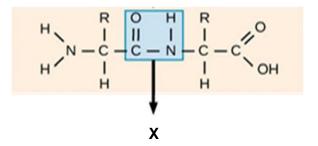
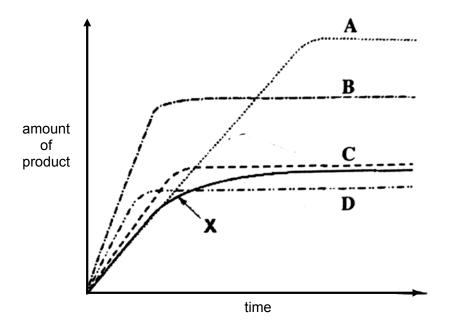
1. The diagram shows a molecular structure.



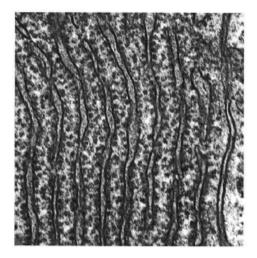
What is enclosed by the box X?

- A Phosphodiester bond
- **B** Glycosidic bond
- **C** Ester bond
- **D** Peptide bond
- 2. Line X shows the activity of an enzyme at 20°C. Lines A to D show the effect of different conditions on the activity of the enzyme.

Which line shows the effect of increasing the temperature by 10°C and adding extra substrate?

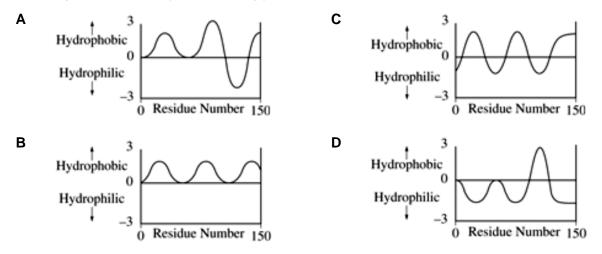


3. The electron micrograph shows part of an organelle in a cell.

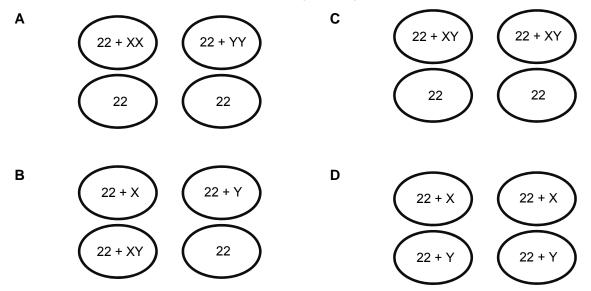


What describes a function of the cisternae in the organelle shown?

- A Moving protein to places where they are covered by phospholipid membranes for secretion outside the cell
- **B** Producing proteins and covering them with phospholipid membranes for secretion outside the cell
- **C** Producing proteins, covering them with phospholipid membranes and moving them for use inside the cell
- **D** Producing ribosomes and proteins and storing them in phospholipid membranes for use inside the cell
- 4. Glycophorin, an integral membrane protein, has a single transmembrane α helix. Which of the following plots most likely represents glycophorin?



5. A human cell with 44 autosomes, and sex chromosomes X and Y, suffers a non-disjunction at the first meiotic division. Which of the following set of gametes could result?

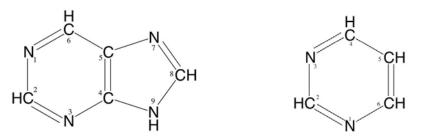


6. The amount of DNA present in the nucleus of a cell at the beginning of interphase is x picograms.

Which of the following combinations reflects the amount of DNA, in picograms, in the nucleus during the various stages of cell division?

	End of interphase	End of mitosis	End of meiosis I	Anaphase II
Α	x	1⁄2X	½X	½X
в	x	1⁄2X	½X	1⁄4X
с	2x	x	x	x
D	2x	X	x	½X

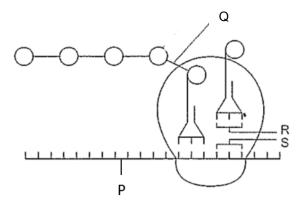
7. The structures of purine and pyrimidine are shown below.



Which of the following correctly shows the number of carbon atoms in the corresponding nucleic acid?

	Molecule	Number of carbons in the molecule
Α	DNA strand with the sequence ATCGAAA	33
в	mRNA molecule with the sequence AUCGAAA on 1 strand	30
с	DNA molecule with the sequence ATCGAAA on 1 strand	33
D	DNA strand with the sequence ATCGAAA	68

8. The diagram below shows the process of translation in a prokaryotic cell.



Which of the following correctly identifies the bonds?

	Р	Q	Between R and S
Α	Peptide	Phosphodiester	Hydrogen
в	Hydrogen	Disulfide	Phosphodiester
С	Phosphodiester	Peptide	Hydrogen
D	Hydrogen	Hydrogen	Peptide

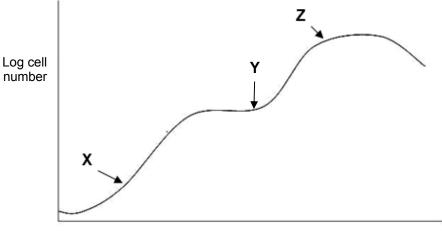
9. The active messenger RNAs (active mRNAs) in tissue cells can be isolated by passing the homogenised cell contents through a fractionating column. The column has short lengths of uracil nucleotides attached to a solid supporting material. Molecules of mRNA that can pass through the column are quickly broken up into small pieces and cannot be translated.

The active mRNAs that attach to the column can be collected subsequently by an appropriate treatment.

Which statements correctly describe active mRNA?

- 1 Active mRNAs are held to the fractionating column by bonds between adenine and uracil bases.
- 2 Active mRNAs can be released from the fractionating column by breaking hydrogen bonds.
- 3 Only mRNAs with polyadenine tailing can be translated.
- 4 Polyadenine tailing stabilises mRNA and prevents it from being broken up.
- **A** 1 and 2
- **B** 1, 2 and 3
- **C** 3 and 4
- **D** 1, 2, 3 and 4
- **10.** A hybrid phage was artificially created by removing the DNA from lambda phage and replacing it with DNA from T4 phage. This hybrid was allowed to infect a bacterium and reproduce. The progeny of the hybrid phage will have the characteristics of a ______.
 - A T4 phage
 - B lambda phage
 - **C** hybrid phage with T4 DNA and lambda proteins
 - **D** hybrid virus with lambda DNA and T4 protein

11. *E. coli* bacteria are grown in a culture of nutrients, which includes glucose and lactose as the main source of carbon-based nutrient. The following growth curve is obtained.



Time

Which of the following corresponds correctly to the region specified on the growth curve of *E. coli*?

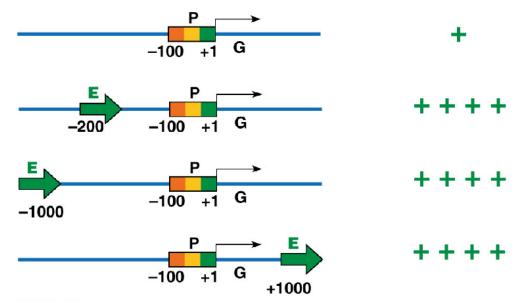
	CAP activated	High amounts of <i>lac</i> polycistronic mRNA	Repressor inactivated
Α	X only	X and Y only	Y and Z only
в	Y only	Y and Z only	X, Y and Z
с	Y and Z only	Z only	Y and Z only
D	Y and Z only	Y and Z only	X, Y and Z

12. Which feature occurs in the life cycle of the influenza virus?

- A Host cell DNA is destroyed by lytic enzymes.
- **B** Viral genome is integrated into the host genome.
- **C** Viral DNA acts as a template for DNA synthesis.
- **D** Viruses enter the host cell by receptor-mediated endocytosis.

- **13.** Which of the following statements describe possible ways by which viruses can cause disease in animals?
 - I They inhibit normal host cell DNA, RNA or protein synthesis in host cell.
 - II They disrupt and inactivate the tumour suppressor genes of the host cell causing uncontrolled cell division.
 - III They disrupt and inactivate the oncogenes of the host cell causing uncontrolled cell division.
 - IV Their viral proteins and glycoproteins on the surface membrane of host cells cause them to be recognised and destroyed by the body's immune system.
 - V They deplete the host cell of cellular materials essential for metabolic functions.
 - A I, II and V
 - **B** I, II and IV
 - **C** II, III and V
 - **D** I, II, IV and V

14. Recombinant DNA techniques can be used to alter the locations of control elements within DNA sequences, so as to study the effects of these changes on the levels of transcription. The diagram shows the various structures of transcription units and the corresponding relative levels of transcription. The promoter, enhancer and coding sequences are represented by letters P, E and G respectively. The number of symbol '+' indicates the relative frequency of transcription.



With reference to the diagram, which of these statements is a valid conclusion?

- **A** The relative distance between promoter and enhancer has no effect on the frequency of transcription.
- **B** The frequency of transcription is increased when the enhancer is located upstream of the promoter.
- **C** An enhancer is required for transcription.
- **D** Orientation of the enhancer does not affect the frequency of transcription.
- **15.** A geneticist determines that a particular human disease is caused by a gene mutation. The mutant allele contains a substitution of cytosine to adenine at position 334. The DNA sequence for bases 301 to 351 from the non-template strand of the normal allele is shown.

5'- ATG TTA CGA GGT ATC ATA CGA ACG GAG CGC GAA CTA GTT ACT CCC ATA AAA - 3'

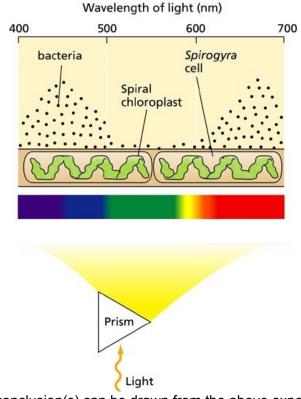
Which of the following statements best describes a consequence of this mutation?

- **A** A nonsense mutation has occurred resulting in no protein product being formed.
- **B** The mutant protein contains fewer amino acids than the normal protein.
- **C** A missense mutation has occurred resulting in a non-functional protein.
- **D** There is no change in length of amino acid sequence due to the mutation occurring outside of the coding region.

16. In 1882, the German botanist T.W. Engelmann performed an ingenious experiment to investigate the effects of different wavelengths of light on the rate of photosynthesis for a suspension of alga *Spirogyra* (a filamentous microorganism containing long, spiral chloroplasts).

In his experiment, a prism was placed between the light source and the alga filament to produce and scatter all colours of the light across the alga filament evenly. Then, aerobic bacteria were added to the alga filament suspension. All the other variables were kept constant.

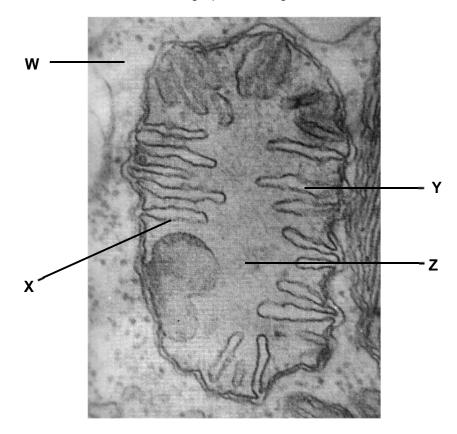
After exposure to light for a certain period of time, the bacteria were found to move towards and accumulate at specific lengths along the alga filament as shown below.



Which of the following conclusion(s) can be drawn from the above experiment.

- I The directional movement of the bacteria is due to oxygen released from *Spirogyra*.
- II Green light is least absorbed, whereas red and blue wavelength of light is efficiently used for photosynthesis.
- III NADPH is the reducing power that drives the formation of glyceraldehyde-3-phosphate.
- A II only
- B I and II only
- C II and III only
- **D** All of the above

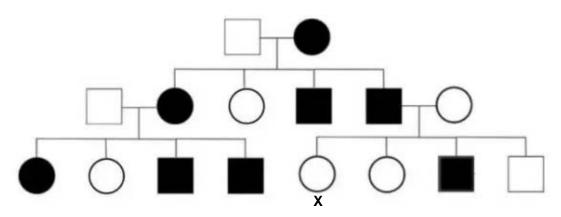
17. The figure below shows an electron micrograph of an organelle.



Which of the following correctly matches the processes with the corresponding structures?

	Formation of pyruvate	Oxidative phosphorylation	Direction of diffusion of H ⁺ ions	Formation of reduced co-enzymes
Α	W	Х	$Z \rightarrow Y$	Z
в	Z	Y	$Z \rightarrow Y$	W and Z
с	Z	Y	$Y \rightarrow Z$	W
D	W	Х	$Y \rightarrow Z$	W and Z

18. The pedigree below shows the inheritance of Marfan syndrome which affects connective tissues in the body.



Individual X is homozygous at the loci for the disease gene. What is the genetic basis of inheritance of the disease?

- A Autosomal dominant
- B Autosomal recessive
- C Sex-linked dominant
- D Sex-linked recessive

19. Coat colour in mice is controlled by two genes, each with two alleles. The genes are on different chromosomes.

One gene controls pigment colour. The presence of allele **A** results in a yellow and black banding pattern on individual hairs, producing an overall grey appearance called agouti. Mice with the genotype **aa** do not make the yellow pigment and are, therefore, black.

The other gene determines whether any pigment is produced. The allele D is required for development of coat colour. Mice with the genotype **dd** produce no pigment and are called albino.

An albino mouse is mated with a black mouse to produce 12 albino mice, 7 agouti mice and 5 black mice.

A χ^2 test was performed to test the significance of the difference between the observed and expected results.

distribution of χ^2				
number of degrees of	probability			
freedom (v)	0.1	0.05	0.01	
1	2.71	3.84	6.64	
2	4.60	5.99	9.21	
3	6.25	7.82	11.34	
4	7.78	9.49	13.28	

$$\chi^{2} = \sum \frac{(O - E)^{2}}{E}$$
O = observed result
E = expected result
y = p-1

Using the equation and the table of χ^2 values, which of the following correctly describes the result of the χ^2 test?

	number of degrees of freedom (v)	probability	significance of difference between observed and expected results
Α	2	< 0.05	significant
в	3	< 0.05	significant
С	2	> 0.05	not significant
D	3	> 0.05	not significant

20. Pure breeding plants of contrasting traits were cross fertilised and the seeds were planted in pots of soil containing equal proportion of fertiliser. The pots were then exposed to different light conditions for 60 days. Throughout the investigation, the plants were watered with equal amount of water, twice daily.

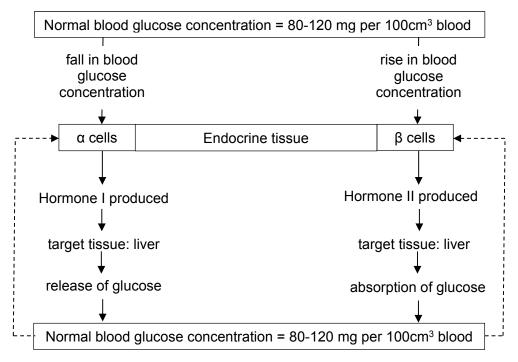
At the end of the investigation, the plants' height, number of leaves, length of leaves and colour of leaves were measured and summarised in the table below.

	No light	Dim Light	Bright light
Height/cm	10.3 ± 0.3	8.1± 0.5	6.6 ± 0.4
Length of leaves/cm	1.7 ± 0.3	1.7 <u>±</u> 0.2	1.6± 0.1
Colour of leaves	Yellow	Pale green	Dark green

Which of the following statement(s) cannot be explained by the data?

- 1 The additive effect of genes is responsible for the continuous variation observed in the height and length of leaves.
- 2 The genes involved in chlorophyll pigment synthesis are activated by light.
- 3 Leaf colour is controlled by a gene locus whereby heterozygotes have pale green leaves.
- A 2 only
- B 3 only
- **C** 1 and 3 only
- D 2 and 3 only
- **21.** Totipotency is demonstrated when _____.
 - A cancer cells give rise to heterogeneous cell types
 - **B** a stem cell can differentiate into placental cells and all cells in an organism
 - **C** a hematopoietic stem cell differentiates into a lymphocyte
 - **D** an embryonic stem cell divides and differentiates

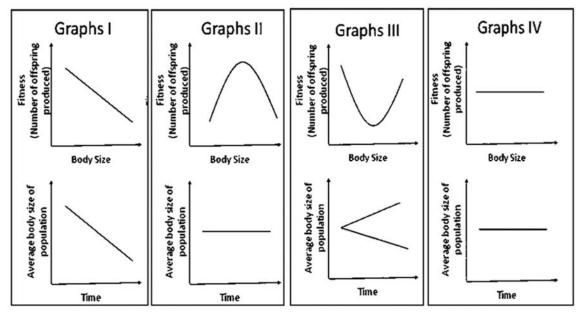
22. The diagram below shows the role of an endocrine tissue in controlling blood glucose concentration.



Which of the following statements are true?

- 1 Small amounts of hormone I and II inducing a large response in liver tissue demonstrates positive feedback.
- 2 Hormones I and II inducing different responses from the same target tissue is due to the hormones binding to different receptors on the liver cell surface membrane.
- 3 The binding of hormone I to receptors on liver cell surface membrane leads to the production of second messenger, cAMP.
- 4 Besides the liver, hormone I will also target muscle tissue to regulate blood glucose concentration.
- A 1 and 2
- **B** 2 and 3
- **C** 3 and 4
- **D** 2 and 4

- **23.** Which of the following statements is false about cell signalling involving tyrosine kinase receptors?
 - A Ligand molecules are mostly hydrophilic in nature.
 - **B** Different activated relay proteins serve to directly amplify the effects of the ligand.
 - **C** Dimerisation serves to initiate auto-phosphorylation.
 - **D** Receptors are transmembrane proteins that are anchored within the cell surface membrane.
- 24. The different forms of natural selection can be distinguished according to their effect on the body size of the pink salmon (*Onchorhynchus gorbuscha*).



Which of the following describes the correct form of natural selection for each of the following sets of graphs?

	Graphs I	Graphs II	Graphs III	Graphs IV
Α	Disruptive selection	Directional selection	Stabilising selection	No selection
в	No selection	Stabilising selection	Directional selection	Disruptive selection
с	Directional selection	Stabilising selection	Disruptive selection	No selection
D	Directional selection	Disruptive selection	No selection	Stabilising selection

25. Bacteria in the genus *Wolbachia* infect many butterfly species. They are passed from one generation to the next in eggs, but not in sperms, and they selectively kill developing male embryos.

During the 1960s in Samoa, the proportion of male blue moon butterflies fell to less than 1% of the population. However, by 2006, the proportion of males was almost 50% of the population.

Resistance to Wolbachia is the result of the dominant allele of a suppressor gene.

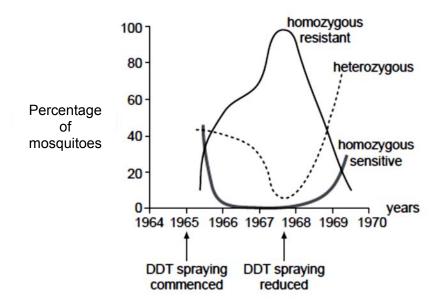
Which statements correctly describe the evolution of resistance to *Wolbachia* in the blue moon butterfly population?

- I Wolbachia acts as a selective agent.
- II The selective killing of male embryos is an example of artificial selection.
- III When infected with *Wolbachia*, male embryos that are homozygous for the recessive allele of the suppressor gene die.
- IV All male embryos that carry the dominant allele of the suppressor gene pass that allele to their offspring.
- V The frequency of the dominant allele of the suppressor gene rises in the butterfly population.
- A I and IV
- B II and III
- **C** I, III and V
- **D** II, IV and V

26. In the mid-1960s, DDT was widely used as an insecticide against mosquitoes. The sensitivity to insecticide in mosquitoes is determined by a single gene that has two alleles.

allele 1 : resistant to DDT allele 2 : sensitive to DDT

Over several years, genotypic frequencies were measured in a population of mosquito larvae. The graph below shows the results.



Analysis of the graph reveals that in the population, _

- A allele 1 confers a selective disadvantage in the absence of DDT.
- **B** heterozygote advantage is demonstrated after DDT spraying is reduced.
- **C** mutant allele 1 emerged as a result of the use of DDT in 1965.
- **D** only one copy of allele 1 is required for resistant phenotype.

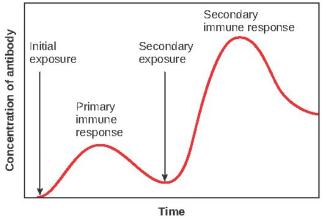
27. The diagram below shows the structure of an antibody.



Which of the following correctly matches the events with the regions in which diversity is generated?

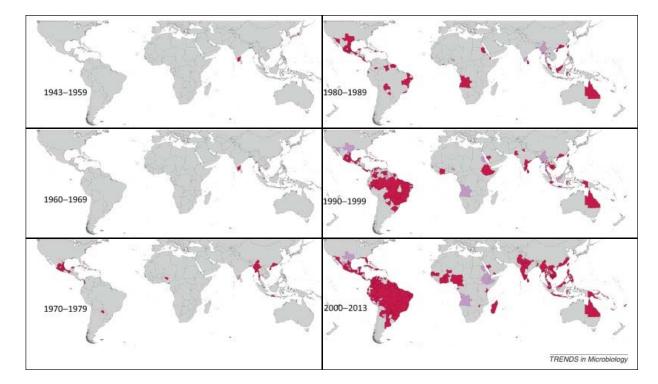
	Somatic recombination	Somatic hypermutation	Class switching
Α	Х	Х	Z
в	Y	Х	Z
С	Y	Y	Z
D	Z	Х	Y

28. The diagram below shows the antibody production during a primary and a secondary immune response.



Which of the following statement(s) is/are correct?

- I Class switching only occurs during the secondary immune response.
- II The secondary immune response is faster and stronger compared to the primary immune response.
- III Class switching results in the production of antibodies with higher binding affinity during the secondary immune response.
- IV Vaccination 'primes' the immune system such that a secondary immune response can be mounted when the body encounters the actual pathogen.
- A II only
- B II and IV only
- **C** I, II and IV only
- D All of the above
- 29. Which of the following describes a positive feedback concerning climate change?
 - A Increased atmospheric temperature result in melting of sea ice which decreases the amount of sunlight reflected back into space.
 - **B** Increased burning of fossil fuels increases atmospheric CO₂ concentration, enhancing the greenhouse effect.
 - **C** Melting of glaciers causes an increase in sea levels.
 - **D** Increase in atmospheric temperature causes many species to move towards increased altitudes to stay within their optimum temperature range.



30. The diagram below shows the distribution of confirmed cases of dengue fever from 1943 to 2013.

Which of the following explain the observed changes in distribution?

- I Increased global human traffic.
- II Increased global temperatures allow mosquitoes to survive better at increased latitudes.
- III Increased global temperatures allow mosquitoes carrying the dengue virus to move northwards.
- IV Increased global temperatures increases the replication rate of the dengue virus in mosquitoes.
- V Increased global temperatures reduces the replication rate of the dengue virus in humans.
- A II and V only
- **B** II, III and IV only
- **C** I, II and IV only
- **D** All of the above

End of Paper -

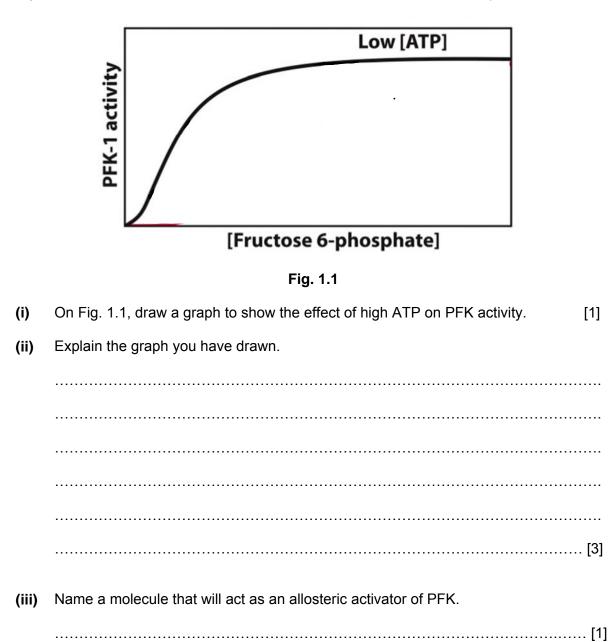
Answers:

1.	D	7.	D	13.	D	19.	С	25.	С
2.	В	8.	С	14.	Α	20.	С	26.	Α
3.	Α	9.	D	15.	С	21.	В	27.	Α
4.	D	10.	Α	16.	В	22.	В	28.	В
5.	С	11.	D	17.	D	23.	В	29.	Α
6.	С	12.	D	18.	Α	24.	С	30.	С

Answer **all** questions.

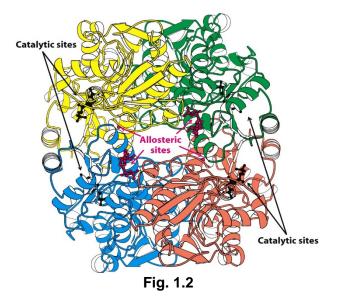
1 Phosphofructokinase (PFK) is an allosteric enzyme made of 4 subunits and controlled by many activators and inhibitors which regulate glycolysis. PFK phosphorylates fructose-6-phosphate to form fructose-1,6-bisphosphate. This enables the cell to increase or decrease the rate of glycolysis in response to the cell's energy requirements. For example, a high ratio of ATP to ADP will inhibit PFK and glycolysis.

Fig 1.1 shows the effect of low ATP on phosphofructokinase (PFK) activity.



(a)

(b) Fig. 1.2 shows a PFK molecule.



(i) Describe the structure of PFK.[4] (ii) Explain how a change in pH may affect PFK activity.[3] [Total : 12]

- For Examiner's Use
- **2** A student wanted to investigate the difference between gene regulation of lactose metabolism in humans and bacteria.

The enzyme, lactase, is responsible for breaking down lactose in humans. *LCT* gene coding for lactase is found on chromosome 2, where its molecular nucleotide location is from base pairs 135,787,840 to 135,837,195.

Deletion constructs of the *LCT* gene were designed with various sequences on chromosome 2 deleted. These nucleotide positions are all upstream of the *LCT* gene, and were selected as they were found to be bound by molecules (A, B and C) which exert influence over *LCT* gene regulation.

Fig. 2.1 shows the map of a particular portion of chromosome 2, where relative positions of these sequences as well as the molecules associated with them are shown.

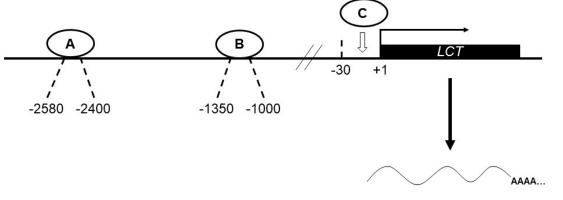


Fig 2.1

In order to quantify the effect of deletion on regulation of *LCT*, the student measured the amount of *LCT* mRNA produced, and the results are summarised in Table 2.1.

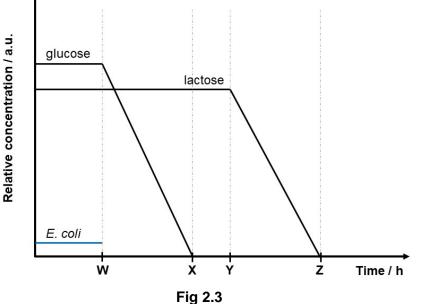
Deletion construct	Nucleotides deleted	Relative mRNA quantity / a.u.			
Wild type	-	268			
1	-30 to 0	0			
2	-1350 to -1000	550			
3	-2580 to -2400	76			
Table 2.1					

- (a) With reference to Fig 2.1 and Table 2.1,
 - (i) identify the type of molecule as well as the type of regulatory sequences studied in the experiment.

	Type of molecule	Position	Type of Regulatory Sequence
Α		-2580 to -2400	
В		-1350 to -1000	
С		-30 to 0	······

[3]

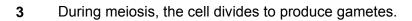
(ii) explain how you arrived at the identity of molecule **B** as well as the type of regulatory sequence at -1350 to -1000. (b) The LCT mature mRNA excluding its poly-A tail is much shorter than the LCT gene. Account for this difference.[2] (c) The lac operon is responsible for lactose metabolism in bacteria. With reference to the lac operon, describe one difference in the regulation of lactose metabolism in bacteria and humans.[1] (d) The student wanted to investigate how glucose and lactose impact gene expression of lac operon and growth of E. coli. Fig. 2.2 shows the utilisation of glucose and lactose as carbon sources in a culture of bacteria.



(c) (i) Complete the graph by showing the concentration of bacteria in culture over time from W to Z.
 [1]

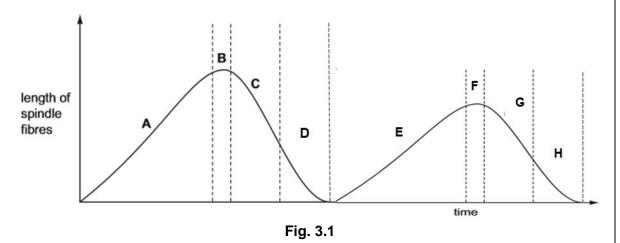
Using your knowledge of the lac operon, explain the shape of the graph for lactose from

(ii)	time W to X , and
	[2]
(ii)	Time Y to Z
	[4]
	[Total : 15]



(a) Discuss the role of centromere in the production of gametes.

(b) The graph in Fig. 3.1 shows the length of the spindle fibres during meiosis.



- (i) In which regions of the graph did the centromeres detach from the spindle fibres?
-[1]
- (ii) Fig. 3.2 shows a particular stage in meiosis

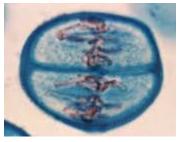


Fig 3.2

Which region on the graph corresponds to the stage shown in the Fig. 3.2.

.....[1]

(ii) Name and identify 2 corresponding stages on the graph in Fig 3.1 that contributes to genetic variation in gametes, and explain how they bring about genetic variation.

Stage
Explanation
Stage
Explanation
[6]

(c) Most human traits as well as medical conditions are under genetic influence.

A gene is found to control a rare disease, Wiskott–Aldrich syndrome (WAS) which is characterised by eczema, low platelet count and immune deficiency.

Another gene that controls hair type has 2 alleles. 1 allele results in straight hair, another codes for curly hair. Presence of both results in wavy hair.

In **couple 1**, a normal female with straight hair married a wavy-haired male suffering from WAS. Predicted phenotypic ratio of their offspring is as follows:

	WAS	Hair
Female	All normal	1 wavy : 1 straight
Male	All normal	1 wavy : 1 straight

For **couple 2**, a wavy-haired female suffering from WAS married a wavy-haired normal man. Predicted phenotypic ratio of their offspring is as follows:

	WAS	Hair
Female	All normal	1 curly : 2 wavy : 1 straight
male	All affected	1 curly : 2 wavy : 1 straight

(i) What is the mode of inheritance of the WAS disease?

.....[1]

(ii) Use suitable symbols to represent the alleles of the gene controlling the WAS disease.

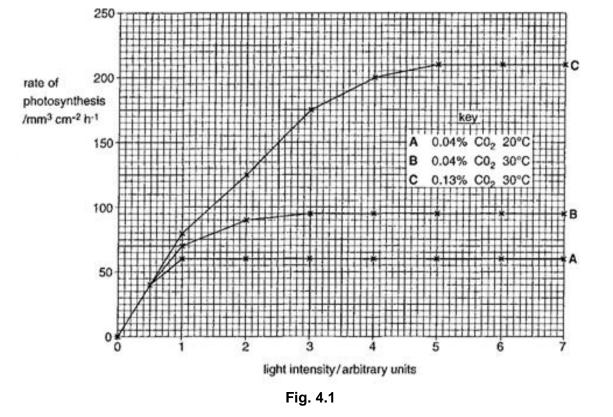
......[1]

(iii) Use a genetic diagram to explain the results of **couple 2**.

[4]

[Total : 17]

4 Fig. 4.1 shows the results of experiments investigating the effect of different light intensities on the rate of photosynthesis of cucumber plants measured as mm³ CO₂ uptake per cm² leaf area per hour. The experiments were carried out at different temperatures and carbon dioxide concentrations.



(a)	(i)	With reference to Fig. 4.1, state the best conditions for the growth of cucumbe plant.	
		[1]	
	(ii)	Define the term limiting factor.	
		[1]	
	(iii)	With reference to Fig. 4.1, explain reasons for the difference between curves B and C .	
		[3]	

(b) Fig. 4.2 shows the absorption spectra of various leaf pigments and the action spectrum for photosynthesis.

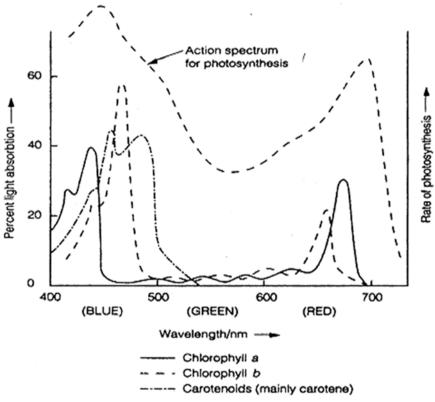


Fig. 4.2

With reference to Fig. 4.2, explain why most plants are characteristically green, and (i)[2] (ii) explain the shape of the action spectrum.[3] In conducting experiments to obtain the action spectrum for photosynthesis, explain why the use of organic solvents such as acetone or ether is necessary to extract photosynthetic pigments.[2]

[Total: 12]

(c)

5 Using two mutant strains of *E. coli* (strain A and strain B) with different nutritional requirements, scientists showed that gene transfer could happen between bacteria. Strain A would grow only if the medium was supplemented with methionine and strain B would only grow if supplemented with threonine. When strains A and B are mixed together, some of the progeny were observed to regain the ability to grow without added nutrients in the medium.

Fig. 5.1 shows what was found when scientists observed the mixture of strains \bf{A} and \bf{B} under a microscope.

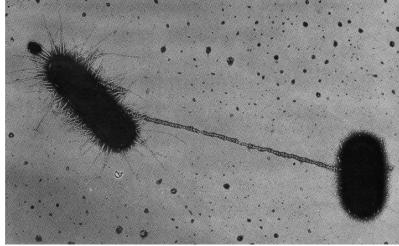
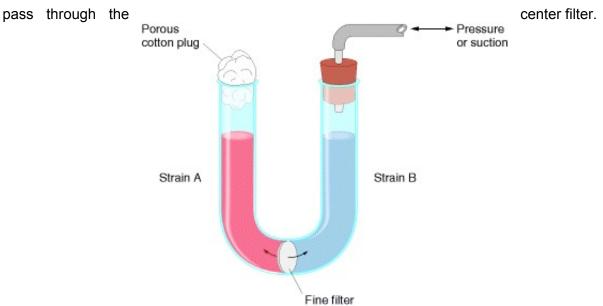


Fig. 5.1

(a) Name and describe the method of gene transfer seen in Fig. 5.1.

 	 [4]

The following experimental set-up was used to investigate more about the method of gene transfer seen in Fig. 5.1. A suspension of strain **A** was placed in one arm of a U-tube and strain **B** was placed in the other arm. Liquid may be transferred between the arms but bacterial cells cannot



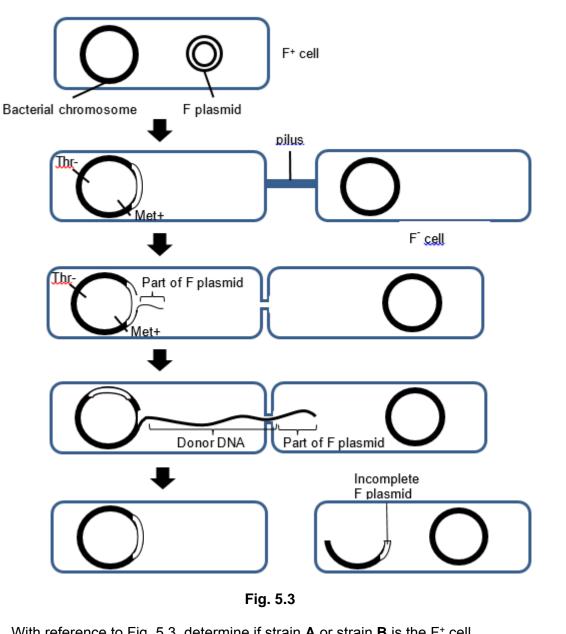


(b) After several hours of incubation, the cells were transferred to minimal medium (without methionine and threonine). Predict and explain if any bacterial growth would be observed.

- (c) It was suggested that the scientists should set up a test-tube containing a mixture of strain **A**, strain **B** and an enzyme nuclease.
 - (i) Predict if the bacterial progeny from this tube can survive in minimal medium without the addition of methionine and threonine.

[1]

(d) Further investigation revealed that the genes controlling methionine synthesis and threonine synthesis are found on the bacterial chromosome. They can be transferred after the F plasmid integrates with the bacterial chromosome. The method of transfer is illustrated in Fig. 5.3.



(i) With reference to Fig. 5.3, determine if strain **A** or strain **B** is the F^+ cell.

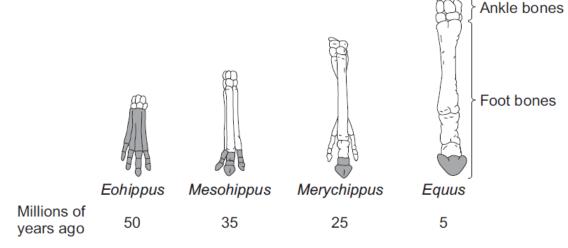
......[1]

[Total: 12]

(ii) In cases of gene transfer shown in Fig. 5.3, the F plasmid-bacterial chromosome is usually not transferred completely to the recipient cell. Random breakage interrupts the process. The recipient cell is unable to go on to donate any genes to other cells.

With reference to Fig. 5.3, suggest if the recipient cell is considered an F^+ or and F^- cell after the transfer. Explain your answer.

6 Fig. 6.1 shows changes in the foot bones of four ancestors of modern horses over the past 50 million years.



Key: The shaded bones are the bones which touched the ground.

Fig. 6.1

(a) Describe two changes in the foot bones of horses that have taken place over the past 50 million years.

- (b) Eohippus lived in swampy areas with soft mud.
 Since then, the ground in the habitat has become drier and harder.
 All of the horse ancestors were preyed upon by other animals.
 - (i) Explain **one** advantage to *Eohippus* of the arrangement of bones in its feet.

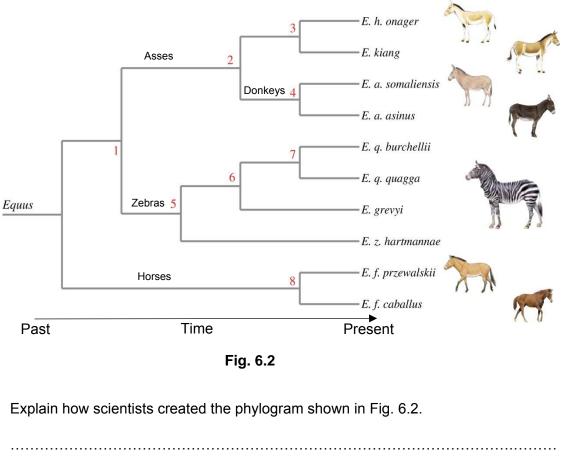
......[1]

(ii) Based on the information given, explain how the changes in the arrangement of the foot bones of horses support Darwin's theory of evolution by natural selection.

(c) (i) Fossils of *Eohippus, Mesohippus and Merychippus* have been unearthed only in the North American continent. Explain how the biogeographical distribution of these fossils support Darwin's theory of evolution.

[3]

(ii) The first "modern" one toed horses Equus also arose in North America, around 5 million years ago, but quickly spread into the Asia and Europe, when the land bridge formed between continental America and Asia. They then diversified into the modern species we know today - horses, donkeys, asses and zebras.







7 The immune system protects the human body against pathogen's infection of the host and subsequent development of disease. Fig. 7.1 shows the action of a macrophage on a pathogenic bacteria.

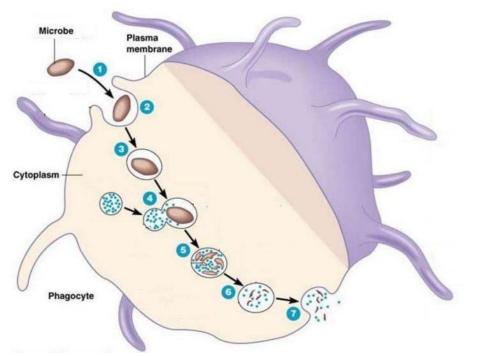


Fig. 7.1

(a) With reference to Fig. 7.1, identify the steps and explain the processes that kills the pathogenic bacteria.

(b) Describe how the humoral immune response enhances the action of macrophages on the pathogenic bacteria.

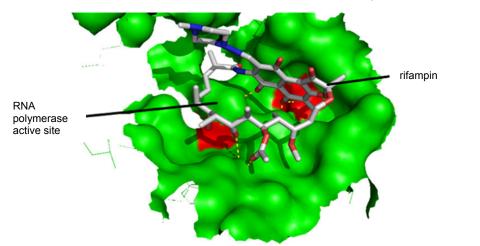
 (c) Tuberculosis (TB) is a highly infectious air-borne disease that affects the lungs, brain, lymph nodes, kidneys and bones. In the recent years, there is a rise in the number of TB cases. In the year 2017, 1,536 new cases of TB infections were reported.

TB is caused by the bacteria, *Mycobacterium tuberculosis*. The process shown in Fig. 7.1 has limited efficacy in clearing *M. tuberculosis* that infected the hosts.

Explain how *M. tuberculosis* protects itself from the host immune system.

.....[2]

- (d) Patients diagnosed with TB are treated with a regime that uses a "cocktail" of drugs which include isoniazid, rifampin, pyrazinamide and ethambutol.
 - Fig 7.2 show the mechanism of action of rifampin on RNA polymerase.





With reference to Fig. 7.2, explain how rifampin acts to achieve bactericidal effects on *M. tuberculosis.*

[Total:10]

[Turn over

8 Sea ice is an integral part of the Arctic Ocean. The extent of area covered by Arctic sea ice is an important indicator of changes in global climate because warmer air and water temperatures are reducing the amount of sea ice present.

Fig. 8.1 shows Arctic sea ice extent for the months of September and March of each year from 1979 through 2016. September and March are when the minimum and maximum extent typically occur each year.

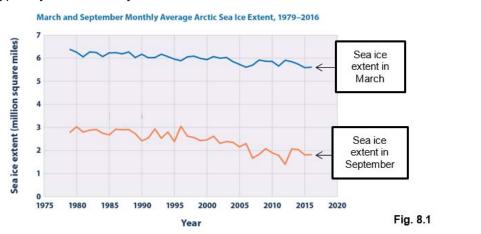


Fig. 8.2 shows the start and end of the sea ice melt season.

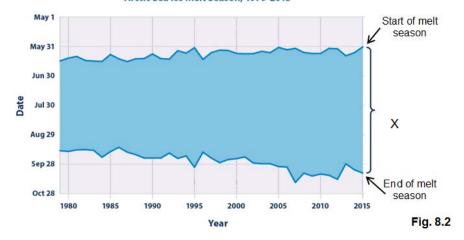
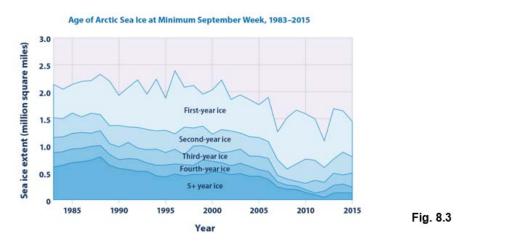


Fig. 8.3 shows the distribution of Arctic sea ice extent by age group during the week in September with the smallest extent of ice for each year.



(a) With reference to Fig. 8.1, suggest why the sea ice extent in March is different from that in September.[1] (b) With reference to Fig. 8.2, identify what X denotes. (i)[1] (ii) describe how X changes from 1979 to 2015.[1] (ii) Explain how this is linked to the changes in sea ice extent as shown in Fig. 8.1 and Fig. 8.3.[3]

(c) A number of animals living in the Arctic are able to change their coat colour as the seasons change.

The ability to change coat colour is a result of their ability to control pigment production in response to variation in lengths of day or night.

These animals are able to detect the lengths of day or night via a light-sensitive receptor known as melanopsin that is found in their retina. Fig. 8.4 shows the structure of melanopsin.

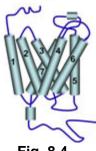
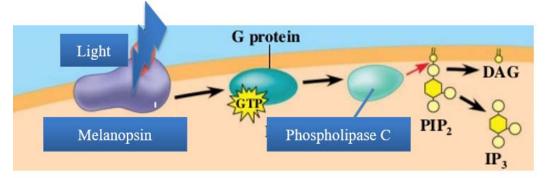


Fig. 8.4

(i) State the class of receptors that melanopsin belongs to.

Melanopsin is known to activate an enzyme known as phospholipase C via the signaling pathway shown in Fig. 8.5.

......[1]





(ii) With reference to Fig. 8.5, describe how phosholipase C could become activated.

[3] [Total : 10]

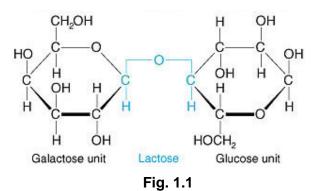
End of Paper

Section A

Answer **all** the questions in this section.

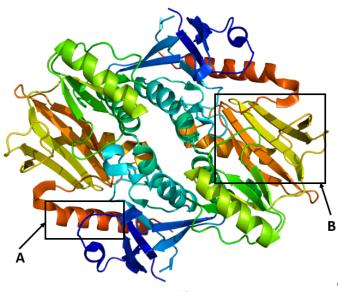
1 Lactose is the major carbohydrate in milk which is a main source of food for infants. Young children almost universally produce lactase, an enzyme which digests lactose.

Fig. 1.1 shows the structure of a lactose molecule.



(a) Show how a lactose molecule is hydrolysed into its monomers in the space provided below.

(b) The structure of lactase is shown in Fig. 1.2.





Compare the structures labelled **A** and **B** in Fig. 1.2.

[3]	

(c) Lactose is digested in humans by lactase in the small intestine, which has an alkaline environment.

People who are lactose intolerant lack the enzyme lactase, which digests lactose. If undigested, lactose reaches the large intestine where it can be fermented by microbes. Lactose fermentation results in the production of volatile fatty acids and gases such as carbon dioxide and hydrogen gas. A part of the hydrogen gas is expired by breath. The hydrogen (H_2) breath test has been developed as a method to identify lactose intolerance.

The fungus, *Aspergillus oryzae*, produces a lactase which converts lactose to glucose and galactose at a pH of 2 to 3.5. This makes the fungal lactase particularly effective to digest lactose in the stomach, which has a highly acidic environment. Therefore it is ideal for use in dietary supplements as a digestive aid.

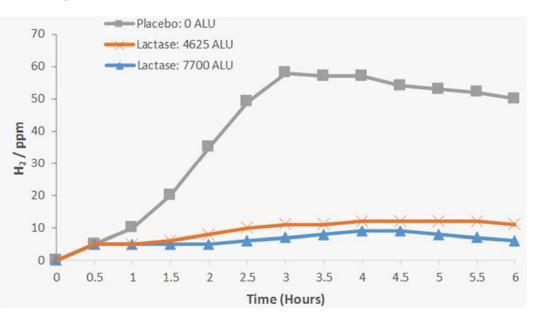
(i) Explain a similarity and a difference in structure between the fungus lactase and the human lactase.

(ii) Explain why the fungal lactase will only work in the stomach but not in the small intestine.

[3]

(d) To determine if the fungal lactase can reduce the symptoms of lactose intolerance, 100 lactose-intolerant participants took part in a H_2 breath test.

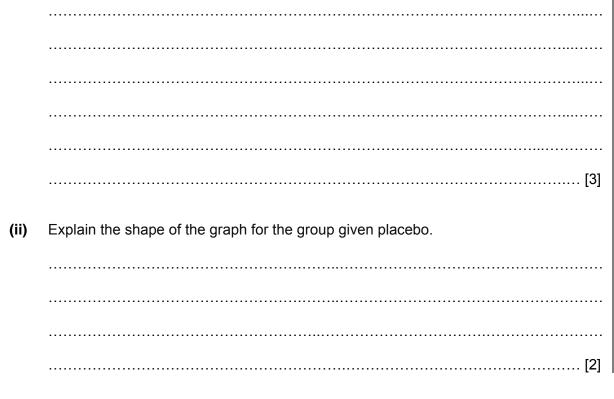
All the participants were given 500 mL of milk during the test. Following that, they were divided into 3 groups: 1 group was given a placebo (0 ALU of lactase). The other 2 groups were given low dosage (4625 ALU) and high dosage (7700 ALU) of lactase, respectively.



The graph in Fig. 1.3 shows the results of the H₂ breath test.

Fig. 1.3

(i) With reference to Fig. 1.3, discuss the effectiveness of lactase treatment in reducing the symptoms of lactose intolerance.



(iii) The participants are required to fast for 12 hours from milk-containing food as a prerequisite for doing the H_2 breath test.

A participant who did not fast from milk-containing food is given the low dosage (4625 ALU) of lactase. He drank milk 4 hours before the H_2 breath test.

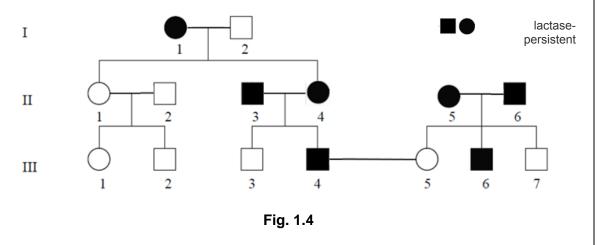
Draw on the graph in Fig. 1.3, the results obtained from this participant's test. [1]

(e) In most mammals, including 65% of humans, the level of lactase mRNA found in the intestinal epithelial cell is greatly decreased in adults.

Describe a mechanism that accounts for this age-dependent lactase regulation during adulthood.

(f) Some individuals, however, are lactase-persistent (LP). They maintain high levels of lactase expression throughout adult life and hence remain tolerant of lactose. LP is thought to have originated from a mutation.

The pedigree in Fig. 1.4 shows the presence of lactase persistence of a family.



.....[1]

(i) State the mode of inheritance of lactase persistence.

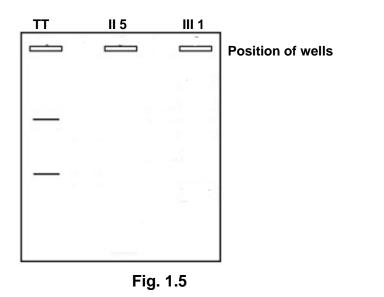
- 7
- (ii) Explain how you arrived at your answer in (f)(i).

(g) A single nucleotide polymorphism (SNP) is found to exist between LP and non-LP individuals. The SNP known as LCT₋₁₃₉₁₀ has 2 alleles, T allele and C allele. The genotype of individuals at that SNP can be used to predict if they are lactase-persistent.

The T allele is found in LP individuals who have higher levels of lactase. Individuals who carry the C allele have lower levels of lactase.

In a RFLP assay of both the genetic variants, polymerase chain reaction was used to amplify both the T and C alleles. Each allele resulted in a 448 bp product. The amplified products were digested with restriction enzyme *BsmF*l. It produced two fragments (351 and 97 bp) for the C allele, and three fragments (253, 98 and 97 bp) for the T allele.

Fig. 1.5 shows a gel with the results of the RFLP analysis for an individual who is homozygous for the T allele. Draw on Fig. 1.5 to show the results of the banding patterns of individuals **II 5** and **III 1** from Fig. 1.4.

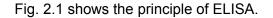


[2]

2 Dengue fever is a mosquito-borne disease caused by the dengue virus. Dengue is transmitted by several species of mosquito, including *Aedes aegypti*. Symptoms such as high fever, headache, vomiting, muscle and joint pains, and a characteristic skin rash, typically begin three to fourteen days after infection.

The disease can develop into the life-threatening dengue hemorrhagic fever.

A number of tests are available to confirm the diagnosis of dengue fever. This includes the Enzyme Linked Immunosorbant Assay (ELISA) which detects the presence of a patient's immunoglobulin (Ig) produced against the dengue virus' non-structural-1 protein (NS-1). This process takes about 3 hours.



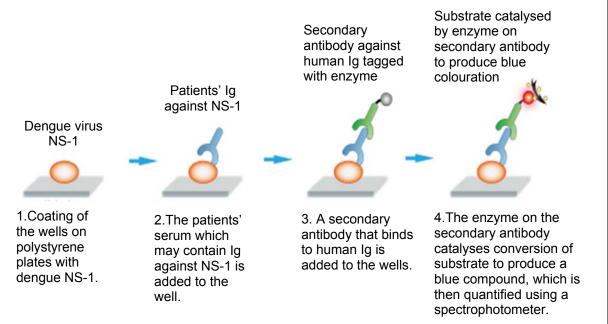


Fig. 2.1

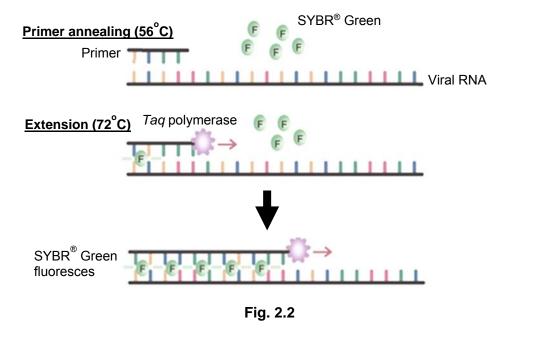
(a) Explain how the structure of the patient's Ig enables its binding to the NS-1 that coats the wells of the polystyrene plates.

(b) ELISA allows detection for the presence of patients' Ig. It also allows the quantification of the concentration of human Ig against NS-1.

With reference to Fig. 2.1 and using your knowledge of enzymes, explain how the process of ELISA allows quantification of the concentration of human Ig against NS-1.

(c) An alternative test is the quantitative polymerase chain reaction (qPCR) that detects the presence of dengue viral RNA. The principles of qPCR is similar to normal PCR except that it uses RNA as a template. In qPCR, SYBR[®] Green is added to the reaction mix. Interaction between SYBR[®] Green with the PCR products produces green fluorescence, which can be detected.

Fig. 2.2 shows the primer annealing and extension phases of qPCR.

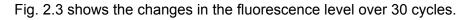


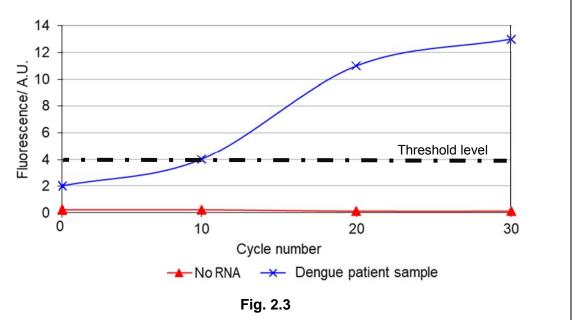
(i) Describe one characteristic of primers used and how it facilitates qPCR.

(ii) Suggest how SYBR[®] green interacts with the PCR products.

.....[1]

(d) qPCR is performed in a thermocycler that measures the fluorescence in the tube over a number of cycles. If the level of fluorescence is above the threshold level (4 A.U.), it is considered a positive test.





(i) Explain the purpose of including the control with no RNA.

.....[1]

(ii) If a typical qPCR cycle takes 3 minutes, calculate the minimum time taken to report a positive result.

Minimum time taken is

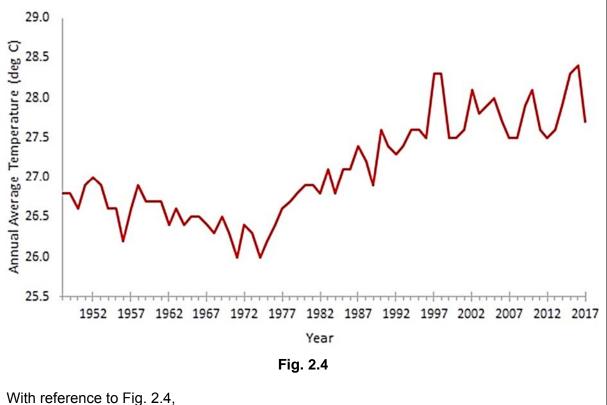
[2]

(iii) Suggest why qPCR is a better test compared to ELISA.



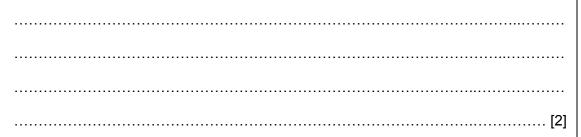
(e) Factors affecting the transmission of dengue are being closely monitored.

Singapore is not insulated from the effects of global warming. Fig. 2.4 shows the annual average temperature in the past 70 years.



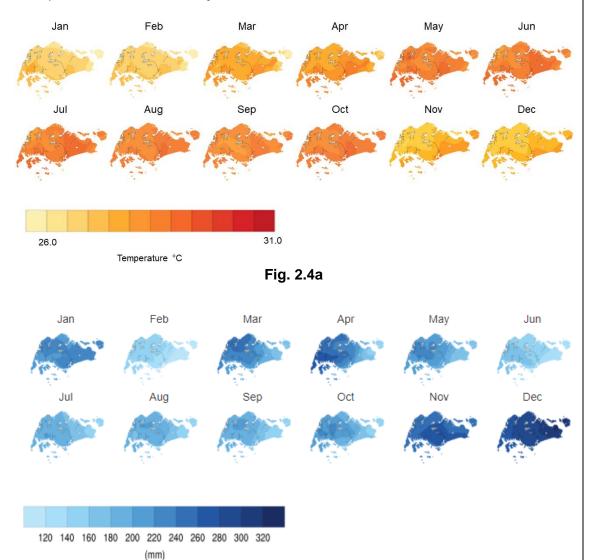


 (ii) explain the impact of climate change on transmission of dengue in Singapore.



As it is situated near the equator, Singapore's climate is characterised by high temperatures, rainfall and humidity all year-round. However, there exists slight variations within the months.

The figures below show the variations in temperature (Fig. 2.4a) and rainfall (Fig. 2.4b) over the months in the year of 2015.





(iii) It has been observed that there are higher incidences of dengue infections reported between June to October.

With reference to Fig. 2.4a and 2.4b, and the information given above, comment on the aspect of climate which has a greater influence on dengue transmission in Singapore.

(f) In order to decrease dengue transmission, studies have been conducted to find a suitable method of control of the *Aedes* mosquito population.

One such method implemented recently was the use of *Wolbachia*, a bacteria common in insect species, to control the mosquito population. *Wolbachia* infection in mosquitoes are maternally transferred, and mosquitoes carrying the *Wolbachia* bacteria are unable to transmit the dengue virus.

Fig. 2.5 shows the crosses of Wolbachia-infected mosquitoes.

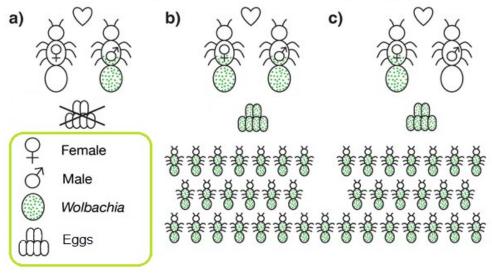


Fig. 2.5

When a male *Wolbachia*-infected mosquito mates with a wild-type female mosquito, the female mosquito lays eggs which are unable to hatch, due to a phenomenon known as cytoplasmic incompatibility. A scientist hypothesised the following to explain cytoplasmic incompatibility: after fertilisation, condensation of paternal chromosomes in the zygote is prevented.

(i) Given the above information, explain how nuclear division may be affected.

(ii) Different *Wolbachia* strategies have been implemented to control the spread of mosquito-borne diseases. Some countries have opted for the release of both *Wolbachia*-infected males and female mosquitoes. However, Singapore has adopted the strategy to release only *Wolbachia*-infected male mosquitoes.

Given the above information, justify Singapore's choice to adopt the release of only *Wolbachia*-infected males.

[Total: 23]

Section B

Answer **one** question in this section.

Write your answers on the separate answer paper 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.

- 3 (a) Account for the importance of hydrogen bonds in biological organisms. [13]
 - (b) Discuss the diverse roles of membranes in a cell.

[Total: 25]

[12]

- (a) Discuss how the complementary shapes between biomolecules enable eukaryotic cells to carry out their diverse functions. [13]
 - (b) Organisms are able to detect and respond to changes in their environments. Discuss this, with reference to specific examples in unicellular and multi-cellular organisms. [12]

[Total: 25]

End of Paper

Raffles Institution 2018 Preliminary Examination H2 Biology

Paper 1

1.	D	7.	D	13.	D	19.	С	25.	С
2.	В	8.	С	14.	Α	20.	С	26.	Α
3.	Α	9.	D	15.	С	21.	В	27.	Α
4.	D	10.	Α	16.	В	22.	В	28.	В
5.	С	11.	D	17.	D	23.	В	29.	Α
6.	С	12.	D	18.	Α	24.	С	30.	С

Paper 2

Fig. 1.1 shows the effect of low ATP on Phosphofructokinase (PFK) activity.

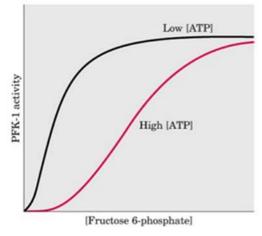


Fig. 1.1

- (a) (i) On Fig. 1.1, draw a graph to show the effect of high ATP on PFK activity [1]
 - (ii) Explain the graph you have drawn. [3]
 - 1) <u>High levels of ATP inhibits PFK activity</u> by lowering its affinity for fructose 6-phosphate.
 - 2) <u>ATP</u>* acts as an allosteric inhibitor which <u>binds to an allosteric site</u> which inhibits the PFK activity
 - 3) [V_{max}]: inhibition can be <u>overcome</u> by <u>high substrate concentrations;</u>
 - 4) [idea of cooperativity] <u>binding of substrate to 1 subunit</u> changes conformation of other subunits such that it <u>becomes easier for substrate to bind</u>;
 - (iii) Name 1 molecule that will act as allosteric activator of PFK activity.[1]

AMP/ADP R: fructose-6-phosphate

- (b) Fig. 1.2 shows a PFK molecule.
 - (i) Describe the structure of PFK. [4]
 - 1) PFK has a *<u>quaternary</u>** structure made up of <u>4 polypeptide chains</u>.
 - 2) Each of which has <u>primary</u> structure which is unique <u>number and sequence of amino</u> <u>acid in a polypeptide linked by peptide bonds.</u>
 - 3) Secondary, tertiary and quaternary structures are hence direct consequences of

primary structure.

- 4) <u>Secondary</u> structure refers to regular coiling and folding/pleating of polypeptide held by <u>hydrogen bonds</u> between <u>CO and NH groups</u> of <u>polypeptide backbone</u>.
- 5) <u>Tertiary structure</u> refers to a single polypeptide chain which is further <u>folded</u> into specific 3D conformation, held by bonds between <u>R-groups within same polypeptide</u>.
- 6) Tertiary structure is maintained by <u>hydrophobic interaction, hydrogen bonds, ionic bonds</u>.
- 7) Overall conformation of PFK is maintained by its primary, secondary and tertiary structure in addition to interaction among the 4 polypeptide chains.
- 8) Presence of *active sites** complementary in shape and charge to substrate.
- 9) Presence of *allosteric sites** complementary in shape and charge to activator/inhibitor.
- 10) PFK is <u>globular/water-soluble</u>, arranged so that most of its <u>hydrophilic amino acid side</u> <u>chains</u> are o <u>external surface of the protein</u> while <u>hydrophobic amino acid side chains</u> are buried in <u>interior</u>.
- (ii) Explain how a change in pH affect the PFK activity.[3]
- 1) Affects ionisation of *<u>R groups</u>** of aa;
- A <u>change in pH will disrupt</u> the <u>ionic and hydrogen bond*</u> that maintain the 3D conformation of active site of PFK; (A: mention of either one of the bonds)
- 3) which will result in a <u>change in 3D conformation</u> of <u>active sites</u>* (R: catalytic sites), <u>no</u> longer complementary in shape and charge <u>to substrate / substrate cannot fit</u>, hence;
- 4) less enzyme-substrate complex* formed hence reducing the PFK activity;

[Total : 12]

2 (a)

- With reference to Fig. 2.1 and Table 2.1,
 - (i) identify the type of molecule as well as the type of regulatory sequences studied in the experiment.[3]

	Type of molecule	Position	Type of Regulatory Sequence
Α	Activator	-2580 to -2400	Enhancer
В	Repressor	-1350 to -1000	Silencer
С	RNA polymerase / General transcription factor	-30 to 0	Promoter

- (ii) explain how you arrived at the identity of molecule B as well as the type of regulatory sequence at -1350 to -1000. [2]
- <correct set of data comparison + quote data> <u>Wild type vs deletion construct 2 / deletion of -1350 to -1000</u>, resulted in an <u>increase</u> of <u>282 a.u.</u> in relative mRNA quantity. (A: deletion construct 2 resulted in increase in relative mRNA quantity of 550 a.u. compared to the wild-type construct of 268 a.u.).
- <u>Repressor* protein is unable to bind to silencer*</u> sequence to lower transcriptional activity. (A: presence of -1350 to -1000 sequence allowed <u>repressor</u>* to bind to a <u>silencer</u>* sequence to downregulate transcription).
- Thus, <u>transcriptional initiation complex</u> / <u>RNA polymerase</u> can assemble (more easily/readily) at <u>promoter*</u>, thus <u>increasing frequency of transcription</u>, which leads to more mRNA formed (marker's note : increase rate of transcription).
 Note to marker: pt 1 is compulsory
- (b) The *LCT* mature mRNA excluding its poly-A tail is much shorter than the *LCT* gene. Account for this difference. [2]

1.Due to non-coding sequences in gene such as *introns**, which are <u>removed</u> during *splicing** by the spliceosome.

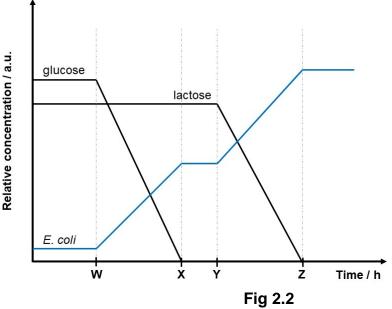
2. The gene includes **promoter*** sequences which are not transcribed by the RNA polymerase.

R: enhancer / silencer (not part of gene)

(c) With reference to the *lac* operon, describe one difference in the regulation of lactose metabolism in bacteria and humans.[1]

	Eukaryotes	Bacteria
Effect of	Each gene* is controlled by a single	Multiple related genes* are controlled
promoter	promoter*;	by the same/single promoter*
		(operon system);
Regulatory	Regulatory sequences e.g. enhancer*	Regulatory sequences, e.g. the
sequences	and silencer* that involved in controlling	operator* that involved in control of
involved	frequency of transcription initiation may	transcription is proximal to the genes
	be <u>distal;</u>	under its control;

(d) (i) Complete the graph by showing the concentration of bacteria in culture over time from W to Z. [1]





Using your knowledge of the lac operon, explain the shape of the graph for lactose from (ii) Time W to X [2]

- 1. Glucose present thus, low cAMP* levels;
- Catabolite Activator Protein* (CAP) not activated, hence no CAP binding to CAP binding site in promoter and thus no up regulation of transcription of lac operon;
- <u>Beta-galactosidase</u>* not expressed to <u>break down lactose</u>, hence <u>lactose</u> <u>amount maintained</u>; OR

Permease* not expressed for lactose uptake, thus lactose level remains

unchanged;

(ii) Time Y to Z [4]

- Lactose is converted to <u>allolactose</u>* which acts as an <u>inducer</u>*, <u>binds</u> to <u>repressor</u>*, repressor is <u>inactivated</u>;
- 2. As binding causes a <u>change in conformation</u> such that <u>repressor no longer binds to the</u> <u>operator</u>*,
- 3. <u>RNA polymerase</u> can <u>bind</u> <u>promoter</u>* and initiate <u>transcription</u>* of the structural <u>genes</u>(Lac Z,Y and A) to take place.
- 4. In <u>absence of glucose</u>, <u>high **cAMP*** levels</u>
- 5. result in <u>cAMP-Catabolite Activator Protein*</u> (CAP) complex which attaches to CAPbinding site on <u>promoter</u> strengthening affinity of RNA polymerase for promoter → <u>upregulation of transcription</u> of the *lac* operon
- 6. <u>Beta-galactosidase</u>* expressed in high amounts to <u>break down lactose</u>, hence <u>lactose</u> <u>amount drops</u>;

OR

Permease^{*} expressed in high amounts for <u>lactose uptake</u>, thus <u>lactose level remains</u> <u>drops;</u>

[Total : 15]

3

- (a) Discuss the role of centromere in the production of gametes. [3]
 - 1. They allow (proteins called <u>kinetochore proteins</u>, and subsequently) <u>spindle fibres, to</u> <u>attach; (MUST HAVE)</u>
 - 2. resulting in proper alignment of bivalents on equator in metaphase I/meiosis I;
 - 3. so that <u>homologous chromosomes can be equally separated to opposite poles</u> during <u>anaphase l/meiosis I;</u>
 - 4. resulting in proper <u>alignment of chromosome</u> singly in <u>on equator</u> in <u>metaphase</u> <u>II/meiosis II</u>;
 - 5. so that <u>sister chromatids / daughter chromosome can be equally separated to opposite</u> <u>poles</u> during <u>anaphase II/meiosis II;</u>

Note : point 1 is compulsory and from points 2-5 any two. Without point 1, maximum marks that can be obtained is 2.

(b) The graph in Fig 3.1 shows the length of the spindle fibres during meiosis.

(i) In which regions of the graph did the centromeres detach from the spindle fibres. [1] D and H

(ii) Which region on the graph corresponds to the stage shown in the Fig 3.2. [1]

F

(iii) Name and identify the 2 corresponding stages on the graph in Fig 3.1 that contributes to genetic variation in gametes, and explain how they bring about genetic variation. [6]

Stage :

- 1. Prophase I, A
- 2. <u>Crossing over*</u> of segments of <u>non-sister chromatids of homologous</u> <u>chromosomes*</u> at prophase I of meiosis I.
- 3. This leads to new combinations of alleles on chromosomes of gametes.

Stage :

4. Metaphase I, B

- 5. Independent assortment* of homologous chromosomes* occurs where arrangement of one pair of homologues at metaphase plate is independent of the arrangement of the other pairs of homologues;
- 6. This results in different combinations of maternal and paternal chromosomes in daughter cells:
- 7. there are 2ⁿ possible combinations of gametes where n is number of homologous pairs. / 2²³ possible combinations of gametes in human ;

A: Metaphase II, F:

only if answer clearly makes ref to;

random arrangement of /independent orientation of non-identical sister chromatids* (R:chromosome) of each chromosome at the metaphase plate & their subsequent separation during metaphase II and anaphase II respectively

- What is the mode of inheritance of the disease WAS? [1] (c) (i) Sex-linked recessive :
 - Use suitable symbols to represent the alleles of the gene controlling the disease WAS (ii) [1]

х

Х

X

 $X^{\underline{W}}$: Dominant allele that codes for **no WAS** on X chromosome

X^w: recessive allele that codes for disease WAS on X chromosome;

Must show both alleles Must have X chromosome on symbol

(iii) Use a genetic diagram to explain the results of **couple 2.** [4]

1. [1] parental phenotype and genotype;

P phenotype: wavy-hair female WAS sufferer X^wX^wH^SH P genotype:

wavy-hair normal male ; X[™]YH^SH^C

YH

Gametes (n): 2. [1] all correct gametes (circled) in P and punnet square

	Male gamete				
X <u>w</u> H _a	YHS	XWH9	YHC		
	X ^w YH ^s H ^s				
X [₩] X ₩H ^S H ^S	WAS	X <mark>₩</mark> XªH ^s H ^c	X ^w YH ^s H ^c		
Normal Straight	sufferer,	Normal wavy	WAS sufferer,		
hair female	straight hair	hair female	wavy hair male		
	male				
	X ^w YH ^s H ^c				
X <mark>₩</mark> XʷH ^s H ^c	WAS	X <mark>™</mark> XªHcHc	X ^w YH ^c H ^c		
Normal wavy hair	sufferer,	Normal curly	WAS sufferer,		
female	wavy hair	hair female	curly hair male		
	male				
3. [1] Punnett square with correct genotypes and related phenotypes					
	Normal Straight hair female X ^W X ^w H ^s H ^c Normal wavy hair female t square with correct g	X ^W X ^w H ^s H ^s X ^w YH ^s H ^s Normal Straight sufferer, hair female straight hair male X ^w YH ^s H ^c X ^W X ^w H ^s H ^c WAS Normal wavy hair Sufferer, female sufferer, sufferer, sufferer, x ^w YH ^s H ^c WAS sufferer, wavy hair female sufferer, wavy hair male t square with correct genotypes and reference	X ^W X ^w H ^S H ^S X ^w YH ^S H ^S X ^W X ^w H ^S H ^S WAS Normal Straight sufferer, hair female straight hair male X ^w YH ^S H ^C X ^W X ^w H ^S H ^C Normal wavy hair female X ^w YH ^S H ^C X ^W X ^w H ^S H ^C WAS X ^W X ^w H ^S H ^C WAS X ^W X ^w H ^S H ^C WAS X ^W X ^m H ^S H ^C WAS X ^M X ^m H ^S H ^C WAS X ^M X ^m H ^S H ^C WAS X ^M X ^m H ^S H ^C WAS All the straight hair Male		

offspring genotypic ratio	X ^w X⁴H ^ṡ H ^s	X <mark>₩</mark> XʷH ^s H ^c	X <u>₩</u> X _w H _c H _c	X ^w YH ^s H ^s	X ^w YH ^s H ^c	X ^w Y H ^c H c
offspring	Normal	Normal	Normal	WAS	WAS	WAS
phenotypic	Straight	wa∨y	curly hair	sufferer,	sufferer,	sufferer,

ratio	hair female	hair female	female	straight hair male	wavy hair male	curly hair male
	1	2	1	1	2	1

4. [1] Correct offspring genotypic and phenotypic ratio

[Total : 17]

- (a) (i) With reference to **Fig. 4.1**, state the best conditions for the growth of cucumber.[1] 0.13% carbon dioxide, 30°C, above 5 arbitrary units of light;
 - (ii) Define the term limiting factor.[1]
 - 1. when a chemical process is affected by <u>more than one factor</u>, its <u>rate is limited by</u> <u>that factor</u> which is nearest its minimum value;
 - It is that limiting factor which <u>directly affects a process if its magnitude is changed</u>; AVP
 - (iii) With reference to Fig. 4.1, explain reasons for the difference between curves B and C. [3]
 - Curve B reaches a plateau at rate of photosynthesis of <u>95 mm³cm⁻²h⁻¹</u> at lower <u>light</u> intensity of 3 units, whereas Curve C reaches a higher maximal rate of photosynthesis of <u>210 mm³cm⁻²h⁻¹</u> at higher light intensity of 5 units; (idea of : max rate being different, with values)
 - 2. due to presence of higher CO₂ concentration at 0.13 (graph C);
 - OR
 - 3. Curve B: reaches max rate at <u>light intensity of 3 units</u>, while Curve C reaches max rate at <u>light intensity of 5 unit</u>
 - 4. <u>light intensity is no longer a limiting factor</u> as any further increase in light intensity does not affect rate of photosynthesis. Hence, <u>CO₂ concentration becomes limiting;</u>

(max 2 from 1 and 2 or 3 and 4)

- 5. Plant C achieve higher rates of photosynthesis as compared to B, because <u>higher</u> <u>CO₂/ substrate concentration</u> during <u>carbon fixation</u>* in Calvin cycle.
- results in <u>higher frequency of effective collision</u>* with enzyme Rubisco (and RuBP), thus <u>more enzyme-substrate complexes</u>* being formed;
- (b) With reference to Fig. 4.2,
 - (i) explain why most plants are characteristically green, and [2]
 - 1. Percentage of <u>green light absorbed</u> by (<u>all 3 pigments marker discretion</u>) is very low at <u>less than 5%;</u>

2. Green light is <u>reflected / transmitted;</u> For reference: Blue ~ 490–450 nm, red ~ 635-700 nm

(ii) explain the shape of the action spectrum. [3]

- 2. <u>Light absorbed</u> by different photosynthetic pigments is used for <u>photoactivation/</u> <u>photosynthesis</u>
- 3. thus <u>higher percentage of light absorption</u> leads to <u>higher rate of photosynthesis</u> in <u>red</u> <u>and blue light</u>; (idea of : chl a peak corresponding to action spectrum peak)
- 4. <u>carotenoids / chl b</u> absorbed at blue and red light, <u>broadening the range of wavelength</u> that can be used for <u>photosynthesis</u>
- 5. Region between <u>500 to 600 nm</u> (R: green) is <u>not an exact match</u> between absorption and action spectra due to role of <u>other accessory pigments;</u>
- (c) In conducting experiments to obtain the action spectrum for photosynthesis, explain why use of organic solvents such as acetone or ether is necessary to extract photosynthetic pigments. [2]
 - 1. Chlorophyll pigments are membrane-bound (A: thylakoid and chloroplast membranes);
 - 2. Only organic solvents can <u>dissolve</u> the membrane, that is composed of a <u>phospholipid</u> <u>bilayer</u>;

[Total: 11]

5

(a) Name the method of gene transfer seen in Fig. 5.1 and describe it briefly. [4]

- 1. Conjugation*;
- 2. **Sex pilus**^{*} of F^+ bacterial cell <u>makes contact</u> with a F^- cell ;
- 3. and <u>retracts / contract</u> to bring the F⁻ cell closer so a <u>mating bridge</u>* is formed between the 2 cells; (R: shorten)
- 4. <u>One of the 2 strands of plasmid DNA in F⁺ cell is **nicked** and <u>transferred from F⁺ cell to</u> the **F⁻ cell** through mating bridge (via rolling circle mechanism);</u>
- 5. as the other DNA strand is used as a template for elongation of nicked strand;
- The single strand F plasmid DNA circularizes in F⁻ cell and is used as a <u>template*</u> to synthesise a <u>complementary strand;</u>
- 7. for a double-stranded F plasmid DNA resulting in F⁺ cell;
- (b) After several hours of incubation, the cells were transferred to minimal medium (without methionine and threonine). Predict and explain if any bacterial growth would be observed.[2]
 - 1. No growth;
 - 2. <u>Filter prevent physical contact</u> between the 2 strains and did not allow for conjugation / did not allow for sex pili from F+ cell to come into contact with F- cell
- (c) (i) Predict if the bacterial progeny from this tube can survive in minimal medium without additional of methionine and threonine.[2]
 - 1. Progeny can survive in minimum medium;
 - 2. DNA <u>enclosed inside</u> cell / mating bridge / not exposed to surrounded, and hence <u>not</u> <u>degraded by nuclease</u>;
 - 3. Conjugation occurred, allowing <u>genes</u> coding for enzymes involved in metabolism of met and threo <u>to be tranfered between the 2 bacteria</u>.
 - R: reference to nuclease needed to nick the F plasmid for conjugation.

(ii) State the purpose of setting up this tube.[1]

To exclude transformation* as a possible method of gene transfer

- (d) (i) With reference to Fig. 5.3, determine if strain A or Strain B is the F⁺ cell.[1] Strain B
 - (ii) In cases of gene transfer shown in Fig. 5.3, the F plasmid-bacterial chromosome is usually not transferred completely to the recipient cell. Random breakage interrupts the process. The recipient cell is unable to go on to donate any genes to other cells. With reference to Fig. 5.3, suggest if the recipient cell is considered an F+ or and Fcell after the transfer. Explain your answer.[2]
 - 1. F-;
 - 2. <u>F factor is not transferred</u>, so the cell is unable to synthesise sex pili to initiate conjugation; (A: incomplete F plasmid transferred)

[Total: 12]

6

- (a) Describe two changes to the bones in the feet of horses that have taken place over the past 50 million years. [2]
 - 1. Bones became larger/longer/thicker;
 - 2. Number of bones became fewer/idea of fewer toes or bones fused;
 - 3. Fewer bones touched ground/lower surface area in contact with the ground; Accept any other valid <u>observable</u> changes
- (b) (i) Explain one advantage to *Eohippus* of the arrangement of bones in its feet. [1]

With a <u>larger surface area (how arrangement of bones confer advantage)</u> in contact with the ground, it can distribute its weight and <u>not sink into the soft mud/ can escape</u> <u>predators</u> (advantage);

(ii) Based on the information given, explain how the changes in the arrangement of the foot bones of horses support Darwin's theory of evolution by natural selection. [4]

Descent from a common ancestor

1. It shows <u>descent with modification</u> from a common ancestor, as basic underlying <u>homologous foot structure</u> was modified over time;

- 2. There was <u>variation in size/number/arrangement of foot bones</u> in population; (context specific)
- 3. <u>Environment changed</u> from swampy to hard and firm ground; (envt change, quote)
- 4. They faced <u>selection pressure of **predation***</u>; (idea of selection pressure, quoting predation) R: if selection pressure is *vague* like "environment" or *wrong* like "hard/soft ground"
- 5. Natural selection <u>selected for</u> individuals who had <u>larger/fewer foot bones</u> as they are; (selected traits)
- 6. Able to <u>run faster</u> on firm ground to avoid predation; (suggest why trait is an advantage) Pt 5 must be correct first.
- 7. Those who **survive***, are able to reproduce and pass on their alleles for larger/fewer bones to the next generation;

With modification

(c) (i) Fossils of *Eohippus, Mesohippus and Merychippus* have been unearthed only in the North American continent. Explain how the biogeographical distribution of these fossils support Darwin's theory of evolution. [3]

Accounting for their geographical distribution and common ancestry:

- Their close geographical proximity to each other suggest that the 3 extinct horses descended from a <u>common ancestor</u>* that originated in <u>North America/same</u> <u>continent</u>*; (idea of center of origin of common ancestor)
- 2. Ancestral population <u>dispersed</u> and spread outwards from center of origin and colonized various habitats but <u>confined by the oceans</u> surrounding North American continent; accept idea of <u>discontinuous distribution</u>; (Distribution: all extinct horse species are only found in N America and nowhere else)

Accounting for descent with modification

- 3. Occupying <u>different niches/environments</u>, with natural selection, they evolve over time to give rise to <u>different species</u>; (Speciation from common ancestor)
- 4. Their <u>fossils/structural foot bone changes</u> demonstrate <u>descent with modification</u> as it changes over time; (descent with modification);

(ii) Explain how scientists created the phylogram in Fig. 6.2? [2]

- 1. They studied <u>DNA sequences</u> of a <u>homologous gene</u> e.g. cytochrome C found in all the organisms;
- 2. Through sequence alignment and <u>comparing difference in nucleotides</u>, they can work out relationship between organisms; R: genotype, analyse
- 3. The <u>fewer nucleotide differences</u>, the <u>more closely related</u> the species and therefore they share a <u>more recent the common ancestor/branch point more recent in time</u>;
- 4. <u>Carbon dating of fossils</u> will tell how long ago an extinct species existed so that you can place them along a timeline;
- 5. Changes in <u>nucleotide sequence accumulate over time with clockwork regularity</u>. We can thus <u>estimate the timeline of speciation events</u>;

[Total :12]

7

- (a) With reference to the steps in Fig 7.1, identify the steps and explain the processes that kills the pathogenic bacteria.[2]
 - 1. Step 4 Fusion of *phagosome* with *lysosome** forming phagolysosome;
 - Step 5 Lysosomal / hydrolytic / digestive enzymes digest pathogen into harmless substance/<u>cuts up antigens</u> into short peptides; Thus bacteria is unable to further infect the host;
- (b) Describe how the humoral immune response enhances the action of the macrophage on the pathogenic bacteria.[3]
 - 1. Plasma cells produce antibodies/immunoglobulins;
 - 2. <u>Agglutination*</u>, where <u>antibodies clumps</u> bacteria to promote <u>phagocytosis*</u> by macrophages;
 - 3. **Opsonisation***, where antibodies tag pathogen to promote **phagocytosis*** by macrophages;
 - 4. Fab region of antibody/immunoglobulins binds to antigens on pathogen;
 - 5. <u>Fc</u> region binds to <u>Fc receptor</u> on phagocytes to promote <u>phagocytosis</u>; (max 3 marks)
- (c) Explain how *Mycobacterium tuberculosis* protects itself from the host immune system.[2]
 - 1. *M. tuberculosis* has cell walls that contain *mycolic acids**; (KIV if MUST HAVE)
 - 2. Protects bacterium from acids / digestive enzymes / lysosomal enzymes / reactive

oxygen species produced by lysosome;

- inhibit the fusion of the phagosome with lysosomes, hence no phagolysosome is formed and no lysosomal enzymes are available to kill the bacteria; (max 2 marks)
- (d) With reference to Fig 7.2, explain how rifampin acts to achieve bactericidal effects on *Mycobacterium tuberculosis.*[3]
 - 1. Rifampin is a competitive inhibitor of RNA polymerase;
 - 2. Structure of rifampin is <u>complementary in shape/conformation to active site</u> of RNA polymerase;
 - 3. <u>Blocks active site</u>*, prevents binding of free <u>ribonucleotides / DNA</u> template/promoter.
 - 4. prevents transcription* of bacterial genes hence no mRNA produced; (R; transcription of mRNA)
 - 5. Prevent expression of essential/housekeeping genes / no proteins produced;

[Total:10]

8

(a) With reference to **Fig. 8.1**, suggest why the sea ice extent in March is different from that in September.[1]

Ice extent was higher in March than Sept, as March is during <u>winter/colder months</u> where <u>sea ice forms/less melting</u> and Sept occurs during <u>summer/warmer months</u> where <u>more ice</u> <u>have melted</u>.

<u>Temperature (hot/cold) / season difference (winter/summer)</u> between March and September \rightarrow <u>More melting / more freezing</u> \rightarrow difference in sea ice extent

(b) (i) Identify X shown in Fig. 8.2.

X: duration of sea ice melt;

- (ii) Describe how X changes from 1979 to 2015. [1]
- 1. X increased by about a month from 1979 to 2015
- 2. <u>Sea ice melt season is starting earlier and ending later</u>, melt season <u>spanning from</u> <u>mid june to mid sept in 1979</u>, to late may to late sept in 2015;
- (iii) Explain how this is linked to the changes in sea ice extent as shown in Fig. 8.1 and Fig. 8.3. [3]

Fig 8.1

- 1. Longer sea ice melt season results in more ice melting, [E]
- 2. lowering minimum sea ice extent observed in September (/min); [O]
- 3. less time is available for formation of sea ice in winter months; [E]
- 4. decrease in maximum sea ice extent seen in March (/max) [O]
- Fig. 8.3
- 5. <u>Longer duration of sea ice melt</u> resulted in <u>less ice remaining frozen</u> past melt season; [E]

6. As seen in <u>decrease in the amount / proportion of multi-year old ice</u> / <u>decrease in extent of sea ice over all ages</u>; [O]
7. Which implies <u>thinning of ice cover over the years</u>; Mark allocation: Max 2 for each Fig Max 2 for Obs across Fig [1]

(c)

(i) State the class of receptors that melanopsin belongs to.[1] G-protein coupled receptor;

- (ii) With reference to Fig. 8.5, describe how phosholipase C could become activated.[3]
- 1. Light causes a conformational change in the cytoplasmic domain of melanopsin;
- 2. Allowing melanopsin to bind to an inactive G protein;
- 3. Causing exchange of its bound GDP for a GTP, activating G protein;
- 4. Activated G protein moves along cytoplasmic side of membrane;
- 5. To <u>bind</u> to and <u>activate phospholipase C;</u>

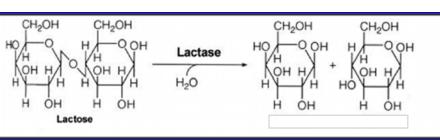
[Total : 15]

Paper 3

Section A

(a) Show how a lactose molecule is hydrolysed into its monomers in the space provided below.

[2]



- 1. <u>Shown</u>* and labelled <u>lactose structure</u> hydrolysed to form correct structure of monomers;
- 2. Shows how water is involved in hydrolysis; [A: if carbon not drawn]
- (b) Compare the structures labelled A and B in Fig. 1.2. [3]
 1. A= alpha helix and B=beta pleated sheet

Similarities

- Both maintained by <u>hydrogen bonds</u>* between <u>NH and CO</u>* of <u>polypeptide</u> <u>backbone</u>*;
- Both consist of <u>amino acids</u>* joined together with <u>peptide bonds</u>*;

Differences

- A is <u>helical in shape</u> whereas B comprised of <u>flat sheet</u> which becomes folded (R: pleated sheet does not describe);
- 5. A is formed <u>within a single region</u> of polypeptide whereas B may be formed between <u>different regions of same polypeptide</u>
- In A, hydrogen bonds are formed between O and CO group of one amino acid and H of NH group of <u>another</u>, <u>4 amino acids away</u>, but in B, hydrogen bonds are formed between CO group of 1 region and NH gp of an <u>adjacent region</u>
 [For full marks, must have 1 similarity + 1 difference]
- (c) (i) Explain a similarity and a difference in structure between the fungus lactase and the human lactase. [4] Similarity [2]
 - <u>Active sites</u>* of both fungal and human lactase have <u>similar 3D conformation</u> and are <u>complementary</u>* in shape and charge to <u>lactose</u>;
 - 2. So as to allow for hydrolysis of glycosidic bonds* in lactose;

Difference [2]

- 3. Consists of <u>different</u> structural <u>amino acids</u> in polypetide of fungal and human lactase (A: primary structure in place of amino acids);
- 4. Both have <u>different 3D conformation</u> due to having <u>different bonds</u>, e.g. ionic / hydrogen bonds;
- 5. Hence they have <u>different optimum pH</u> because the <u>bonds are stable at</u> <u>different pH;</u>
- (ii) Explain why the fungal lactase will only work in the stomach but not in the small intestine. [3]
 - 1. <u>Stomach</u> has an <u>pH of acidic around 2</u>, which is <u>within pH range</u> of fungal lactase; Or

<u>Small intestine</u> has an alkaline environment, which is out of <u>pH range</u> of fungus lactase;

- Results in <u>change</u> in <u>specific 3D conformation/shape</u> of enzyme's <u>active site</u> or results in causing <u>enzyme</u> to be <u>denatured</u>*;
- (d) (i) With reference to Fig. 1.3, discuss the effectiveness of lactase treatment in reducing the symptoms of lactose intolerance. [3]
 - 1. Lactase treatment was <u>effective</u> in <u>decreasing amount of H₂ in breath test;</u>
 - 2. <u>**Compare**</u> placebo and lactase-treatment, e.g. steep/sharp vs gradual/slight increase, highest vs lowest amount;
 - 3. Little / slight difference between high dosage and low dosage of lactase;
 - 4. + data quoted to support points 2 and 3, e.g. max value, difference in H2 amount etc;
 - (ii) Explain the shape of the graph for the group given placebo. [2]
 - 1. From <u>0 to 3 hours</u>: **sharp / steep*** increase in amount of H₂ produced from 0 to 56 ppm ; (shape and paired values required)
 - 2. Due to lack of lactase resulting in <u>large amount of lactose</u> reaching large intestine, hence <u>high rate of lactose fermentation</u>;
 - 3. At <u>3 hours</u>, <u>max</u> reached at <u>56 ppm</u> / from <u>3 to 6h</u>, decrease <u>slightly / gradually*</u> to 53 ppm; (shape and paired values required) R: plateau

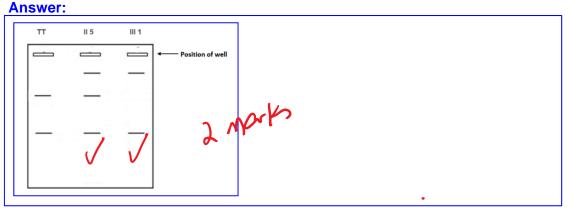
4. Since no more H_2 produced as all lactose was used up in fermentation;

- Must have 2 or 4 to get max 2
- (iii) Draw on the graph in Fig. 1.3, the results obtained from this participant's test. [1]
 0 to 3 hours: start at higher +
 3 to 6 hours: follows trend of lactase or plateau (R: gradual increase that follows placebo);
- (e) Describe a mechanism that accounts for this age-dependent lactase regulation during adulthood. [2]
 - 1. DNA methylation / histone deacetylation / chromatin remodelling complex, <u>condensation</u> of DNA / chromatin;
 - Prevents access of transcription factors and RNA polymerase / transciption initation factors to promoter, decrease frequency of transctiption;
 R: repressors binding to silencers since this is not long-term control of gene expression
- (f) (i) State the mode of inheritance of lactase persistence. [1] Autosomal dominant;
 - (ii) Explain how you arrived at your answer in (f)(i). [4] Dominant
 - 1. **Individual <u>II5 and II6 are both LP</u>. However, some of their children, <u>II5 and II17</u> <u>are non-LP</u>; (must clearly state the individuals and their phenotype)
 - 2. Shows that <u>II5 and II6 are heterozygous</u> where <u>non LP allele is masked</u> by presence of LP allele;
 - 3. If it is recessive, then all their children will have LP;

Autosomal

- 4. **<u>Father with LP (II6)</u> produce <u>daughter who is non LP (III5);</u> (must clearly state the individuals and their phenotype)
- III5 inherited <u>X chromosome</u> from II6 but is <u>non LP</u>. Therefore LP allele is not found on X chromosome; Or

If it <u>sex-linked</u> and dominant, <u>father with LP</u> would produce <u>daughters that are all</u> <u>LP</u>;



- 2 Fig. 2.1 shows the principle of ELISA.
- (a) Explain how the structure of the patient's Ig enables its binding to the NS-1 that coats the wells of the polystyrene plates. [2]
 - 1. Each Ig contains 2 antigen binding site*;
 - 2. Complementary in shape and charge to antigen/epitope of NS-1;
- (b) With reference to Fig. 2.1 and using your knowledge of enzymes, explain how the process of ELISA allows quantification of the concentration of human Ig against NS-1. [3]
 - 1. As concentration of human Ig against NS-1 bound to NS-1 on the well increases;
 - 2. <u>concentration of secondary antibody</u> tagged with <u>enzyme increases;</u>
 - 3. <u>Increase in enzyme concentration</u> results in increase <u>frequency of effective</u> <u>collision/enzyme substrate complex formation/results in increase in rate of reaction</u> between <u>chromogen</u> and active site o<u>f enzyme</u>;
 - 4. Increase in <u>intensity of blue product formed</u> within <u>fixed time</u> (idea: rate), which is detected using a spectrophotometer;

(c) (i) Describe one characteristic of primers used and how it facilitates qPCR. [2]

1. Single-stranded;

2. <u>Complementary to</u> 3' end viral <u>RNA sequence</u> so that they can <u>anneal</u> to viral RNA;

3. Contain a free 3'OH end so that

4. <u>Taq polymerase</u> can extend the DNA/<u>synthesize complementary DNA</u> strand/form phosphodiester bonds between adjacent deoxyribonucelotides;

- (ii) Suggest how SYBR[®] green interacts with the PCR products. [1]
 - Sybr green <u>intercalates/bind</u> itself in space between the <u>base pairs</u>; Reject reference to formation of hydrogen bonds with nitrogenous bases/complementary base pairs.
 - 2. Structure of Sybr green is <u>complementary in shape</u> to grooves of eg: DNA-RNA hybrid;
- (d) (i) Explain the purpose of including the control with no RNA. [1]
 - 1. This is a negative control to show <u>base line fluorescence level</u> if there is <u>no</u> <u>amplification of viral RNA/PCR product formed;</u>

AVP; ORA;

- (ii) If a typical qPCR cycle takes 3 minutes, calculate the minimum time taken to report a positive result.
 - Minimum time taken is

- [2]
- 1. 3 min x 10/11 cycles; (Ans must show working and number of cycles needed)
- 2. 30/33 minutes;

- (iii) Suggest why qPCR is a better test compared to ELISA. [2]
 - 1. Allowing earlier detection of dengue infection;
 - 2. <u>Viral RNA is immediately present</u> in infected patient whereas it <u>take some time</u> <u>before lg</u> is produced;
 - 3. Faster than ELISA;
 - 4. with reference to 30 min vs 3 hour;
 - 5. AVP
- (e) (i) describe and explain the temperature trends in Singapore from 1974 to 2017, [2]
 - 1. Annual <u>temperatures increased</u> from <u>26.0°C</u> in <u>1974</u> to <u>27.7°C</u> in <u>2017</u> (A: +/- 0.1°C);
 - 2. Globally, industrialisation / burning of fossil fuels for electricity/ deforestation led to increased release of CO₂;
 - 3. <u>Increased greenhouse gas emissions (GHG)</u> led to <u>increased trapping of</u> <u>radiation/heat</u> causing temperatures to rise; (R: radiation reflected from/to earth)
 - (ii) explain the impact of climate change on transmission of dengue in Singapore. [2]
 - 1. Global warming results in <u>higher temperatures</u>, thus conditions become more <u>suited for survival of mosquitoes / shortens the mosquito's life cycle ;</u>
 - <u>Transmission of dengue virus (DENV)</u> will <u>increase</u> if mosquito <u>vector</u>, *Aedes* aegypti, that transmits dengue is <u>present</u> / increases as the virus reproduces within the mosquito;
 - 3. <u>Faster replication</u> of dengue <u>virus in the mosquito</u>; (ref: shorter incubation period in mosquito)
 - (iii) With reference to Fig. 2.4a and 2.4b, and the information given above, comment on the aspect of climate which has a greater influence on dengue transmission in Singapore. [2]
 - 1. <u>Temperature</u> has greater influence over dengue transmission;
 - 2. During June to October, dengue infections were high, which <u>correlates with higher</u> <u>temperatures May to Oct</u>, where as <u>rainfall was low/moderate in those months</u>.
- (f) (i) Given the above information, explain how nuclear division may be affected. [2]
 - 1. <u>Paternal chromosomes did not condense</u> during *prophase**;
 - 2. <u>Mitosis is arrested</u>, and thus unable to produce daughter cells;
 - Chromosomes are <u>unable to align</u> singly at the <u>metaphase plate</u> during <u>metaphase</u>*;
 - <u>Non-disjunction</u> occurs / sister chromatids of paternal chromosome are <u>unable to</u> <u>separate</u> during <u>anaphase</u>*;
 - 5. <u>Paternal chromosomes</u> may get <u>entangled</u> during <u>**anaphase**</u>*, and thus resulting in <u>DNA damage</u>;
 - R: meiosis, and cannot form gametes.
 - (ii) Given the above information, justify Singapore's choice to adopt the release of only *Wolbachia*-infected males. [2]
 - 1. <u>Male mosquitoes do not bite</u>, whereas release of female mosquitoes will increase mosquito bites;
 - 2. It helps to <u>bring down mosquito population</u>, as the cross of infected male with wild type does not result in new mosquito offsprings;
 - 3. Conversely a <u>cross with infected female mosquitoes will still result in propagation</u> <u>of mosquitoes</u> albeit they are unable to transmit dengue;
 - 4. Idea of unable to control mosquito population if there is undesirable outcome eg: ecological damage;
 - 5. AVP;
 - R: prevents the transmission of dengue

Section B

3 (a) Account for the importance of hydrogen bonds in biological organisms. [13]

Context	Importance
Protein	Ref to levels of protein structure
structure	 Secondary structure * maintained by hydrogen bonds between <u>CO* and NH* groups of peptide backbone;</u> <u>Tertiary structure</u>* maintained by hydrogen bonds <u>between R</u> <u>groups* of same polypeptide</u> to allow for protein to fold into functional conformation; <u>Quaternary structure</u>* maintained by hydrogen bonds between <u>R groups between polypeptides</u>* allowing for interaction between different subunits of same protein; Relate structure to function Ref. to specific example: Collagen: hydrogen bonds between R groups of adjacent polypeptide in a tropocollagen → high tensile strength; Note: tensile strength (pt5 and pt 10 – 1 mark] A: General description 3D conformation eg. Enzyme active site Ref. to → crucial for function eg binding to substrate complementary in shape and charge to catalyse reaction Ref to co-operativity eg: allosteric enzyme, haemoglobin
Cellulose/Carh ohydrates	 9. Cellulose: <u>hydrogen bonds between cellulose chains to produce cellulose fibres;</u> 10. that has <u>high tensile strength;</u>
Solubility	 In a largely aqueous environment, <u>ability of molecules to form</u> <u>hydrogen bonds with water;</u> a. <u>enables solubility</u> so that molecules can <u>e.g. (give 1 e.g.)</u>: transport, diffuse through, metabolised; b. lack of hydrogen bonds result in insolubility – relate to function eg. Structural.
DNA structure	 13. Hydrogen bonds <u>between complementary bases;</u> 14. causing DNA to be a <u>stable double-stranded</u> molecule;
DNA replication / transcription	 15. Hydrogen bonds between <u>complementary bases (A: idea of 2 strands)</u> are <u>weak bonds;</u> 16. hence it is <u>easy break and separate DNA into 2 strands</u> to allow for <u>DNA replication / transcription;</u>
	 17. DNA <u>replication</u> uses 1 strand as a template to synthesise new strand: <u>Free nucleotides / deoxyribonucleoside triphosphate (dNTPs)</u> form <u>hydrogen bonds</u> with bases on DNA strand; 18. <u>Transcription</u>: same as above but <u>free ribonucleotides / ribonucleoside triphosphate</u> form <u>hydrogen bonds</u> with DNA strand;
	 For 17 and 18, should identify product as either DNA or RNA; 19. Function: to use 1 strand as <u>template</u>* to synthesise a <u>complementary strand;</u>
tRNA and Translation	 20. tRNA has <u>intramolecular</u> hydrogen bonds; 21. maintain <u>3D conformation</u> of tRNA so that tRNA can <u>fit</u> into <u>active site*</u> of <u>aminoacyl-tRNA synthetase</u> to be joined to its corresponding amino acid;

	 22. Translation: <u>anticodon*</u> on <u>tRNA*</u> forms hydrogen bonds with <u>complementary</u>* <u>codon</u>* on <u>mRNA</u>*; 23. Allows polypeptide synthesised to have <u>amino acid sequence</u> <u>corresponding to codon sequence</u> on mRNA;
Ligand- receptor interactions	 24. <u>Ligand-receptor</u> interactions may also involve hydrogen bonds allow ligand to <u>bind</u>, cause conformational changes and trigger <u>cell signalling</u>; 25. As hydrogen bonds are <u>weak interactions, it will</u> allow for ligand to <u>bind and dissociate</u> (A: bind temporary) from receptor;
Enzyme- substrate complex	 26. Interaction involving enzyme <u>active site*</u> and <u>substrate</u> may also involve hydrogen bonds 27. which would help to <u>bind to substrate</u> and hold them in <u>correct orientation</u> for reaction;
Transpiration	 28. Hydrogen bonds form <u>between water molecules;</u> 29. allow for <u>water uptake</u> in plants via <u>transpirational pull/ capillary</u> <u>action;</u>

AVP; QWC: ref to 4 different context;

- (b) Discuss the diverse roles of membranes in a cell. [12]
 - Membranes are made up <u>phosphoslipid bilayer</u> with <u>hydrophobic core</u>;
 <u>Protein being embedded</u> within bilayer;

Context	Roles
Selectively	3. Being selectively permeable, membrane only allows some
permeable	molecules to cross directly without assistance;
/ S	4. <u>Non-polar/uncharged</u> (ignore: hydrophobic) molecules are able to
	dissolve and <u>diffuse</u> through;
	5. <u>lons/charged</u> and/or most <u>polar</u> molecules (ignore: hydrophilic)
	are <u>repelled;</u>
O a rear a ret	(Generic principle cannot be replaced by H ⁺)
Compart-	 Formation of <u>unique environment</u> for <u>highly specialised activities;</u> e.g. lysosomes maintain an acidic environment that favours its
mentalisation / C	 e.g. <u>lysosomes</u> maintain an <u>acidic</u> environment that favours its enzymes to work;
/0	enzymes to work,
	8. localization of enzymes for reactions to take place so that they are
	 <u>localization</u> of enzymes for <u>reactions</u> to take place so that they are not suppose to catalyse reactions that they are not supposed to;
	 e.g.: <u>enzymes</u> in Calvin Cycle in <u>stroma;</u>
	5. c.g.: <u>chzymes</u> in odivin oyole in <u>sitoma,</u>
	10. accumulation of ions to generate a concentration gradient;
	11. e.g. H ⁺ in intermembranal space in mitochondria/within thylakoid
	space in chloroplasts establishes a proton gradient for
	chemiosmosis* and formation of ATP;
	12. <u>Storage</u> of <u>food source</u> ;
	13. e.g. starch in plant cells are stored in amyloplasts;
	R: starch grain, make sure it is membrane bound
Localisation	14. Allows <u>functionally-related proteins</u> to be <u>grouped</u> together to
of proteins	enhance <u>sequential biochemical</u> processes;
of a related function	15. e.g. enzymes and proteins are grouped into photosystems II and I
TUNCTION	on thylakoid membrane of chloroplast so that electrons from

/ L	photosystem II are shuttled to photosystem I during photophosphorylation;
Increase surface area / I	 16. <u>Highly folded increases surface area</u> to hold <u>more;</u> 17. e.g.: <u>electron transport chains/ ATP synthase</u> in <u>cristae</u> of <u>mitochondria;</u>
Cell-cell recognition and adhesion / R	 18. <u>Glycoproteins</u> / <u>glycolipids</u> are involved in cell-cell <u>recognition</u>; 19. (any 1 e.g.) <u>eg: T cell receptor</u> on membranes of <u>naïve T cells</u> + how it helps to recognize <u>peptide: MC</u> on <u>antigen presenting cells</u>;
Signal transduction / T	 20. Some transmembrane proteins serve as cell surface <u>receptors</u> to transfer <u>information</u> from <u>environment</u> into cell when specific molecules (ligands) bind to them; 21. E.g. <u>glucagon</u> (hormone) bind to <u>glucagon receptor</u> in membrane which triggers a cascade of chemical reactions leading to <u>hydrolysis of glycogen to glucose;</u> E.g. insulin
QWC: ref to 3	different contexts

4 (a) Discuss how the complementary shapes between biomolecules enable eukaryotic cells to carry out their diverse functions. [13]

Category	Description
Transport protein – molecule to be transported / T	 A <u>transport protein</u> has a <u>binding site</u> complementary in shape to <u>specific for molecule</u>, E.g. <u>pyruvate</u> – <u>pyruvate translocase</u> / <u>water</u> - <u>aquaporin</u>
Enzyme – substrates / E	3. <u>Substrates</u> have complementary <u>conformation</u> * and <u>charge</u> * to <u>active site</u> (conformation is a more accurate word than 'shape');
	 <u>DNA polymerase</u> – active site can bind to <u>deoxyribonucleotides</u>, catalyses <u>formation of phosphodiester bonds</u> between <u>adjacent DNA nucleotides</u> to synthesise new strand (DNA replication); <u>DNA ligase</u> – active site can bind to <u>deoxyribonucleotides</u>, <u>forms a phosphodiester bond between two DNA fragments/ sealing nick;</u> RNA polymerase – active site can bind to <u>ribonucleotides;</u> and <u>catalyses formation of phosphodiester bonds</u> between adjacent RNA nucleotides of newly synthesised strand; <u>Amino acyl RNA synthetase</u> – active site complementary to an
Destain	 <u>amino acid</u> and <u>tRNA</u> molecule; 11. in order to join them to form an amino acyl tRNA molecule, to help form peptide bonds between <u>amino acids;</u> 12. Drataine may have DNA binding demaine (sites that are an an
Protein	12. Proteins may have DNA binding domains / sites that are

– nucleic acid / PN	complementary in shape and charge to specific DNA sequence;				
	 13. <u>RNA polymerase</u> – <u>promoter</u> sequence; 14. <u>Initiating RNA synthesis/transcription</u> at <u>correct position</u> / in frame to produce functional protein; 				
	 15. <u>Transcription factors</u> - <u>specific sequence of DNA</u> adjacent to the genes that they regulate; 16. Activators - specific transcription factors known as <u>activators*</u> bind to <u>enhancer nucleotide sequences</u> 17. to <u>increase frequency of transcription;</u> / promoting assembly of <u>transcription initiation complex;</u> 18. Repressors - specific transcription factors known as <u>repressors</u>* bind to <u>silencers*</u> nucleotide sequences 19. to <u>decrease frequency of transcription</u>/ inhibiting assembly of <u>transcription initiation complex;</u> 				
Protein	 20. <u>Spliceosome</u> recognises <u>DNA sequence at intron-exon</u> boundary, 21. to <u>excise intron and join exons to form mature mRNA;</u> 22. <u>DNA-binding proteins</u> (e.g. transcriptional repressors, histone deacetylases and repressive chromatin remodeling complexes) recognize and bind to <u>methylated DNA</u> 23. to <u>condense chromatin</u> (A: converse) results in <u>gene silencing</u>/ no gene expression; 24. Proteins may have <u>protein binding domains / sites</u> that are 				
– protein / PP	<u>complementary in shape and charge</u> to other <u>protein</u> molecules;				
	 25. Activators – specific transcription factors known as <u>activators*</u> bind to <u>general transcription factors</u> at enhancers; 26. <u>promoting assembly of transcription initiation complex;</u> 27. Repressors – specific transcription factors known as <u>repressors</u>* bind to <u>general transcription factors</u> at silencers; 28. <u>inhibiting assembly of transcription initiation complex;</u> 				
Transport Or enzyme Or enzyme complex to link	 29. <u>H</u>⁺ must fit into <u>ATP synthase</u> 30. so that can <u>diffuse</u> through <u>ATP synthase</u>, so that ADP can be <u>phosphorylated</u> to form ATP; 				
Ligand - receptor / L	31. <u>Ligand</u> has a <u>complementary shape</u> that allows it to fit precisely into (extracellular domains_of) <u>receptor</u> molecules on <u>target cells</u> ;				
	32. in order to transfer <u>information</u> from <u>environment</u> into cell when specific molecules (ligands) bind to them / convert extracellular signal to an intracellular signal;				
	 33. Eg. Cell surface receptors: has an <u>extracellular <i>ligand-binding site</i></u> binding to <u>hydrophilic ligand</u>, 34. Eg. <u>Binding of ligand</u> causes <u>conformational change</u> in 				

	intracellular domain of receptor; can ref to specific receptor. 35. Eg. <u>GPCR</u> : Ligand binding to GCPR causes <u>conformational</u> <u>change</u> in intracellular domain of receptor allowing it to bind to and <u>activate G protein</u> ;			
	or <u>RTK: conformational change</u> <u>activation of kinase region</u> to result in <u>cross-phosphorylation</u> ;			
Immune system / I	36. <u>Ligand</u> has a <u>complementary shape</u> that allows it to fit precisely into (extracellular domains_of) <u>receptor</u> molecules on <u>target cells</u> ;			
	 37. <u>Naïve T cell</u> has specific <u>T cell receptor</u> that fits and binds to <u>peptide:MHC complex</u> presented on antigen presenting cells; 38. Helps to trigger clonal expansion and differentiation of naïve T cells; 			
	39. <u>T helper cells</u> has specific <u>T cell receptor</u> that fits and binds to specific <u>B cell receptor</u> on <u>naïve B cells</u> ;			
	40. Helps to trigger clonal expansion and differentiation of naïve B cells;			
	41. Antibodies have unique <u>antigen binding site</u> that can bind to <u>antigens/ pathogens</u>			
	42. causing opsonisation or neutralisation;			
Function point (eg: are given).	points 5, 14 can only be given if named of interacting molecules			
AVP – any valid points are awarded depending on importance of interacting molecules				
and depth of description.				
QWC: 3 different categories + overacrching principle for each category				

- (b) Organisms are able to detect and respond to changes in their environments. Discuss this, with reference to specific examples in unicellular and multi-cellular organisms. [12]
 - Unicellular organisms eg bacteria: <u>responds to changes in the nutrients</u> available in the environment <u>in order to optimise use of resources / produce enzymes only when</u> <u>necessary</u> / WTTE;
 - Specific example: <u>*lac operon*</u>*: <u>genes involved in lactose metabolism</u> under control of a <u>single *promoter*</u>* and <u>*operator*</u>*;
 - 3. <u>Lac I codes for active lac repressor</u> which <u>binds to operator</u> region to <u>prevent</u> <u>expression</u> of structural genes in *lac* operon;
 - 4. <u>Presence of lactose</u> results in production of allolactose which binds to <u>lac repressor</u> to <u>inactivate</u> it;
 - 5. Allowing for <u>structural genes to be transcribed</u> and translated to produce bgalactosidse, permease and transacetylase
 - 6. Allows bacteria to take up lactose from its surrounding and break down lactose into glucose and galactose;
 - 7. Presence of glucose results in <u>low cAMP</u>. Hence <u>catabolite activator protein (CAP)</u> is <u>not activated</u>;
 - 8. And is therefore <u>unable to upregulate / increase</u> the frequency of transcription of the structural genes;
 - Specific example 2: trp operon: <u>genes involved in synthesis of tryptophan</u> under the control of a <u>single *promoter*</u>* and <u>operator</u>*; (promoter and operator can be in pts 2 or 7)
 - 10. <u>*Trp*</u> *R* codes for <u>inactive trp</u> repressor which <u>cannot bind</u> operator, <u>allowing for</u> <u>transcription</u> of structural genes in *trp* operon;

- 11. Tryptophan binds to *trp* repressor to activate it;
- 12. Tryptophan synthesis is thus stopped when bacteria is able to obtain sufficient amounts of tryptophan (from its environment);
- 13. Multicellular organsms eg humans have mechanisms to maintain a <u>constant internal</u> <u>environment despite changes in the external environment;</u>
- 14. Specific example: maintenance of blood glucose concentration;
- 15. Intake of food results in an increase in blood glucose concentration
- 16. which stimulates production of *insulin** by _-cells* in the islets of Langerhan*;
- 17. Insulin binds to insulin receptors on muscle cells* and liver cells*;
- 18. Causes increase uptake of glucose into cells and conversion of glucose to glycogen;
- 19. Lack of food results in low blood glucose concentration as glucose;
- 20. which stimulates production of glucagon* by a-cells* in islets of Langerhan*;
- 21. Glucagon binds to glucagon receptors on liver cells included
- 22. Causes breakdown of glycogen into glucose and release of glucose into blood;

(Signal transduction)

- 23. Receptor allows transduction from extracellular to intracellular;
- 24. <u>Multiple steps</u> in pathway allowing for many pts of control / <u>different pathways in</u> <u>different cells</u> to produce a coordinated response;
- 25. signal amplification to produce a large response;

QWC: at least 1 ref to unicellular and 1 to multicellular; Cap unicellular to 7, multicellular to 7 A: details on nervous system

Paper 4

1 (a)

(b)

- Start time / end time did not reset stopwatch between readings, record in e.g. 00:30, 02:15;
 - Computation of time taken, in whole numbers;
 - Table 1.1: correct trend of data; in ascending order of time taken W2, 4, 1, 5, 3;
 - Table 1.2: correct trend of data; in ascending order of time taken W2, 4, 1, 5, 3;
- (c) Prepare and complete a table in the space below to show the mean time taken to reach the end-point for each glucose solution. [4]
 - 1. Heading (glucose solution, mean time taken with correct units (<u>s not sec</u>))
 - 2. Correct Calculation of mean;
 - 3. Mean time taken recorded to nearest second / 1 d.p.;
 - 4. Table must show all grid lines and border, drawn using ruler.
- (d) A sixth boiling tube should be set-up. Describe how a control is set-up and explain the reason for this. [2]
 - 1. With all other variables kept constant, replace glucose solution is with distilled water;
 - 2. This is to show that <u>glucose is responsible</u> for reduction and hence the <u>decolourisation /</u> <u>colour change</u> of permanganate;
- (e) Present the above data in a graphical format. [5]
 - 1. S Scale must be appropriate and both axis must cover at least 1/2 of the space given.
 - 2. A correct choice of axes + correct labels and units + correctly labelled intervals
 - 3. P all points plotted correctly
 - 4. L all points joined by line of best fit and
 - 5. E no extrapolation beyond plotted points
- (f) Estimate the concentration of,
 - (i) solution **W1** by using the graph

Concentration of solution W1 :[1]

Read off graph + show dotted line on graph and values on x and y axes + Value should be within teachers' range.

Without extrapolating the graph, estimate the concentration of solution W5 by :

(ii) Using the formula y=mx + c,. Show your working clearly, on how the values of m and c are calculated.

Concentration of solution U :[3]

Use values from graph to calculate gradient ;
 m: Gradient = 115 - 10

2) Use gradient (m) and y value from graph to calculate C ; Reject if candidate use y = 0

$$Y = -5.25x + C$$

C :

$$88 = -5.25(10) + C$$

C = 140 5

3) Use calculate gradient (m), value of C and time taken for wW to turn from pink to colourless to calculate concentration. Values of time take for W5 to turn colourless must be within teachers' value.

X : substituting y = mx + C ,
77.5 =
$$-5.25x + 140.5$$

X = 12 %

- 2 You are given a prepared slide showing a section of a root tip showing different stages of mitosis.
- (a) Examine the slide. Identify 2 cells at different stages of mitosis and make a large labelled drawing of each cell. Indicate the stage of mitosis for each of the cells drawn. [6]
 - 1. Drawing of chromosomes matched with label of stage and accurately represented (cell 1)
 - Drawing of chromosomes matched with label of stage and accurately represented (cell 2)
 * Reject if individual chromosomes are drawn or if drawing is inaccurate. E.g. nuclear enveloped has not disintegrated in Metaphase or Anaphase.
 * Reject interphase. Not part of mitosis.
 - 3. Sharp and smooth continuous line. Reject fuzzy lines, or messy drawing with blunt pencil
 - 4. Proportion: Thin cell wall of consistent thickness + size of chromosomes
 - 5. Labels: cellulose cell wall, plasma membrane, chromosome, cytoplasm (any 3 labelled)
 - 6. Large: 2/3 of space provided
- (b) Calculate the actual length of the chromosome shown in Fig. 2.1 in μ m.

.....μm [3]

10 eyepiece graticule division = 0.02mm = 20μ m; (conversion of mm to μ m) 1 eyepiece graticule division = $20/10 = 2\mu$ m; (calibrate eyepiece graticule) A: alternative definitions of a "division"= smallest division as 1 according to diagram or smallest division = $\frac{1}{4}$ 1 division = 20/40 = 0.5um

Length of chromosome = 1.5 eyepiece graticule = 3μ m; (measurement of chromosome)

- (c) Using the prepared slide, count the number of cells in each stage of the mitotic cell cycle, using 100 cells from a randomly chosen field of view of the microscope. Repeat the count for another chosen field of view.
 - (i) Prepare and complete a table in the space below to show the percentage of cells in each stage of the mitotic cell cycle. [5]

Stage	Num	Percentage of cells				
	Count 1	Count 2	Average	in stage / %		
Interphase						
Prophase						
Metaphase						
Anaphase						
Telophase						

- 1. Appropriate headings with units
- 2. All stages listed
- 3. Cells out of 100 + average recorded to nearest whole number
- 4. Percentage of cells to whole number
 - A and %: up to 1 d.p.
- 5. Correct trend: Interphase most, Meta/Ana least
- (b) The percentage of cells observed in each stage can be used to infer the duration of the stage.
 - (i) State the longest stage of the cell cycle and explain how you arrived at your conclusion. [2]
 - 1. Interphase; (ecf based on student's results)
 - 2. <u>Majority / Higher percentage</u> of cells in stage <u>implies</u> that the <u>stage took the</u> <u>longest duration to complete</u> hence highest probability of finding a cell in that stage;
 - (ii) A student commented that a vital piece of information is required to determine the exact duration of each stage. Suggest what is this vital piece of information. [1]

(iii) Another student commented that this method of determining the duration of each stage is inaccurate.

Other than the absence of 3 replicates, suggest why it is so and describe how you can improve on the accuracy of the results obtained. [2] Error:

• <u>Small numbers (100) of cells counted /</u> small number cells in metaphase / anaphase / telophase / not all cells in each stage may be observed, hence not accurate;

How to improve:

- Count <u>larger number of cells</u> eg. 200 (must specify at least 200) / different root samples or slides ;
- (c) Using the formula above and the probability table given, what conclusion can be drawn from this t-test? [3]
 - 1. Degree of freedom = 10 + 10 2 = 18, from table: p<0.001;
 - 2. Since p<0.05, at 5% level of significance,
 - 3. the difference between T1 and T2 is significant and not due to chance;
 - 4. Rate of cell division is higher at higher temperature;
- 3 It is known that Q, an organic compound can act as an inhibitor of phosphatase. Using the list of apparatus below, design an experiment to determine if Q is a competitive or a non-competitiveinhibitor. [14]

Aim

To determine if Q is a competitive or a non-competitive inhibitor of phosphatase activity.

Theory

- T1. Substrate, phenolphthalein diphosphate (XP) <u>complementary in shape and charge</u> (must have both shape and charge) to <u>active site</u>* of phosphatase;
- T2. Intensity of colour/pink compound can be <u>measured</u> using a spectrophotometer over a <u>fixed period of time</u> is an indication of <u>phosphatase activity</u>;
- T3. If Q is a <u>competitive</u> inhibitor, it competes with <u>substrate for *active site*</u>*;
- T4. If Q is a <u>non-competitive</u> inhibitor, it <u>binds to a site other than active site</u>, <u>causing a</u> <u>change in active site</u>;

[Theory T1, T2, max 2 marks]

<u>Trend</u> (Expected results / how to make conclusion)

- Tr1. If Q is a <u>competitive</u> inhibitor, <u>same maximum absorbance</u> reached <u>at high [XP]</u>, compared to without inhibitor;
- Tr2. If Q is a <u>non-competitive</u> inhibitor, <u>lower maximum absorbance</u> <u>at high [XP]</u>, compared to without inhibitor

[Trend Tr, max 1 mark]

<u>Variables</u>

	Description	How it is controlled			
Independent variable	IV1: Presence and absence of inhibitor	Conduct experiment, <u>with and without</u> <u>inhibitor Q</u> .			
	IV2: <u>5 concentrations</u> of XP	• Dilution table (5 regular [XP]).			
Measurable variable	MV: <u>Measure</u> absorbance	 <u>Measure 1 cm³ of solution (XP +</u> <u>phosphatase + sodium carbonate)</u>, add it into a <u>cuvette</u>. 			
Dependent variable	DV: <u>Rate of</u> phosphatase activity	 obtained by measuring <u>absorbance</u> of pink colouration divided by <u>time</u> (idea of fixed time). 			

_						
-						
[Independent variable – IV1: with and without inhibitor Q, IV2: 5 concentrations of XP in						

dilution table]

[Measurable variable – state + how it is measured (MV)]

[Dependent variable – state + how it is measured (DV)]

Conduct a pilot experiment to determine suitability of apparatus, range of independent variables (e.g. range of concentration of inhibitor Q), duration of experiment & amount of materials used.

Numbered steps

Preparation of inhibitor solution

1. Using 10 cm³ syringe, prepare <u>5 different concentrations of XP</u> solution by following dilution table below:

Concentration of XP /%	Volume of 10% XP used /cm ³	Volume of distilled water used /cm ³		
2	2.0	8.0		
4	4.0	6.0		
6	6.0	4.0		
8	8.0	2.0		
10	10.0	0.0		

Preparation of enzyme reaction

2. Set up a water bath of <u>37^oC</u>, <u>using hot and cold water</u>, <u>and use a thermometer to</u> <u>monitor temperature</u>.

(constant variable: temperature

+ how: using <u>hot and cold water, use a thermometer to monitor temperature</u>
+ why: temp. affect rate of enzyme catalyzed reactions)

- 3. Place <u>substrate XP</u>, <u>enzyme phosphatase</u> and inhibitor Q, <u>separately</u> into water bath. Allow <u>1 min of equilibration time</u>, so that solutions <u>can attain temperature</u> of water bath. [Equilibration E]
- 4. Using a syringe add 1 cm³ of 2%, 4%, 6%, 8% and 10% XP to test tubes.
- 5. Add 1 cm³ of 5% inhibitor Q to each test tube.
- Add 1 cm³ of pH buffer 7 to each test tube. (constant variable: pH + how: using <u>buffer/ fix pH eg pH7</u> + why: pH changes denatures enzymes)
- Add 1 cm³ of 5% phosphatase to each test tube. Mix well and incubate mixture in water bath. (constant variable: concentration of enzyme / volume of enzyme
 - + how: using syringe / fix vol. eg 1 cm³
 - + why: affect total amount of enzyme / final [enzyme] , so affects rate of reaction
- Prepare a "<u>blank</u>" fill a cuvette with 1 cm³ distilled <u>water</u> and measure its absorbance value using a spectrophotometer. This acts a <u>blank</u> / absorbance should be at <u>0%</u> / if it is not 0% then spectrophotometer should be <u>re-zeroed</u>.
 [Blank state how + why, B]
- 9. After 5 minutes, add 1 cm³ of mixture with 1 cm³ sodium carbonate to get a pink colouration.

(constant variable: reaction time)

10. Transfer <u>1 cm³</u> of reaction mixture into a cuvette and measure absorbance value/colour intensity of pink solution using a spectrophotometer.

11. Repeat Step 3-10 using distilled water instead of inhibitor Q.

12. Record colour intensity in absorbance value (A) for each concentration of XP.

13. Plot a graph of colour intensity in absorbance value/A against concentration of XP.

[Constant variable: state any 2]

[Constant variable: how + why it is controlled CV1]

[Constant variable: how + why it is controlled CV2]

<u>Control</u>

- Keep all variables constant, set up control experiment using boiled and cooled phosphatase/ distilled water instead of inhibitor Q.
- This is to <u>obtain baseline results</u>, in <u>absence of inhibitors</u>/ to show <u>phosphatase enzyme</u> is responsible for dephosphorylation of XP.

[Control: state + purpose, Co]

Repeats & replicates

- Conduct 3 replicates for each condition to check that no anomalies are present.
- Repeat entire experiment twice to <u>check for reproducibility</u>. [Repeat and replicates: state how + why purpose, RR]

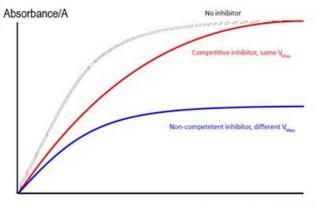
Data Recording and Processing

Record and process data: (table and graph should have same heading / unit)

Concentratio	Absorbance /A							
	With inhibitor Q			Without inhibitor Q				
of XP /%	Trial 1	Trial 2	Trial 3	Avera	Trial 1	Trial 2	Trial 3	Avera
				ge				ge
2								
4								
6								
8								
10								

[Table, Ta]





Concentration of XP/%

[Graph, G: shows expected trend]

Risks & precautions

1. Wear gloves and <u>goggles</u> when handling XP solution, inhibitor Q solution and phosphatase solutions as they may be toxic.

2. Do not touch electrical switch of the spectrophotometer with wet hands to prevent electrocution.

3. Wear oven mittens / insulating gloves when handling hot water to avoid scalding. [Risk and Precautions, RP: state 1 risk + precaution]

Compulsory point to get max 14m: Answering the question