Taq polymerase. |

12. One PCR cycle consists of 3 steps: Heating & Denaturation of DNA strands (93°C), Cooling & Annealing of Primer to DNA strands (55°C) and Replication & Extension of Primer (72°C)
13. DNA mixture is heated to 93°C to separate the double-stranded DNA into 2 complementary single-stranded DNA. This is done by breaking the hydrogen bonds holding the strands together.
14. The DNA mixture is subsequently cooled to 55°C to allow complementary primers to hybridize with / anneal to 3' ends of single-stranded DNA template
15. Primers provide the free 3'OH required by Taq polymerase to add deoxy ribonucleotides to the 3' end of the primer.
16. Replication and extension of the DNA in 5' to 3' direction occurs rapidly at 72°C
17. Each cycle is repeated 30-40 times. Each time the cycle is repeated, the amount of DNA is doubled. Cycle is repeated to yield exponential increase of target DNA sequences as product.
- [1] mark for brief description of PCR including process and temperature.
- Step 18 - 24 : Restriction digest followed by gel electrophoresis
18. A suitable restriction enzyme is chosen based on the presence of the appropriate restriction sites on the DNA samples, as well as the ability to produce distinct and differentiating number and length of restriction fragments between the specimens. [1]

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| <p>19. The amplified DNA fragments from both specimens are then subjected to separate Restriction enzyme digestion, using the same restriction enzyme, to yield restriction fragments. [1]</p> <p>20. The various restriction fragments are loaded into the different wells of an agarose gel and gel electrophoresis is carried to separate the DNA fragments. [1]</p> | <p>21. The agarose gel is a cross-linked matrix and functions as a 'molecular sieve' where the matrix forms little pores (holes) through which DNA can travel through.</p> <p>22. The negatively charged DNA molecules will migrate toward the positive end of the field (anode). [1] from 22. to 24.</p> <p>23. The DNA molecules are pulled towards the positive end by the current, but are separated according to their molecular size.</p> <p>24. The smaller molecules are able to move through the agarose gel faster than the larger one, so they will travel further down the gel than the larger molecules.</p> | <p>Step 25 - 30: Southern Blot</p> <p>25. Once gel electrophoresis is completed, the restriction fragments in the agarose gel are transferred to a piece of nitrocellulose membrane by capillary action (Southern blotting).</p> <p>26. The nitrocellulose membrane will contain DNA fragments in a pattern that is a replica of the agarose gel.</p> <p>27. The DNA in the membrane is also denatured into single-strands by adding alkaline solution (NaOH). This causes the hydrogen bonds between the complementary base pairs of double helix to break. [1]</p> <p>28. The membrane is then incubated with multiple radioactively labeled DNA probes which is</p> |
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- complementary to the target sequences. [1]
- 29. The hybridized probe is detected by autoradiography. Bands containing the DNA that hybridize with probe can be visualized by laying a sheet of photographic film over the nitrocellulose filter. [1]
 - 30. Exposure to X-ray will yield an image corresponding to specific radioactively labeled DNA bands. [1]

[Result analysis]

- The banding patterns between the specimens are compared
 - If the banding patterns of the confiscated rhino specimen are similar with that of the wildlife sanctuary, then it can be concluded that there is high possibility it came from the wildlife sanctuary.
 - If the banding patterns are different, then it is not.

[1]

[Safety precautions]

- Electrical appliances – handle with dry hands and switch off the power when not in use. Clean up any spillage near the equipment immediately.
- Radioactive probes – wear protective clothes and gloves when handling radioactive substances
 - Alkaline solution (NaOH) – irritant and corrosive to skin and respiratory system. Avoid direct contact with skin and mouth. Rinse thoroughly with lots of water when contact occurs.