BIOLOGY DEPARTMENT
JC2 PRELIMINARY EXAMINATIONS
2016 Higher 2

CANDIDATE NAME

CLASS

BIOLOGY 9648/01

Paper 1 Multiple Choice **22 September 2016**

1 hour 15 minutes

Additional Materials: Multiple Choice Answer Booklet

READ THESE INSTRUCTIONS FIRST

Write in a soft pencil.
Do not use any staples, paper clips, highlighters, glue or correction fluid.
Write your name, Centre number and candidate number on the Answer Sheet in the spaces provided.

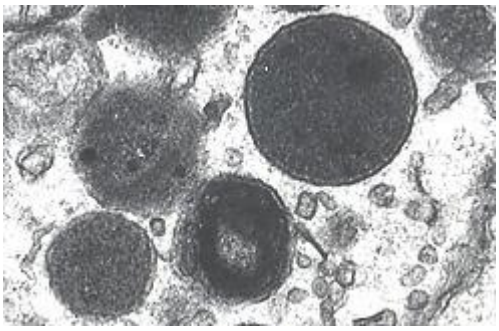
There are **forty** questions on this paper. Answer **all** questions. For each question there are four possible answers **A, B, C** and **D**.
Choose the **one** you consider correct and record your choice in **soft pencil** on the separate Answer Sheet.

Read the instructions on the Answer Sheet very carefully.

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

1. The electron micrographs of several structures of a liver cell are shown below.

1



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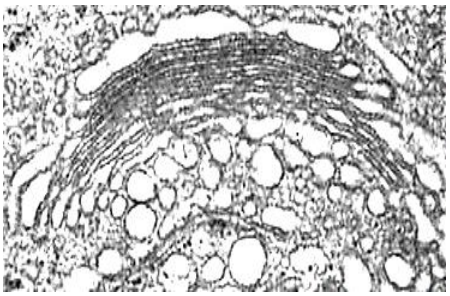

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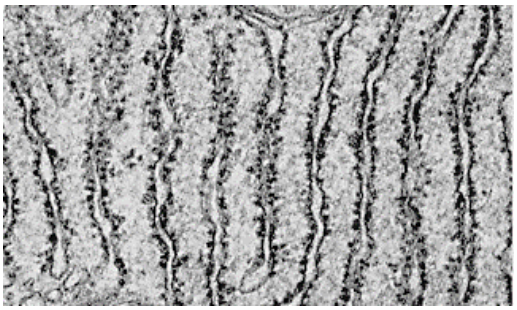

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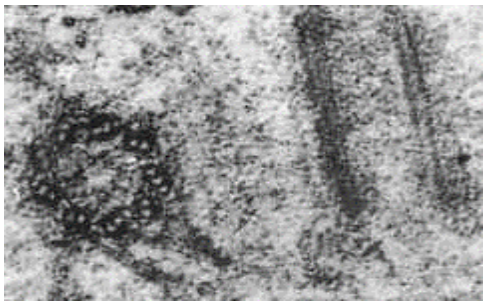
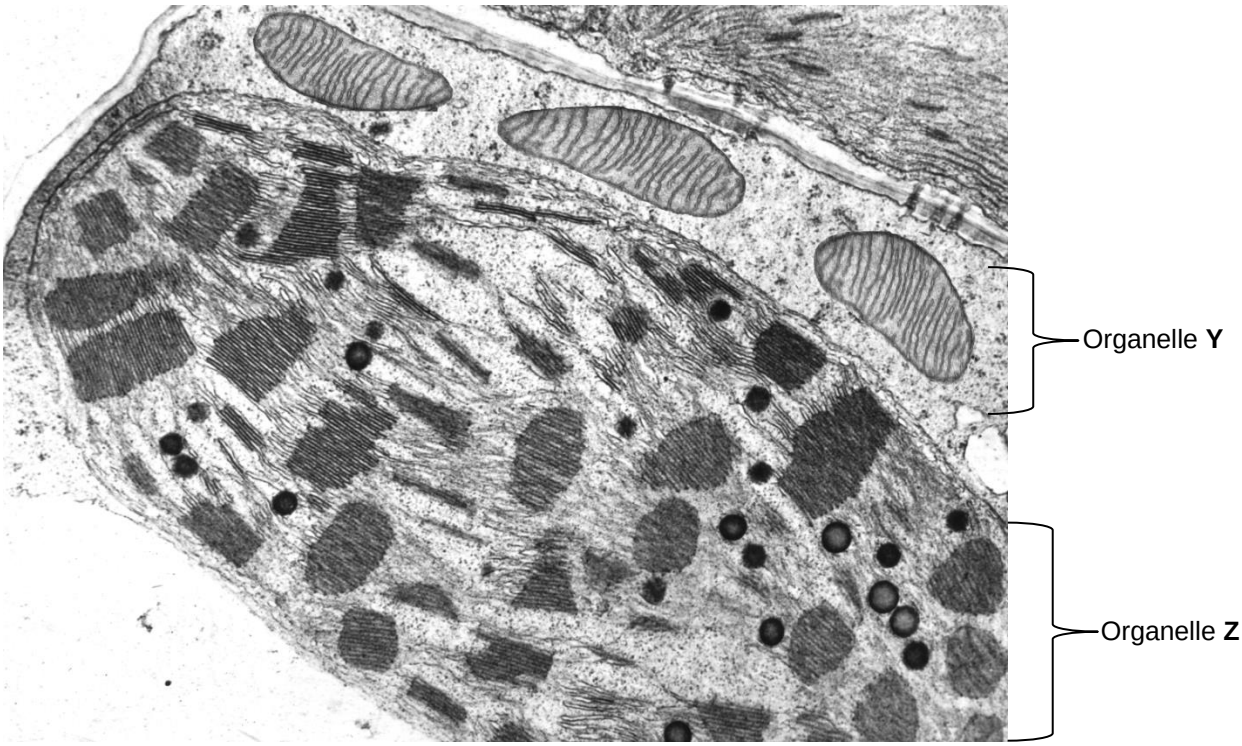


Radioactive amino acids were introduced into the cell to trace the path taken in the formation of hydrolytic enzymes.

Which of the following options correctly show the time taken, in minutes, for radioactivity to be detected in the structures **1 – 4**.

	Time taken, in minutes, for radioactivity to be detected in structure			
	1	**2**	**3**	**4**
A	3	10	20	30
B	30	20	3	-
C	10	20	30	3
D	20	10	3	30

2. The electron micrograph below shows two organelles **Y** and **Z** in a leaf mesophyll cell of a plant.



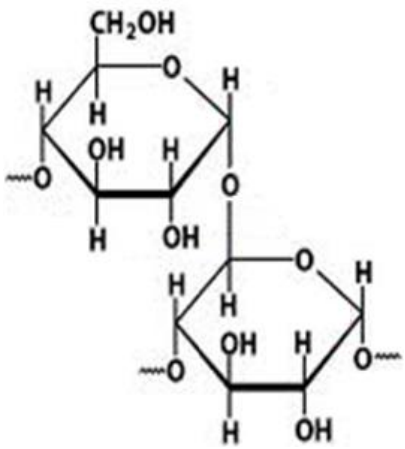
Which of the following statements are **not** true about organelles **Y** and **Z**?

1 Organelle **Z** utilises transporters to export ATP to organelle **Y** to drive cellular activities.
2 Oxygen released by organelle **Z** is used in organelle **Y** during glycolysis.
3 Transcription and translation occurs in both organelles.
4 Organelle **Y** has electron transport chain proteins but organelle **Z** does not.

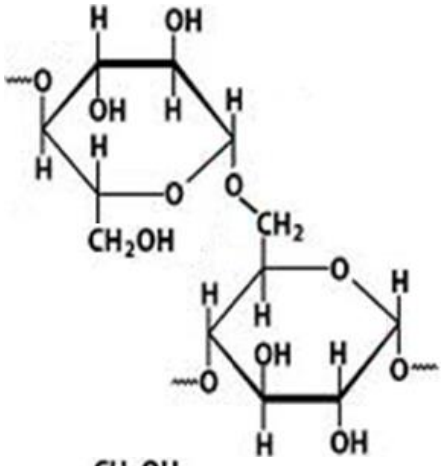
A 1 and 2 only
B 3 and 4 only
C 1, 2 and 4
D All of the above

3. Which of the following correctly shows an α 1,6 glycosidic bond found in amylopectin?

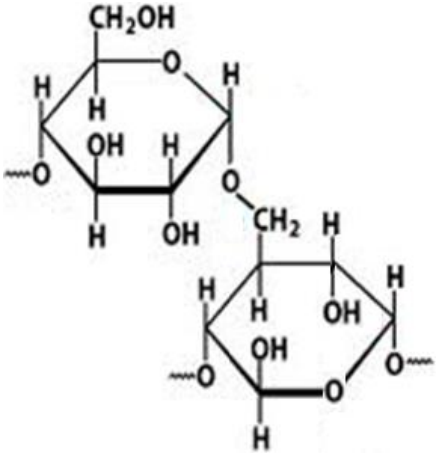
A



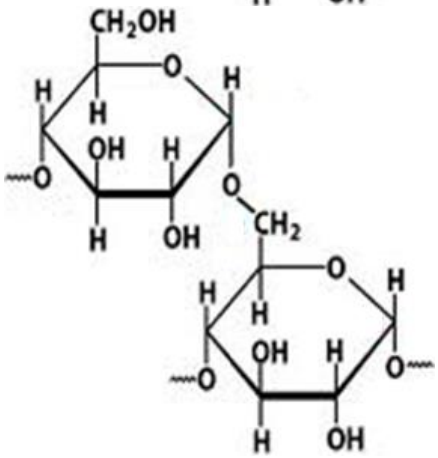
B



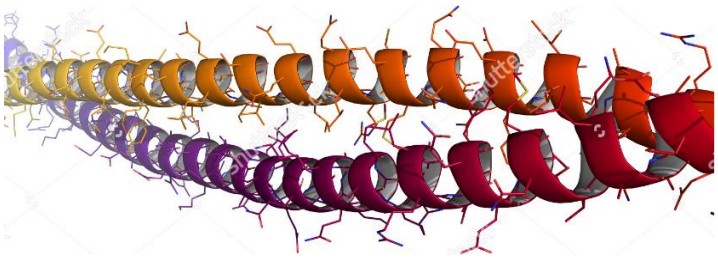
C



D



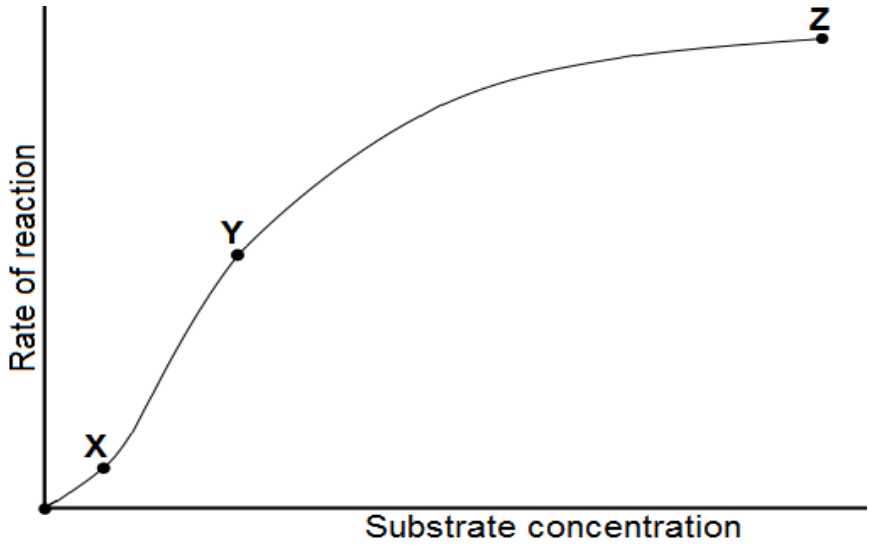
4. The diagram below shows the structure of a protein found in the hair, claws and horns of many animals.



Which of the following is true about the protein?

A The polypeptides are arranged in a staggered manner to increase in stability.
B The protein is insoluble in water due to hydrophobic R groups on the exterior.
C The secondary structure of the protein is maintained by hydrogen bonds between R groups.
D Every third amino acid is a proline.

5. The graph below shows the effect of increasing substrate concentrations on the activity of an allosteric enzyme under optimum conditions.



Which of the following statements is correct?

A There is low kinetic energy at **X** to overcome the activation energy, thus resulting in a low rate of reaction.
B Rate of reaction increases at a faster rate at **Y** as the allosteric activator outcompetes the allosteric inhibitor to bind to the allosteric site.
C At **Z**, enzyme molecules are in the active state and active sites are saturated.
D Substrate concentration is the limiting factor at **X** and **Y** but temperature is the limiting factor at **Z**.

6. Which of following statements regarding the fluid mosaic model are correct?

1 Fluidity of the membrane is a result of hydrophilic and hydrophobic interactions between components of the membrane.
2 Cholesterol maintains the fluidity of membrane by preventing the two layers of phospholipids from moving too far away from each other.
3 The attachment of different carbohydrates to the components of the membrane gives the look of a mosaic.
4 Fluidity of the membrane allows for the entry and release of influenza viruses.
5 Cholesterol increases membrane fluidity by binding to the phospholipid tails which causes the tails to bend.

A 1 and 4
B 2 and 3
C 1, 4 and 5
D 3 and 5

7. An experiment was conducted to determine the mode of entry of a drug into

animal cells. Cells which did not contain the drug were placed into separate containers with different concentrations of the drug. The concentrations of the drug inside the cells at the end of 10 minutes were obtained. The experiment was conducted in 2 different temperatures. The results are shown in the tables below.

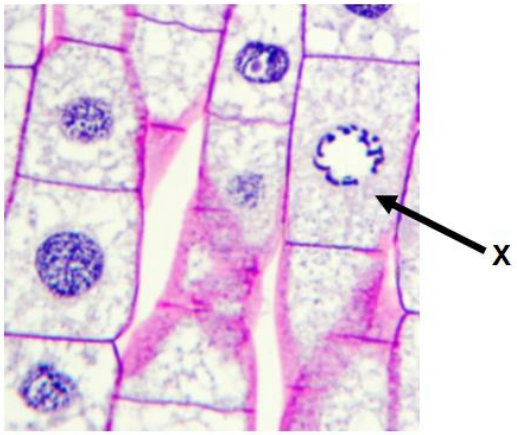
Experiment conducted in 20^{o}C	
Concentration of drug in the container / moldm^{-3}	**Concentration of drug inside the cells after 10 minutes / moldm^{-3}**
0	0
10	4
20	7
30	11
40	13
50	13
60	13

Experiment conducted in 30^{o}C	
Concentration of drug in the container / moldm^{-3}	**Concentration of drug inside the cells after 10 minutes / moldm^{-3}**
0	0
10	5
20	9
30	14
40	20
50	20
60	20

Which of the following statements is incorrect?

A The drug molecule is hydrophilic and water-soluble.
B A drastic change in extracellular pH will decrease rate of drug entry.
C No ATP is required for the entry of the drug.
D Increasing membrane fluidity results in faster drug entry.

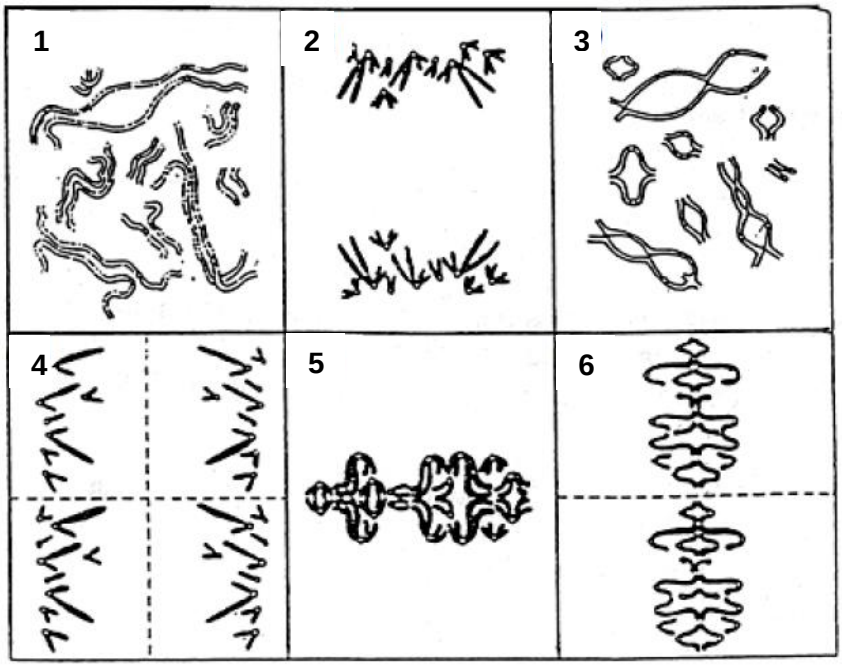
8. X shows a cell at a particular stage of cell division.



For the next stage in this nuclear division, what would be correct?

	Centrioles	Nuclear envelope	Paired chromatids
A	present	breaking down	absent
B	absent	reforming	present
C	present	absent	present
D	absent	absent	absent

9. The figure below shows 6 stages of the process of meiosis occurring in a plant cell. (2n = 18)



What is the correct order of these 6 stages?

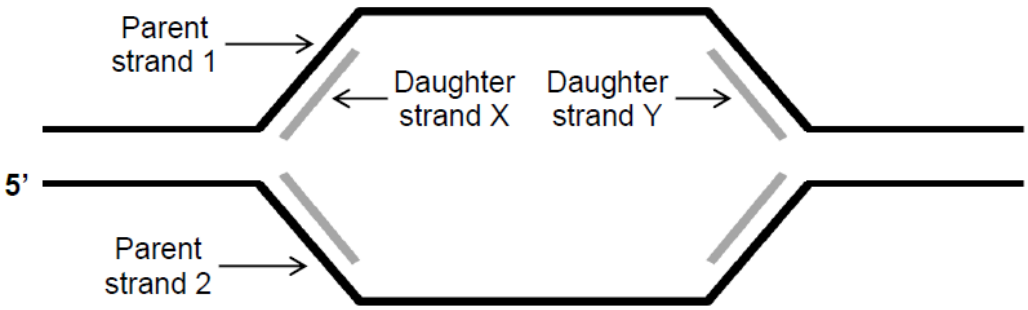
A 3, 5, 2, 6, 4, 1
B 3, 1, 5, 2, 6, 4
C 2, 3, 1, 5, 6, 4
D 1, 3, 5, 2, 6, 4

10. The table below shows the events that occur at different stages of the cell

cycle. Which row shows the correct event for the respective stages?

	Late interphase	Prophase I	Metaphase I	Anaphase II
A	DNA replication	condensation of chromosomes	alignment of chromosomes at the equator	separation of chromosomes
B	DNA replication	pairing of bivalents	alignment of bivalents at the equator	separation of sister chromatids
C	protein synthesis	crossing over	alignment of bivalents at the equator	separation of sister chromatids
D	replication of organelles	pairing of bivalents	alignment of chromosomes at the equator	separation of chromosomes

11. A simplified representation of a replication bubble is shown in the figure below. Parental strands 1 and 2 and the growing daughter strands X and Y are indicated.



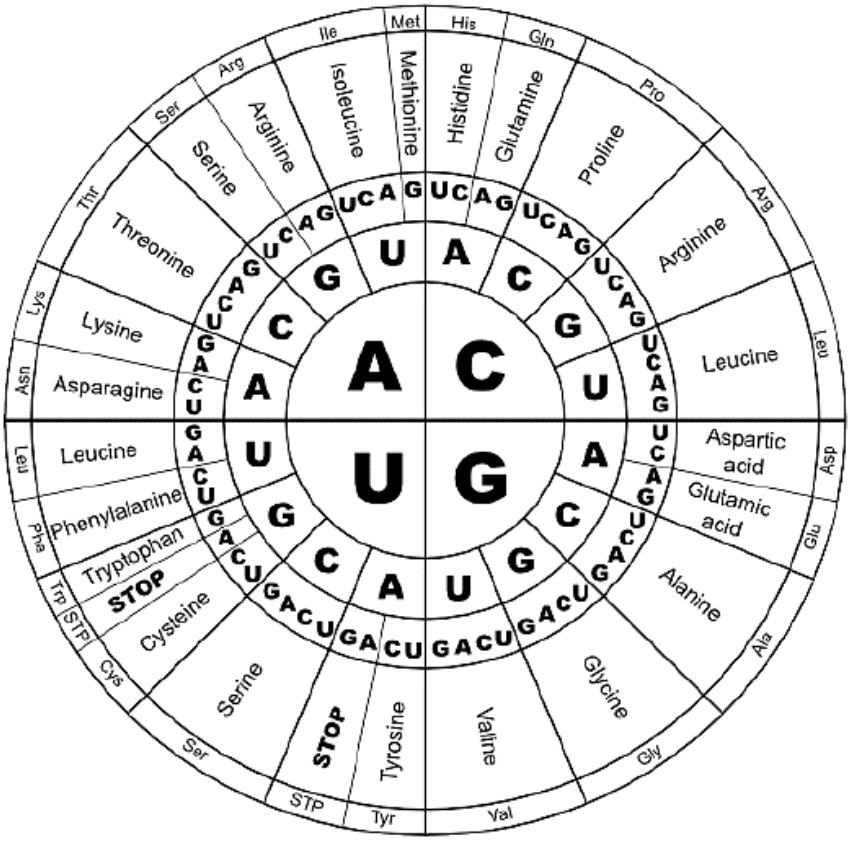
Which of the following statements about the syntheses of daughter strands X and Y is correct?

A Daughter strands X and Y are synthesised away from their respective replication forks.
B Daughter strand X is synthesised continuously while daughter strand Y is synthesised in the form of Okazaki fragments.
C Daughter strand X is synthesised in the 5' → 3' direction while daughter strand Y is synthesised in the 3' → 5' direction.
D DNA ligase will eventually catalyse the fusion of daughter strand X with daughter strand Y.

12. The first five DNA triplets that code for a particular protein is shown below:

3’ CAC GGA AGC CCA GAA 5’

The genetic information in the sequence above is eventually converted into a specific amino acid sequence according to the figure below.

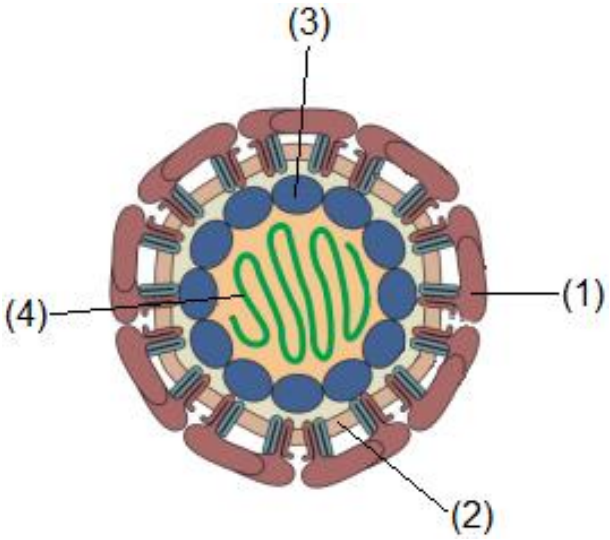


With the aid of figure, what is the sequence of the protein encoded by the DNA sequence above?

A His – Gly – Ser – Pro – Glu
B Val – Pro – Ser – Gly – Leu
C Lys – Thr – Arg – Arg – His
D Phe – Try – Ala – Ser – Val

13. Zika virus, formerly a neglected pathogen, has recently been associated with microcephaly in foetuses, and with Guillian–Barré syndrome in adults. Recent research into its structures and replication cycle has aided medical scientists in designing potential vaccines against the virus.

The diagram below shows the structure of Zika virus.



Which of the following shows correctly the components of each labelled structure in the diagram?

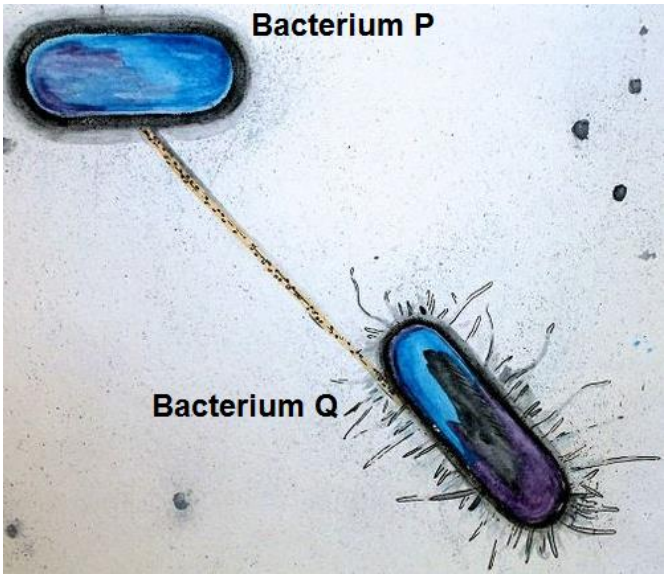
	(1)	(2)	(3)	(4)
A	Lipids	Phospholipids	Protein	DNA
B	Protein and carbohydrates	Phospholipids and cholesterol	Protein and carbohydrates	DNA
C	Protein and carbohydrates	Phospholipids and cholesterol	Protein	RNA
D	Carbohydrates	Phospholipids	Protein and carbohydrates	RNA

14. Which of the following are valid comparison between the replication cycles of a lambda phage and HIV?

1 Both replication cycles involve uncoating to release viral genome into the cytoplasm.
2 The protein involved in receptor binding for HIV is attached with short carbohydrate chains but not lambda phage.
3 The synthesis of viral proteins in both viruses involves transcription of viral DNA and translation.
4 Both involve the insertion of viral DNA into host genome and may cause insertional mutagenesis leading to uncontrolled cell division.
5 The replication cycle of HIV involves enzymes not coded by the host genome but not lambda phage.

A 1 and 4
B 2 and 3
C 2, 4 and 5
D 1, 3, and 5

15. The following shows a process taking place among prokaryotic cells.



Which of the following is false regarding the process?

A The ability of a bacterium to carry out the process is conferred by genes present on a plasmid.
B RNA primers are needed to provide free 3' OH ends for DNA replication in both bacteria.
C The DNA is linear as it is being transferred between the two bacteria via the cytoplasmic bridge.
D Genes transferred from bacterium P to bacterium Q are not essential for survival normally but are beneficial under stressful conditions.

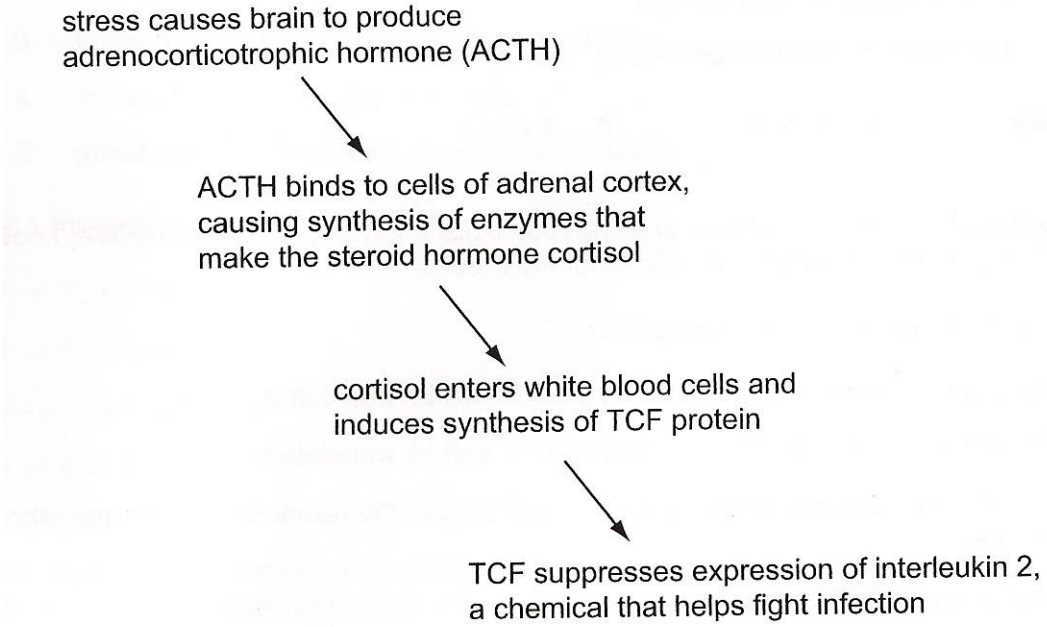
16 Some steps involved in bacterial binary fission are listed below.

1 Breaking of hydrogen bonds in DNA
2 Formation of cell membrane and cell wall between DNA
3 Attachment of DNA to mesosome
4 Bidirectional DNA replication
5 Separation of DNA due to cell elongation

Which of the following shows the correct sequence of events in binary fission?

A 3 → 1 → 4 → 5 → 2
B 3 → 4 → 5 → 1 → 2
C 1 → 4 → 3 → 5 → 2
D 1 → 3 → 5 → 4 → 2

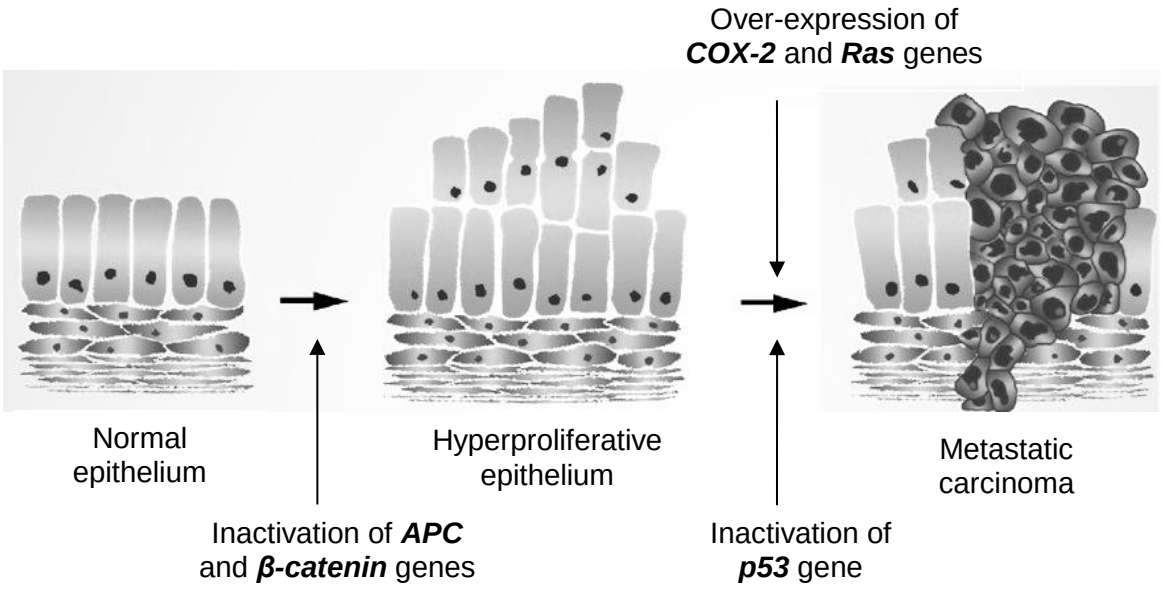
17. When a person undergoes a stressful experience, their immune system can be depressed and they become more susceptible to infection. Some of the elements involved in this chain of events are shown in the diagram below.



Which combination correctly shows the genes that are likely to have transcription-enhancing factors bound to their control elements during the above sequence of events?

	Gene for ACTH	Gene for TCF	Gene for interleukin 2
A	✓	X	X
B	X	✓	✓
C	✓	✓	X
D	X	X	✓

18. The diagram below illustrates the development of colorectal cancers.



Which of these statements can be inferred from this multistep model of carcinogenesis?

1 Cells whose *APC* and *β-catenin* genes are inactivated have lost contact inhibition and can form a tumour mass.
2 *APC* and *β-catenin* genes are tumour suppressor genes
3 High levels of *Ras* protein are produced only when both copies of *Ras* gene are mutated.
4 Two copies of normal *p53* alleles must be present to inhibit cell division
5 Gain-of-function mutation in *COX-2* gene is a pre-requisite for the formation of metastatic carcinoma.

A 1 and 3
B 2, 3 and 4
C 1, 2 and 5
D 2, 3 and 5

19. Which of the following **do not** provide a possible mechanism for the production of a wide number of types of antibody proteins from a small number of genes?

1 gene amplification
2 alternative splicing
3 crossing over and random segregation
4 deletions and random translocation of DNA segments

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

20. The fur colour of hamsters is controlled by a gene with 3 alleles. The phenotypes are black, brown and white fur. 4 crosses were repeated many times. The crosses and the outcomes of these crosses are shown in the table below.

Cross	Parents	Offspring phenotype and ratio
1	black x black	3 black : 1 white
2	brown x white	1 brown : 1 white
3	black x black	3 black : 1 brown
4	white x white	all white

From the data, it is possible to conclude that

A brown fur is recessive to white fur.

B all of the white fur offspring are heterozygous.

C two thirds of the black fur offspring in cross **3** are heterozygous.

D the black fur parents in cross **1** have the same genotype as the black fur parents in cross **3**.

21. Fruit flies *Drosophila* homozygous for long wings, were crossed with flies homozygous for vestigial wings. The F_1 and F_2 generations were raised at three different temperatures.

At each temperature, the F_1 generation all had long wings.

The table below shows the results in the F_2 generation.

Temperature	***Result***
21°C	¾ long wings, ¼ vestigial wings
26°C	¾ long wings, ¼ intermediate wing length
31°C	all long wings

Which statement explains these results?

A Wing length is under polygenic control.
B Long wing and vestigial wing illustrate codominance at 26°C.
C Heterozygous flies have vestigial wings only at 21°C or below but have long wings at 31°C or above.
D Vestigial wing allele is recessive but causes a vestigial wing phenotype only at lower temperatures.

22. In cattle, the gene responsible for normal development of hair and teeth, ectodysplasin 1 (*ED1*) is located on the X chromosome. Mutations in the *ED1* gene result in a rare genetic disorder, anhidrotic ectodermal dysplasia. Another character, the presence of horns, is determined by a gene on an autosome. The allele for the absence of horns (**H**) is dominant and the allele for the presence of horns (**h**) is recessive.

A horned bull with anhidrotic ectodermal dysplasia was mated on several occasions to the same female. A large number of offspring consisting of males and females in equal numbers in all combinations of phenotypes are shown in the table.

Offspring phenotypes
No anhidrotic ectodermal dysplasia, horns present
No anhidrotic ectodermal dysplasia, horns absent
Anhidrotic ectodermal dysplasia, horns present
Anhidrotic ectodermal dysplasia, horns absent

If $\mathbf{X^E}$ represents an X chromosome carrying the normal *ED1* allele and $\mathbf{X^e}$ represents an X chromosome carrying the *ED1* allele for anhidrotic ectodermal dysplasia, what is the genotype of the female parent?

A $\mathbf{X^EX^EHH}$

B $\mathbf{X^EX^EHh}$

C $\mathbf{X^EX^eHH}$

D $\mathbf{X^EX^eHh}$

23. Which of the following statements are true?

1 Continuous variation is controlled by the additive effects of polygenes.

2 Continuous variation is always affected by the environment.

3 Discontinuous variation is sometimes affected by the environment.

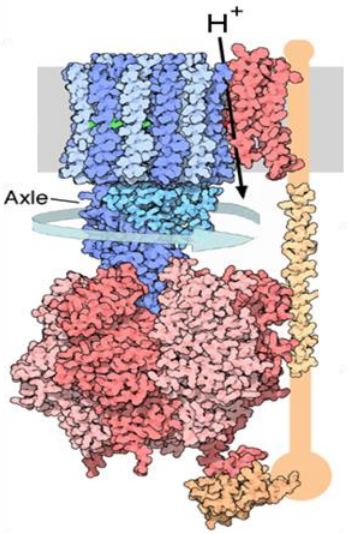
4 Discontinuous variation exhibits a normal distribution curve.

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

24. A man and a woman, both with normal colour vision, have a colour-blind boy together. The woman is pregnant for a second time, and the doctor tells her she is carrying twins of one boy and one girl. What is the chance that both twins will have normal colour vision?

A 0%
B 25%
C 50%
D 100%

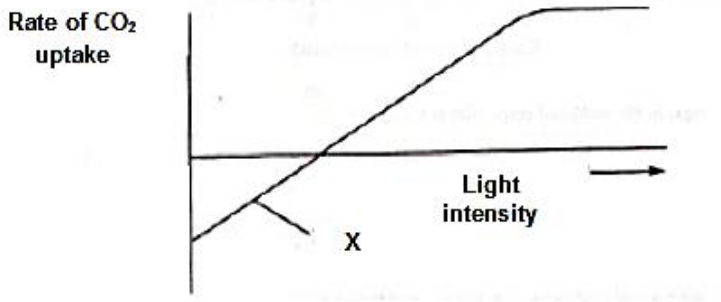
25. The diagram below shows a transmembrane protein that is involved in photophosphorylation



Which of the following statements are true

A It utilises the energy of ATP to do work.
B Its activation results directly in the production of water
C It carries out an oxidation reaction
D It transports ions through it via facilitated diffusion

26. In the graph below, the rate of CO_2 uptake by green algae cells is shown to vary with increasing light intensity.



Which of the following is true at point **X**?

A The algae cells are photosynthesising.
B Rate of carbon fixation by the calvin cycle equals rate of respiration.
C CO_2 is a limiting factor.
D There is not enough light for photosynthesis to have commenced.

27. A mitochondria suspension obtained from liver cells is prepared for investigations of the products of respiration. Acetyl-CoA is added to the suspension.

Which of the following correctly matched the products of Krebs cycle for every oxidation of one glucose molecule in this mitochondria suspension?

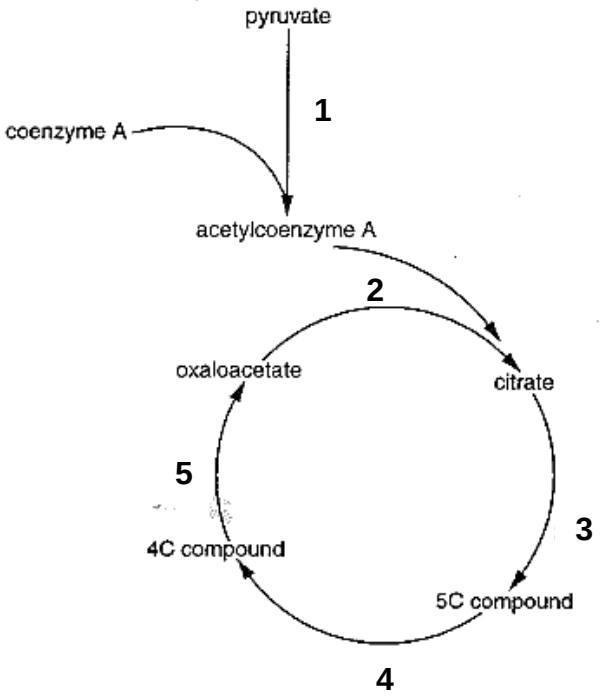
	Product	**Krebs cycle / glucose**
A	ATP	1
	Reduced NAD	3
	Reduced FAD	1
	CO_2	2

	Product	**Krebs cycle / glucose**
B	ATP	2
	Reduced NAD	6
	Reduced FAD	2
	CO_2	4

	Product	**Krebs cycle / glucose**
C	ATP	4
	Reduced NAD	4
	Reduced FAD	2
	CO_2	2

	Product	**Krebs cycle / glucose**
D	ATP	4
	Reduced NAD	6
	Reduced FAD	2
	CO_2	4

28. The diagram shows a process in a cell.



At which numbered stages does decarboxylation take place?

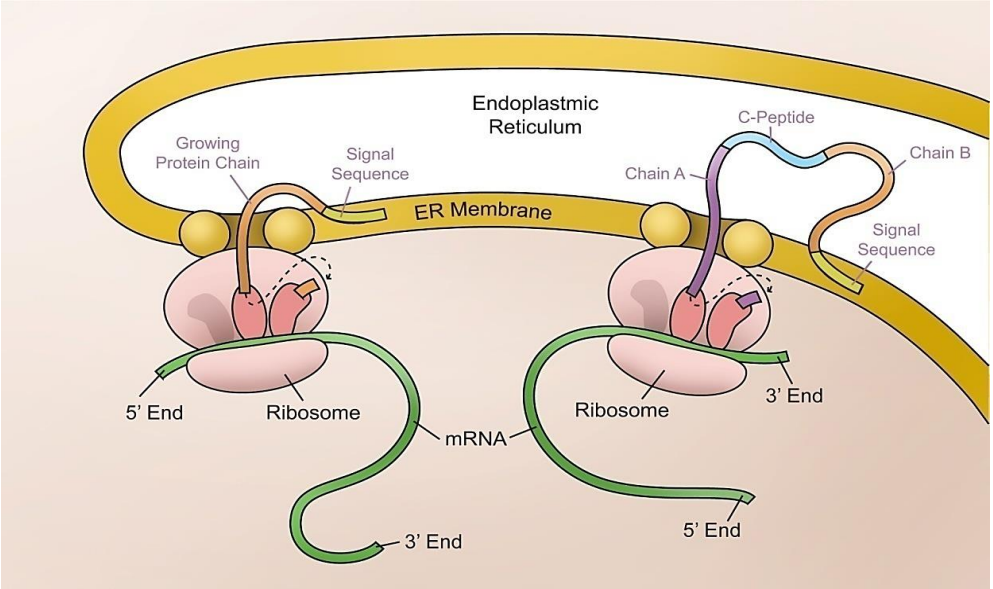
A 1 and 3
B 1, 3 and 4
C 2, 3 and 4
D 3, 4 and 5

29. Tetrodotoxin, a puffer fish toxin, blocks voltage-gated sodium channels. Black widow spider's venom causes the voltage-gated calcium channels to be constantly open. Crotoxin binds irreversibly to acetylcholine receptors.

What will happen to the nerve transmission if each toxin is applied?

	Tetrodotoxin	**Black widow spider's venom**	**Crotoxin**
A	block action potentials along axon	reduce transmission of impulse across synapse	increase transmission of impulse across synapse
B	increase transmission of impulse across synapse	reduce transmission of impulse across synapse	block action potentials along axon
C	block action potentials along axon	increase transmission of impulse across synapse	reduce transmission of impulse across synapse
D	reduce transmission of impulse across synapse	block action potentials along axon	increase transmission of impulse across synapse

30. The diagram below shows part of the insulin synthesis pathway in the pancreas.



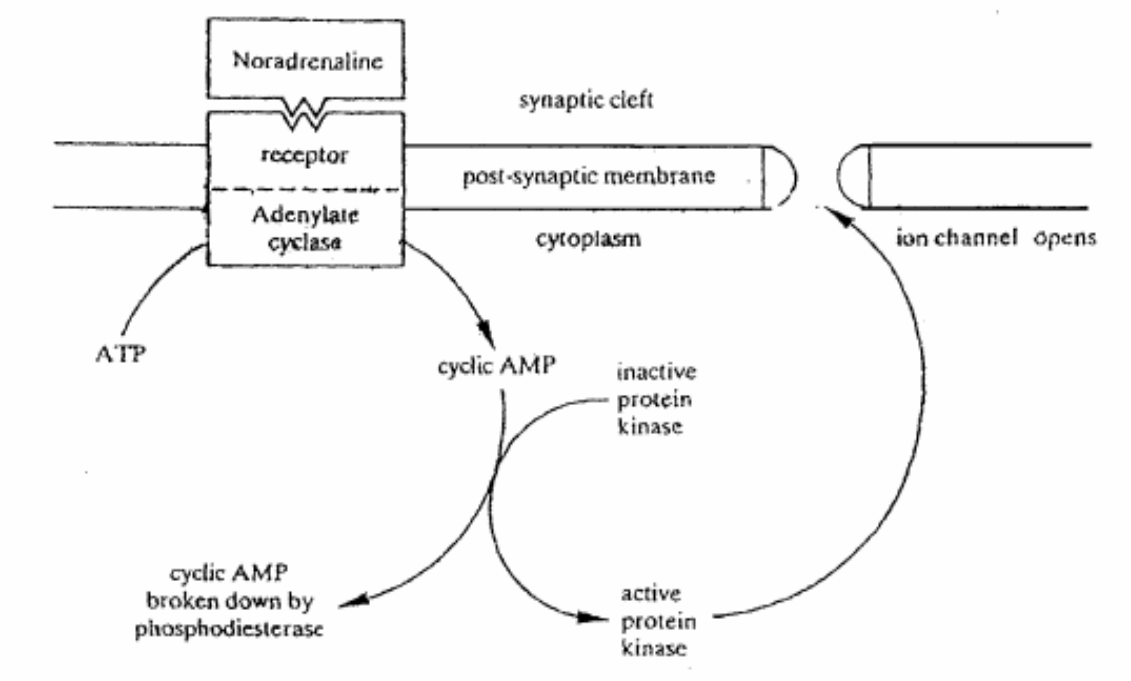
Which of the following statements is correct?

1 The islets of Langerhans are the effector cells in the regulation of blood glucose levels.
2 The signal peptide sequence (in the diagram above) that is synthesised in beta cells is made up of many amino acids with hydrophobic R groups.
3 The functional insulin hormone, made up of an A chain and a B chain held together by disulfide bonds, is formed in the rER lumen.
4 In the beta cells, pro-insulin will be converted to insulin after proteolytic cleavage of the C peptide from pro-insulin and the A and B chains are joined in the correct conformation.
5 Two separate ribosomes synthesise the A chain and the B chain in the above beta cell.
6 Negative feedback to prevent further synthesis of insulin occurs when blood glucose levels rise above norm.

A 1, 2 and 6
B 1, 3, and 4
C 2, 3 and 4
D 3, 5 and 6

31. Noradrenaline stimulates the activity of an enzyme adenylate cyclase located on

the post-synaptic membrane of a neurone. This initiates the sequence of reactions shown in the diagram, causing the opening of channels in the membrane through which ions can pass

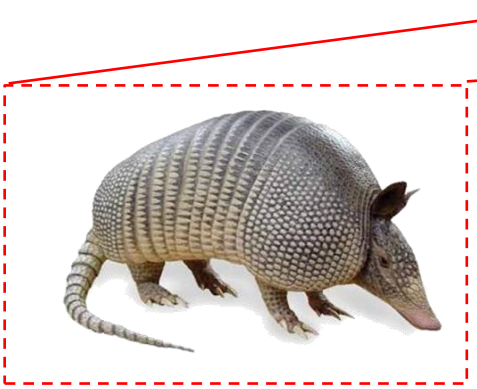


Caffeine causes the ion channels to remain open. A possible explanation is the inhibition of

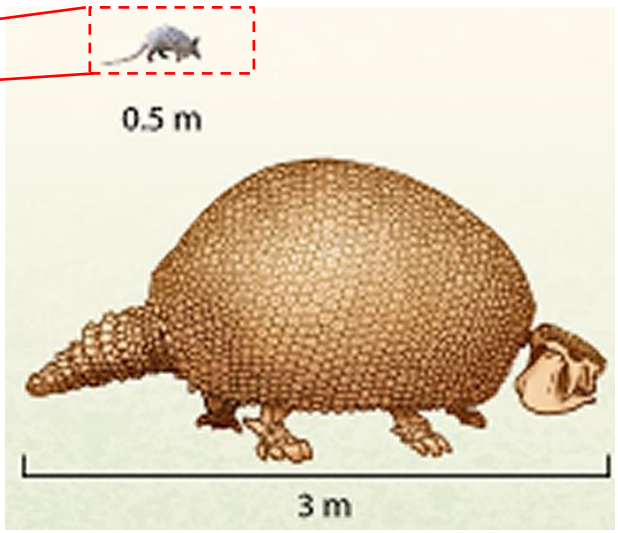
A ATP production
B phosphodiesterase
C cyclic AMP production
D adenylate cyclase

32. Armadillos are medium-sized mammals with tough bony covering that

protects the body. When harassed, armadillos will coil under their shield to minimise the amount of exposed flesh. They are insectivores, feeding on adult and larval forms of ants and termites. Once the prey is detected, armadillos use their claws to dig rapidly to tear into the ant and termite mounds. Their sticky tongues effectively lap up the scurrying insects. Fossils of a recent extinct species of giant armadillo were found to be similar to another smaller species of armadillo presently inhabiting the same region where it is discovered.



Living armadillo



Extinct armadillo

Which of the following statement supports the theory of natural selection?

A Some environmental conditions remained similar whereas other conditions changed between the past and the present.
B The similar characteristics of both species are due to the result of divergent evolution.
C The difference in size of both species is due to the result of constant rate of mutation of a particular gene affecting the growth rate.
D The extinction of the larger armadillo species was due to a chance event.

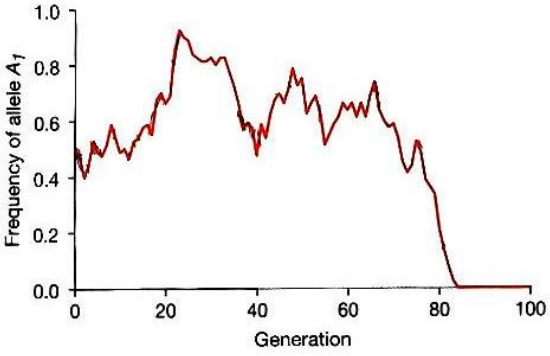
33. Cabbage, *Brassica oleracea* (2n = 20), and radish, *Raphanus sativus* (2n = 18), are different species of the Brassicaceae family. When these plants are crossed, a hybrid is produced. Two cells from the hybrid plant are fused to form a single cell which is propagated using tissue culture technique.

Which of the following is true?

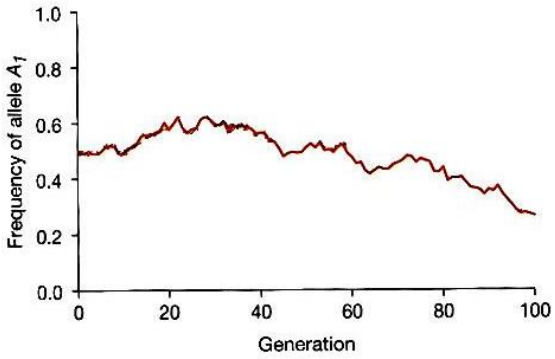
A The hybrid plant is a polyploid.
B The hybrid plant can produce gametes.
C The single fusion cell may eventually result in a new plant species.
D The plant that arises from the cultured cell may be crossed with either *Brassica oleracea* or *Raphanus sativus* to produce a viable and fertile offspring.

34. The graphs show the frequency of allele A1 of two fruit fly populations on an island and on a nearby mainland over time.

Island population size: 40



Mainland population size: 400,000



Based on the information, which of the statement(s) is/are true?

1 Selective pressures for gene A in the mainland changed over time.
2 Genetic drift and natural selection contributed to the change in frequency of allele A1 in the mainland population.
3 The loss of allele A1 from the island population could be due to a spontaneous mutation in the allele sequence.
4 Random chance could result in the fixation or loss of allele A1 in the island population.

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

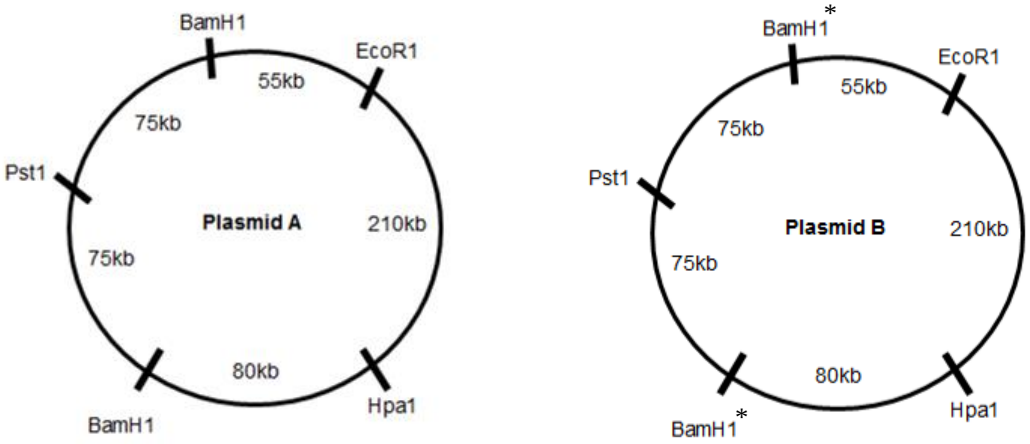
35. Which of the following statements are true for DNA libraries in cancer research?

1 The amino acid sequence of a mutated p53 protein can be determined from only a genomic DNA library.
2 Only a genomic DNA library can be used for the study of a strong enhancer sequence of a proto-oncogene.
3 The expression of specific proto-oncogenes and tumour suppressor genes in a type of cancer can only be determined from a cDNA library.
4 The identity of a specific regulatory protein affecting the expression of a tumour suppressor gene in a type of cancer can only be determined from a cDNA library.

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

36. The figure below shows 2 plasmids (A and B) and the respective restriction sites of various restriction enzymes.

In plasmid B, mutations were deliberately introduced and affected restriction sites are denoted by *****. The lengths of the plasmid DNA between consecutive restriction sites are also indicated.



A scientist carried out a series of 2 experiments by adding different restriction enzymes to the plasmids. Each tube was then left to incubate at 37ºC for about 60 minutes (sufficient time for complete digestion to take place). The results are shown in the table below.

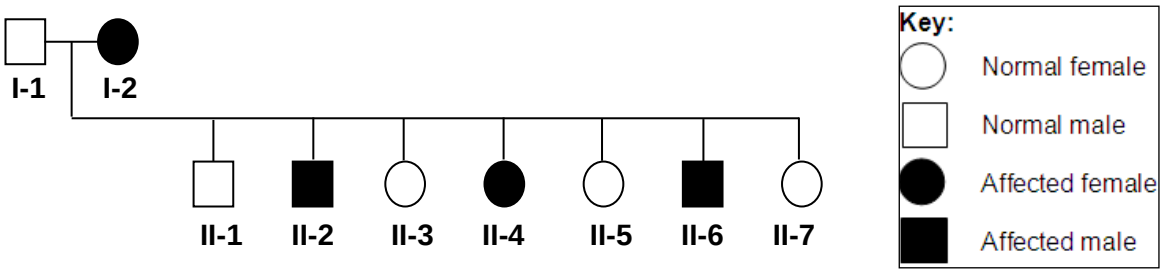
Tube number	Components	Fragment sizes/ kb
1	Plasmid A + 2 restriction enzymes	80, 150, 265
2	Plasmid B + 2 restriction enzymes	155, 340

Identify the enzymes that were added to tubes 1 and 2.

	Tube number	Restriction enzymes
A	1	HpaI , EcoRI
	2	EcoRI , HpaI
B	1	BamHI , PstI
	2	PstI , EcoRI
C	1	BamHI , HpaI
	2	HpaI , PstI
D	1	BamHI , EcoRI
	2	HpaI , BamHI

37. Use the information below for Questions 37 and 38.

Adult polycystic kidney disease (APKD) is inherited in an autosomal dominant manner. In an investigation to determine the chromosomal locus of APKD, linkage analysis of the APKD gene was carried out on members of one family. Three RFLP loci, **P**, **Q** and **R**, located on the non-coding regions of three different chromosomes, were used. The results of the linkage analysis are shown in the figure.



	RFLP alleles present at various individuals' RFLP loci								
	I-1	**I-2**	**II-1**	**II-2**	**II-3**	**II-4**	**II-5**	**II-6**	**II-7**
RFLP locus P	2 , 6	1 , 6	1 , 2	2 , 6	1 , 6	6 , 6	1 , 2	6 , 6	1 , 6
RFLP locus Q	1 , 5	2 , 7	5 , 7	2 , 5	1 , 7	1 , 2	5 , 7	1 , 2	1 , 7
RFLP locus R	4 , 8	5 , 8	5 , 8	8 , 8	5 , 8	4 , 8	5 , 8	4 , 8	5 , 8

Based on the information, which RFLP allele(s) will reveal if an individual has APKD?

	RFLP locus P	**RFLP locus Q**	**RFLP locus R**
A	-	2	-
B	6	2	8
C	-	1 , 2 , 5 , 7	-
D	1 , 2 , 6	1 , 2 , 5 , 7	4 , 5 , 8

38. Once the RFLP allele(s) associated with the disease allele is/are identified and sequence is determined, how could one check if a child may be suffering from APKD? Assume the sequence of APKD is not known.

1 Obtain genomic DNA → restriction digestion → gel electrophoresis → ethidium bromide staining
2 Obtain genomic DNA → restriction digest → gel electrophoresis → southern blot → autoradiography
3 Obtain genomic DNA → PCR → restriction digestion → gel electrophoresis → ethidium bromide staining
4 Obtain mRNA → cDNA → restriction digestion → gel electrophoresis → ethidium bromide staining

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

39. Which of the following correctly describes the role of stem cells in adult tissues and organs?

A Stem cells are undifferentiated cells found amongst differentiated cells and they take over the function of the tissue when the overlying cells become damaged or worn out.
B Stem cells are embryonic cells that persist in the adult, and can give rise to all of the cell types in the body.
C Stem cells are partially differentiated cells that have yet to express the genes and proteins characteristic of their differentiated state, and do so when needed for repair of tissues and organs.
D Stem cells are undifferentiated cells that can divide asymmetrically, giving rise to one daughter cell that remains a stem cell and one daughter cell that will differentiate to replace damaged and worn out cells in the adult tissue or organ.

40. What are the arguments against the use of genetically modified organisms (GMOs)?

1 Insufficient testing of genetically modified crop for their side effects
2 Unforeseen long-term effects of genetic manipulation
3 Accidental genetic recombination in gut bacteria as a result of consuming food derived from GMOs
4 Control of food supply by a small number of companies that have access to genetic engineering technology

A 1 and 2 only
B 2 and 3 only
C 1, 2 and 3 only

D All of the above

ANSWERS

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

BIOLOGY DEPARTMENT
JC2 PRELIMINARY EXAMINATIONS
2016 Higher 2

CANDIDATE NAME

CLASS	1	5	S		

EXAM NUMBER								

BIOLOGY 9648/02

Paper 2 Core Paper **14 September 2016**

Additional Materials: Answer Paper **2 hours**

READ THESE INSTRUCTIONS FIRST

Write your Class, exam number and name on all the work you hand in.
Write in dark blue or blue pen.
You may use a soft pencil for any diagrams or graphs.
Do not use any staples, paper clips, highlighters, glue or correction fluid.

Section A
Answer **all** questions in the spaces provided on the question paper.

Section B
Answer any **one** question on the writing paper provided.

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

At the end of the examination,
1. hand in sections A and B separately;
2. fasten all your work securely;
3. enter the question number of section B that you have answered in the grid opposite.

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

For Examiner's Use	
Section A	
1	
2	
3	
4	
5	
6	
7	
8	
Section B	
Total	

Section A: Structured questions
Answer **all** the questions. All answers must be written on the spaces provided and nowhere else.

1 (a) **Fig. 1.1** shows the electro-micrograph of a T-helper cell found in the bloodstream of a healthy individual. **Fig 1.2** shows the how a region of the T-helper cell looks like when magnified.

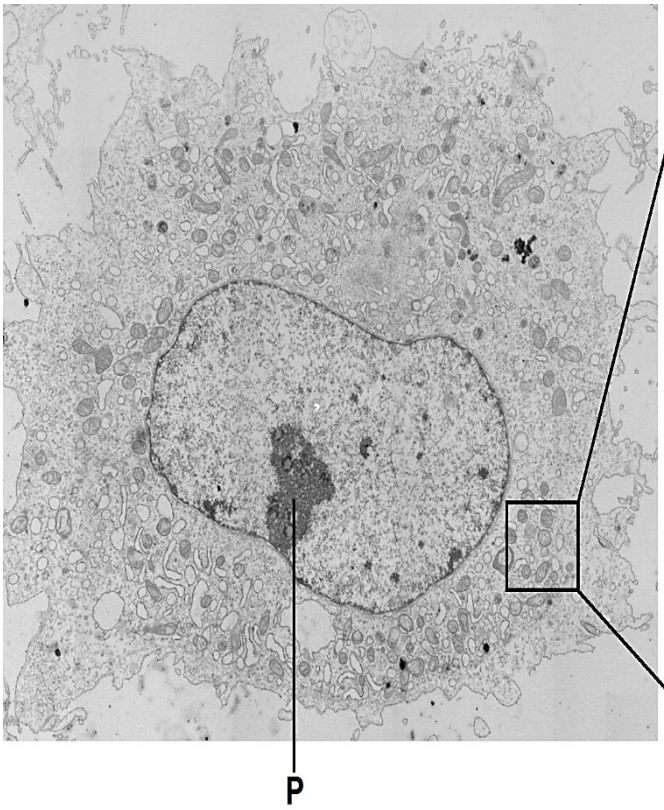


Fig. 1.1 EM of T helper cell

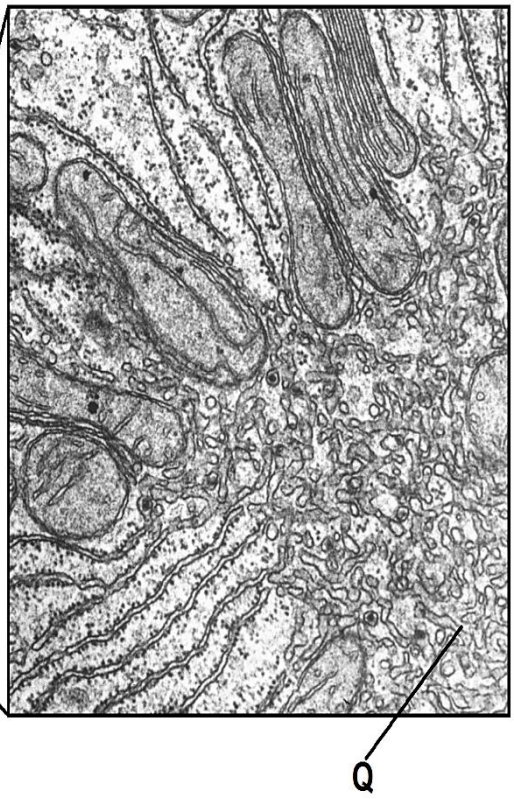


Fig. 1.2 Magnified region of a part of the T helper cell

(i) Identify structures **P** and **Q** and describe briefly their functions.

[3]

(ii) Contrast the structure of a lysosome with structure **P**.

[2]

(b) The cytoplasm of T-helper cells contains different proteins and enzymes, one of which is phosphofructokinase involved in the metabolism of glucose to produce energy necessary for cell survival and functions. In order to ensure a constant supply of energy, most organisms have evolved the use of storage molecules to store excess glucose.

(i) Name a carbohydrate that functions as a storage molecule for T-helper cells.

[1]

(ii) Describe **three** structural differences between the carbohydrate named in **(b) (i)**. and cellulose.

[3]

(iii) Explain how the presence of two types of bonds in amylopectin enables it to carry out its function.

[3]

(c) Phosphofructokinase is an allosteric enzyme. Explain how the presence of an allosteric inhibitor affects the enzymatic activity of an allosteric enzyme.

.....

.....

.....

.....

[2]

[Total: 14]

2 The Fig. 2.1 shows an enzyme involved in the activation of tRNA for translation in prokaryotes.

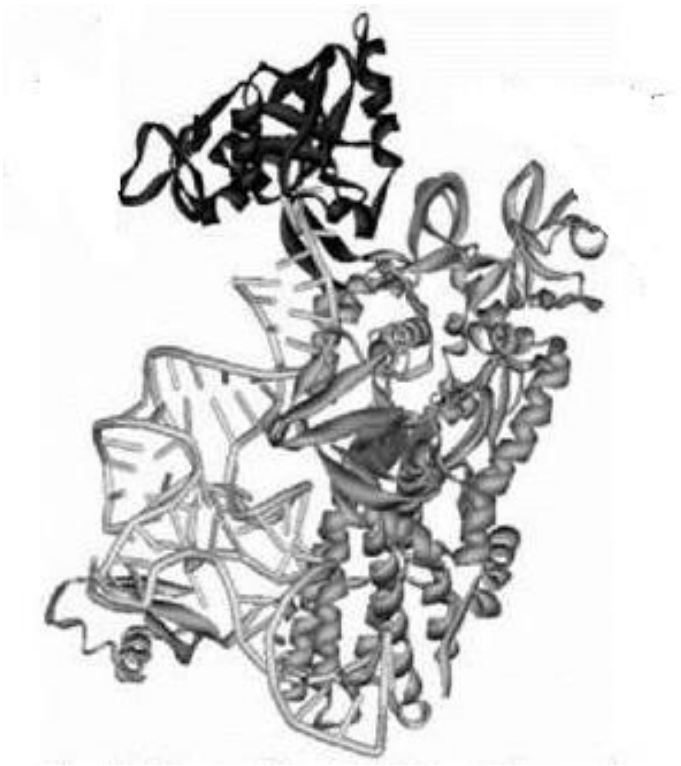


Fig 2.1

(a) (i) Explain the mode of action of this enzyme.

[3]

(ii) Explain the significance of having more than one type of the enzyme named in **(a) (i)** in the cell.

[2]

(b) How does the order of nucleotides in a gene encode the information that specifies the primary structure of a polypeptide? Include two features of the genetic code in your answer.

[3]

(c) Explain how different polypeptides can be synthesised simultaneously from a single mRNA is produced from an operon in prokaryotes.

[2]

[Total: 10]

3 Fig. 3.1 shows the *arg* operon found in *Escherichia coli*. In the absence of arginine, the operon is in the active state. In the presence of arginine, the expression of the structural genes decreases.

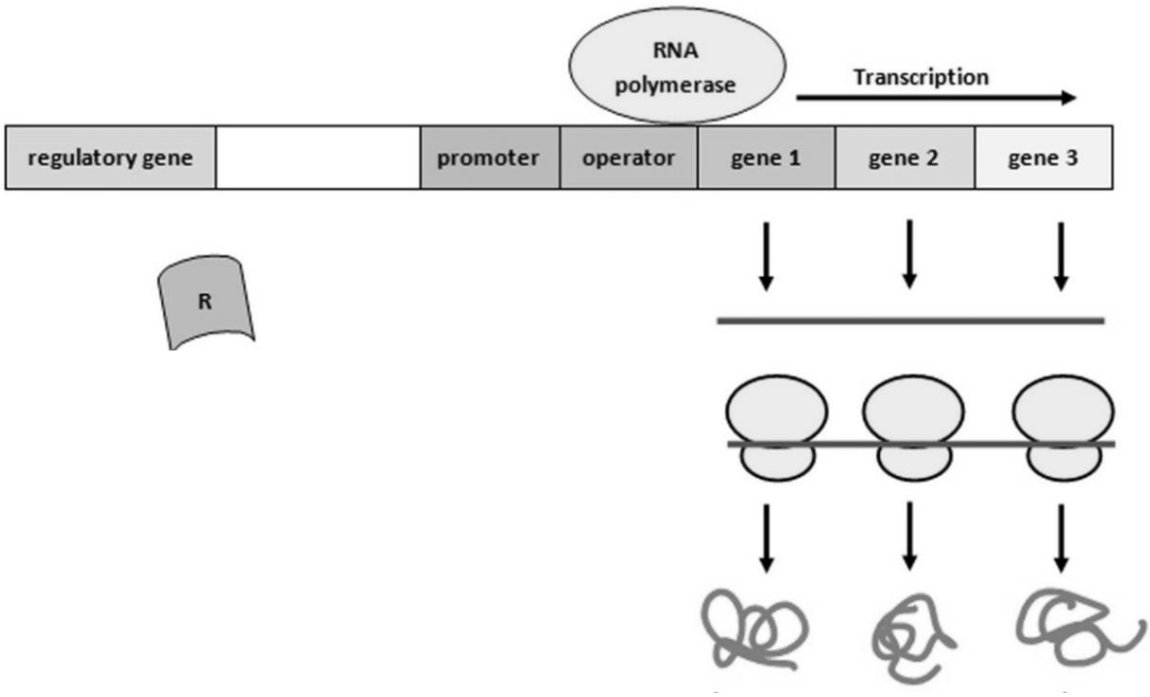


Fig 3.1

(a) Using **Fig 3.1** explain the mode of control of the Arg operon.

[2]

(b) Explain why it is useful for a bacterial cell to decrease expression of the structural genes when arginine is present.

[2]

Besides having operons, bacteria can have other means to enhance their adaptability to the changing environment through gene transfer. Fig. 3.2 shows one way in which bacteria can acquire new genetic material.

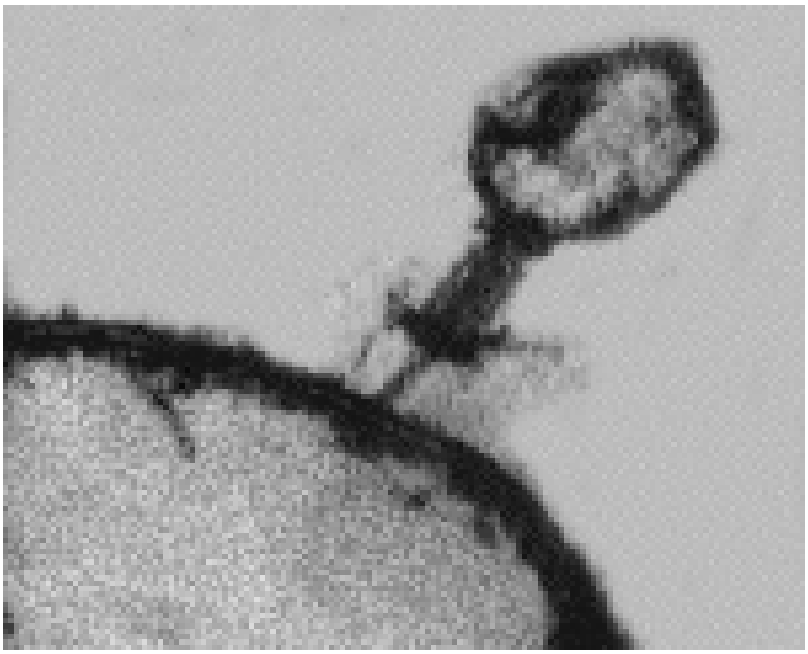


Fig. 3.2

(c) Name and describe the process which can result in this population of bacteria acquiring the same allele needed to increase their likelihood of survival.

[3]

Fig. 3.3 shows a classic experiment used to show that physical contact between bacterial cell is necessary in order for conjugation to happen

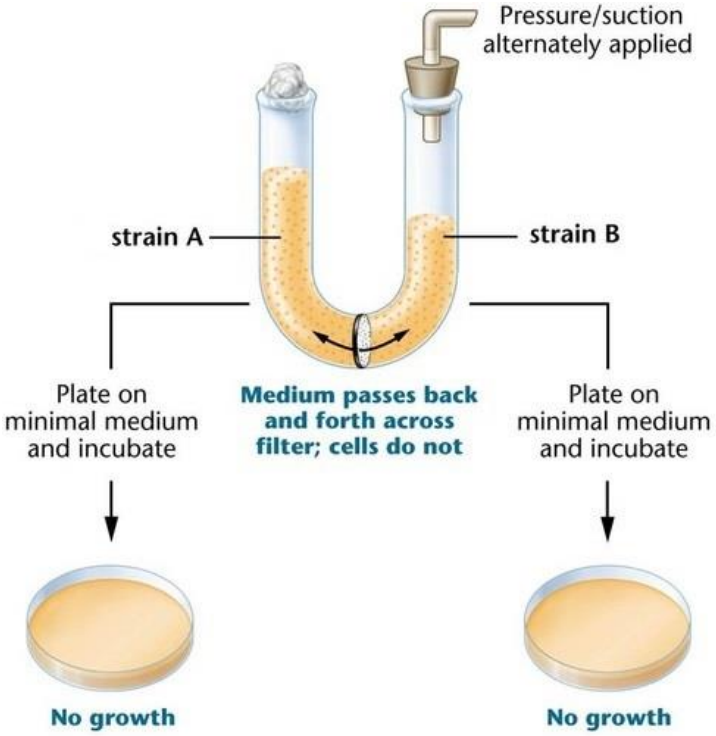


Fig. 3.3

(d) A student tried to replicate the experiment but did not get the result shown in Fig. 3.3. Instead, he observed a few bacterial colonies which are hybrids of strains A and B. He later realized that he had accidentally forgot to add in DNAase when carrying out the experiment.

(i) Briefly describe the role of DNAase in this experiment

[1]

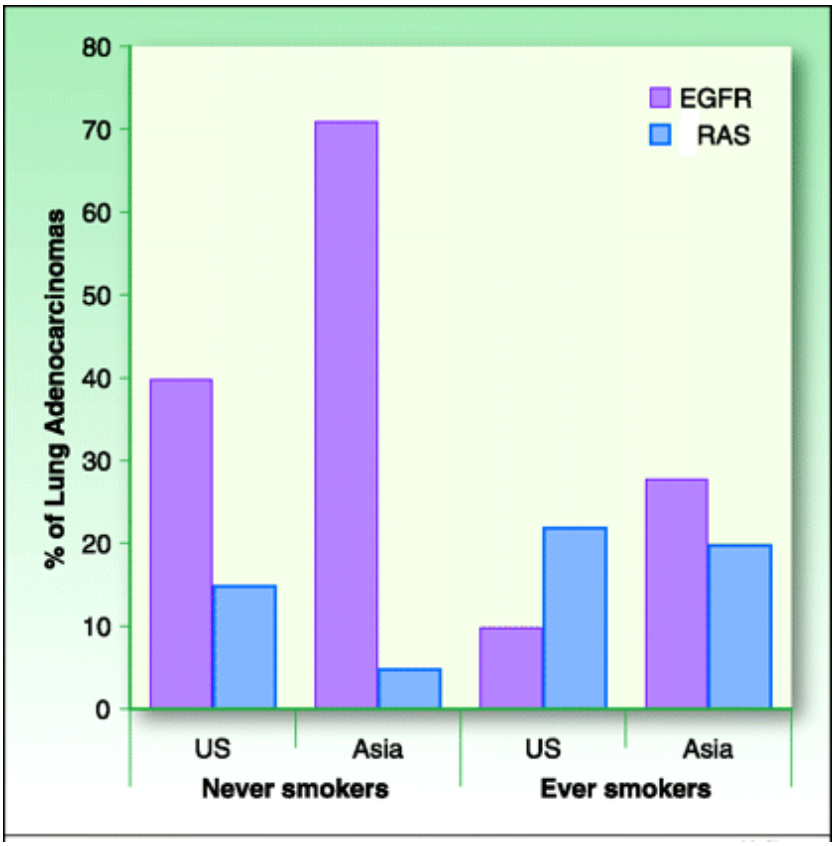
(ii) How does the lack of DNAase in the experiment result in the growth of the hybrid bacterial colonies?

[2]

[Total: 10]

4 The majority of lung cancers are caused by long term exposure to the several classes of carcinogens present in tobacco smoke. However there are instances of lung cancers arising in the absence of detectable tobacco exposure. Analysis of the different mutations in individuals who smoked (ever-smokers) and those who did not (never-smokers) suggest that lung cancers in never smokers may follow a very different cellular and molecular pathway of malignant transformation.

The Fig. 4.1 shows the differential frequencies of gene mutations of the epidermal growth factor receptor (EGFR) and Ras reported in lung adenocarcinomas in Asia versus United States, in never-smokers and ever-smokers.



http://clincancerres.aacrjournals.org/content/15/18/5646

Fig. 4.1

With reference to Fig. 4.1,

(a) (i) describe the effect of Ras and EGFR mutations on the development of lung cancer in never-smokers and ever –smokers in Asia.

.....

[1]

(ii) Suggest the molecular basis behind why never-smokers in Asia develop lung cancer.

[2]

Data emerging over the past several years show that activating mutations in the epidermal growth factor receptor (EGFR) gene may underscore the development of a distinct class of lung cancers. EGFR signalling is triggered by the binding of growth factors resulting in the dimerization of EGFR.

(b) Suggest the role of the *EGFR* gene in relation to the development of cancer.

[3]

(c) Small molecule inhibitors of the tyrosine kinase enzymatic activity to inhibit cross-phosphorylation and signalling of the EGFR have been used in clinical treatment in the United States. Results found that the success of treatment of lung cancers is higher for never-smokers than ever-smokers. Based on Fig. 4.1 suggest why this is so.

[2]

(d) 50% of never-smokers with lung cancer also have mutations in the p53 gene. Explain how mutations in the *p53* gene may lead to the development of lung cancer.

[3]

[Total: 11]

5 In the Korean clover plant, the development of petals is determined by two genes on separate autosomes. Gene A has two alleles - the dominant allele **A** results in the development of normal petals, while the recessive allele, **a**, results in small petals. On another chromosome, the dominant allele **B** of another gene has no effect on petal development. However, allele **b** hinders petal development and so results in the formation of fused petals regardless of the nature of the allele at gene A.

A Korean clover plant was self-pollinated and obtained the following offspring:

Normal petals	46
Small petals	14
Fused petals	20

(a) (i) State the genotype and phenotype of the clover plant that was self-pollinated.

Genotype:

Phenotype: [1]

(a) (ii) Use a genetic diagram to illustrate the phenotypic ratio of the offspring from this cross.

[3]

(b) A scientist decided to study the inheritance of flower colour and plant height in another plant species. The alleles for these traits are shown below.

T: Allele for tall plants **t:** allele for dwarf plants

Y: allele for yellow flowers **y:** allele for white flowers

He carried out a cross between a heterozygous tall, yellow-flowered plant with a homozygous recessive dwarf, white-flowered plant and obtained a large number of offspring. Table 5.1 shows the results.

Phenotype	**Number of offspring**
Tall, yellow-flowered plant	78
Tall, white-flowered plant	22
Dwarf, yellow-flowered plant	20
Dwarf, white-flowered plant	80

Table 5.1

(i) State the expected phenotypic ratio of the offspring in Table 5.1.

[1]

(ii) Use the chi-squared (X^2) test and the table of probabilities shown in Table 5.2 to find the probability of the results of this cross departing significantly by chance from expectation. Show your working. [2]

$$X^2 = \sum \frac{(\text{Observed Value} - \text{Expected Value})^2}{(\text{Expected Value})}$$

Key to symbols

s = standard deviation	n = sample size (number of observations)	E = expected 'value'
Σ = 'sum of'	v = degrees of freedom	
x = observation	c = number of classes	
$\bar{x}$ = mean	O = observed 'value'	

Distribution of X^2

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

Table 5.2

X^2 value = ...

number of degrees of freedom = ...

probability = ...

(iii) State what conclusions may be drawn from the probability found in (b) (ii).

.....

.....

.....

[2]

(iv) Explain the observed phenotypic ratio in Table 5.1.

.....

.....

.....

[2]

[Total: 11]

6 Myasthenia gravis is a disease of the neuromuscular junctions which causes muscular weakness. It develops because the muscle's response to repeated nerve signals declines with time, and the muscles become weak and tired.

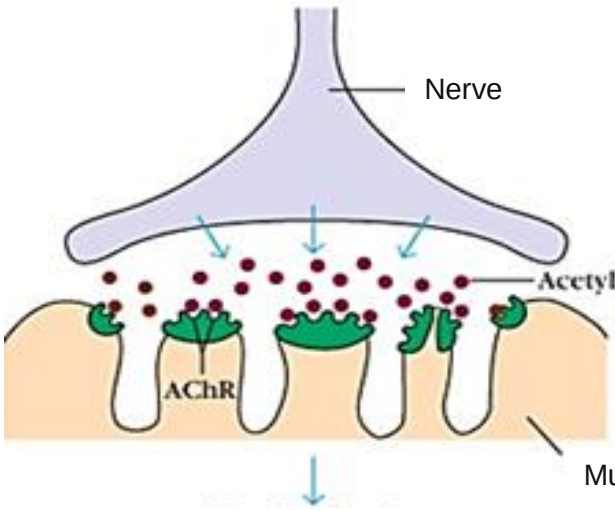


Fig. 6.1: Normal neuromuscular junction

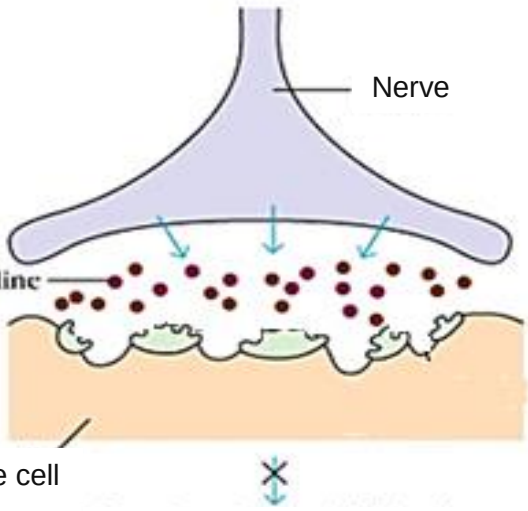


Fig. 6.2: Myasthenic neuromuscular junction

(a) (i) State one similarity in the structure of a normal and myasthenic neuromuscular junction as seen in Fig. 6.1 and 6.2 and explain how it aids in synaptic transmission.

[2]

(ii) State one difference in the structure as seen in Fig. 6.1 and 6.2 and explain how it affects synaptic transmission.

[2]

(b) Describe the mechanism that ensures unidirectional movement of nerve impulses along the axon of a neurone.

[2]

(c) Fig. 6.3 shows action potentials generated along an axon over a fixed time, X.

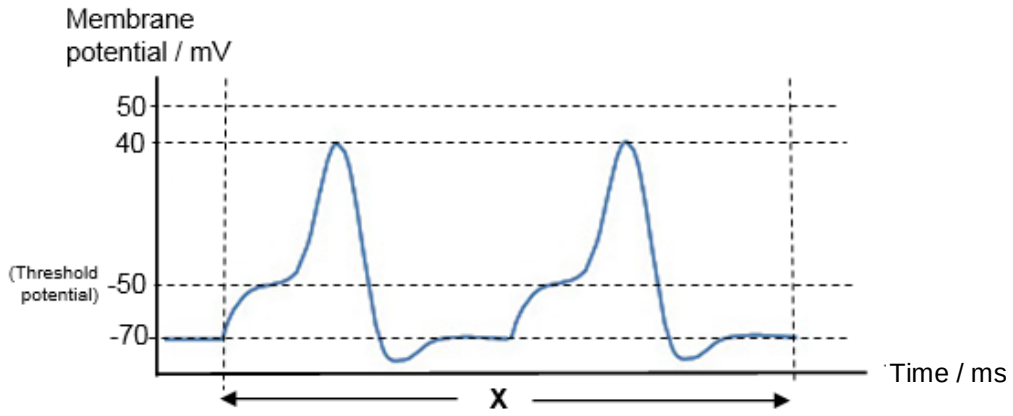
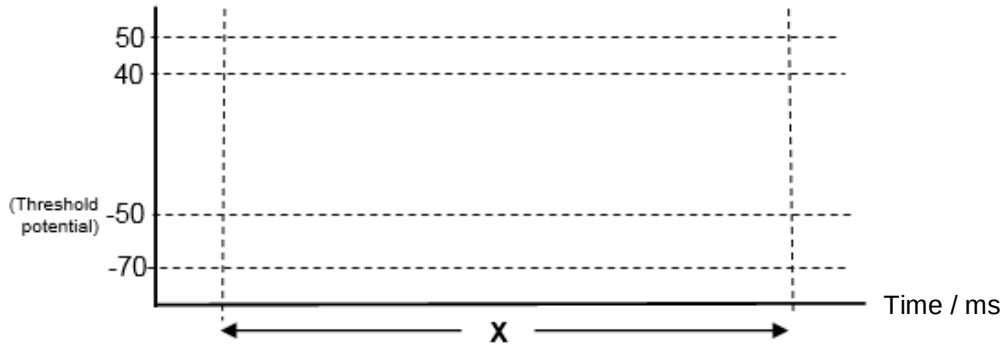


Fig 6.3

In the space provided, draw the action potentials generated over the same time period X if the stimulus is more intense. [2]



[Total: 8]

7 Glucagon plays a critical role in maintaining glucose homeostasis. The molecular mechanisms for glucagon-mediated glucose regulation are shown in Fig. 7.1.

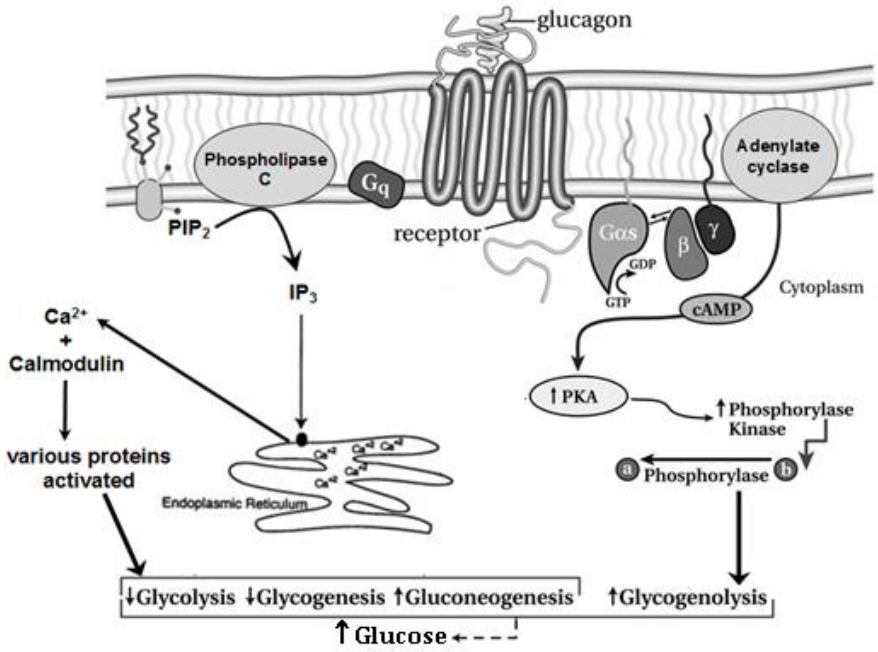


Fig. 7.1

(a) State the type of receptor that glucagon binds to and explain how this receptor is fully activated.

[2]

(b) With reference to Fig. 7.1, briefly explain how cAMP can lead to an increase in blood glucose concentration.

[3]

[Total: 5]

8 Members of the family Rhopalidae include the soapberry bugs which are brightly-coloured fruit-eaters, comprising of three genera and about 65 species. These bugs are specialists on plants in the soapberry family (Sapindaceae) in which they obtain the nutrients from the fruits by piercing the skin using their sharp beaks.

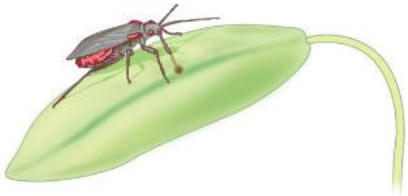


Fig. 8.1: A soapberry bug feeding on a fruit

Jadera haematoloma is a soapberry bug found in Florida following the introduction of a non-native soapberry plant in the 1920s which out-competed the native plant in some locations. At such locations, measurement of the beak length of individual bugs was also carried out (Fig. 8.2). Analysis of the fruit of the non-native plant also showed that it has thinner skin as compared to the fruit of the native plant.

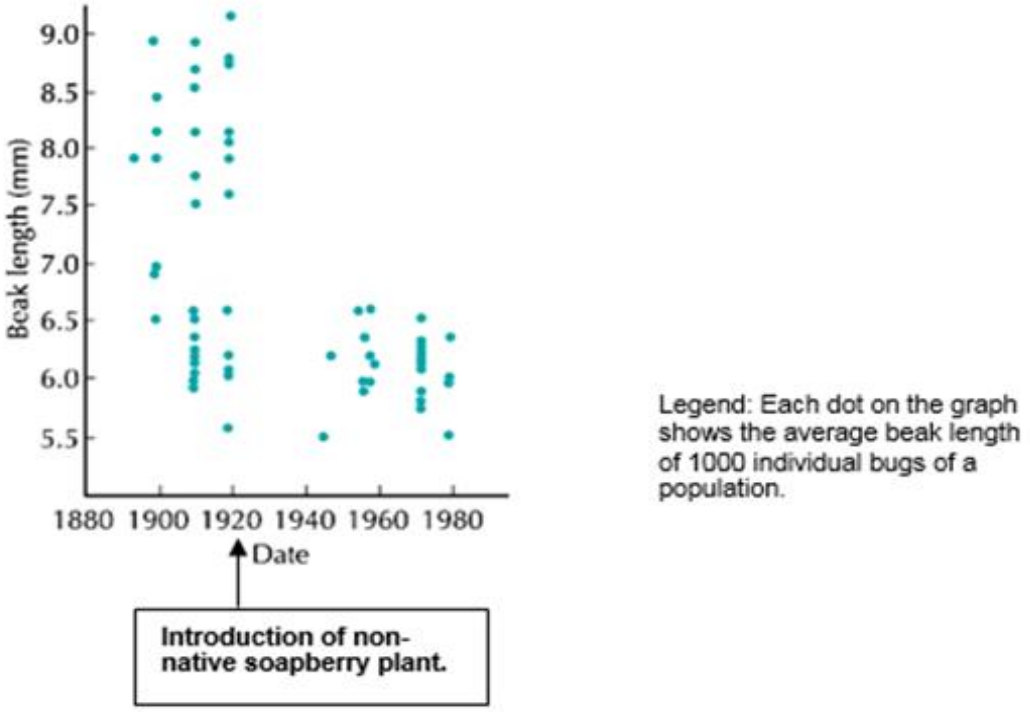


Fig. 8.2: Beak lengths of soapberry bugs (1880-1980)

(a) Explain why the evolution of *Jadera haematoloma* after the introduction of a non-native soapberry plant in the 1920s is not considered a form of divergent evolution.

.....

[1]

(b) Account for the beak lengths over time.

[4]

(c) In 1980, 4000 soapberry bugs at one sampling site were caught and transported to island X with a variety of soapberry plants with thick and thin skinned fruits. The bugs were allowed to populate the island in the absence of predator. In 1990, the beak lengths of bugs were measured and showed a wide variety of beak lengths.
At the same time in 1980, 40 individuals were taken and released to an isolated island Y with the same conditions as island X. In 1990, the beak lengths of bugs in both islands were also measured but the beak lengths were all short (below 6mm) although both islands were similar to each other.

(i) Suggest a likely explanation for the result seen in island Y.

[2]

(ii) In 2000, both islands were invaded by a plant viral disease that destroyed the soapberry plants with thin-skinned fruits. The outcome of/effect on the bugs in both islands in terms of the beak length variety and total number of bugs over time are shown in Fig. 8.4.

Island	**X**	**Y**
Total number of bugs	Remains about the same	Nil (extinction)
Variety of beak length	Only long beaks	-

Fig.8.4

Explain the results in Fig. 8.4.

[2]

(c) Analysis of the DNA sequences of the soapberry bugs in both islands before the viral invasion revealed differences that could not be explained by the theory of natural selection. How may the neutral theory of molecular evolution account for the differences?

[2]

[Total: 11]

Section B

Answer **one** question.

Write your answers on separate writing paper.

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

Your answer must in continuous prose, where appropriate.

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

Begin your answers to each part (a), (b) and (c) on a new sheet of writing paper.

9 **(a)** Explain how molecular and anatomical homology supports Darwin's theory of natural selection [6]

(b) Describe the response of the muscle cell to insulin with respect to cell signaling [8]

(c) Define control elements and explain how they interact with other factors to influence transcription [6]

10 **(a)** Explain how recessive alleles may be preserved in a natural population [6]

(b) Explain the advantages and significance of having a cell signaling system [6]

(c) Describe binary fission and explain how it differs from bacterial conjugation. [8]

[20]

BIOLOGY DEPARTMENT
JC2 PRELIMINARY EXAMINATIONS
2016 Higher 2

BIOLOGY 9648/02

Paper 2 Core Paper Answers **14 September 2016**

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1 (a)

(i) Identify structures **P** and **Q** and describe briefing their functions. [3]

- P – Nucleolus [1/2]
- Transcription of ribosomal RNA [1/2]
- Site of ribosome assembly [1/2]
- Q – Smooth endoplasmic reticulum [1/2]
- Site of synthesis of lipids [1/2]
- Detoxification of drugs and poisons [1/2]
- Stores calcium ions required for contraction in muscle cells [1/2]

(ii) Contrast the structure of a lysosome with structure **P**. [2]

Lysosome	P (Nucleolus)
• Membrane-bound [1/2]	• Not membrane-bound [1/2]
• Contains hydrolytic enzymes [1/2]	• Contains DNA coding for rRNA [1/2]

(b)

(i) Name a carbohydrate that functions as a storage molecule for T-helper cells.

- Glycogen [1]

(ii) Describe **three** structural differences between cellulose and the carbohydrate in **(bi)**. [3]

Cellulose	Glycogen
• Made up of β-glucose	• Made up of α-glucose
• Joined by β 1,4 glycosidic bonds	• Joined by α 1,4 glycosidic bonds and α 1,6 glycosidic bonds
• Unbranched, straight chains	• Branched brush-shaped
• Alternate subunits rotated 180^{o}	• Alternate subunits in the same orientation
• Inter-chain hydrogen bonds present	• No cross-linkages between adjacent chains

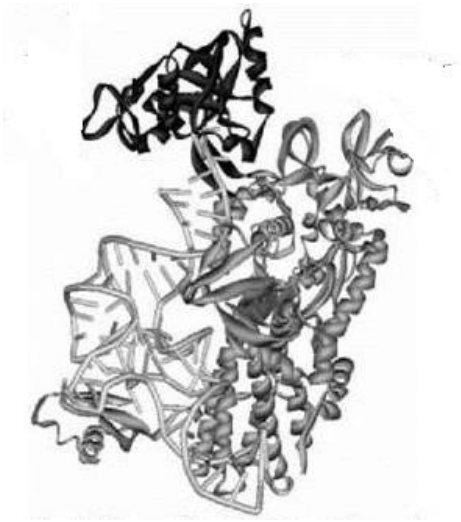
(iii) Explain how the presence of two types of bonds in amylopectin enables it to carry out its function. [2]

- α 1,4 glycosidic bonds between subunits within a branch [1/2]
- α 1,6 glycosidic bonds at branch points [1/2]
- form branched helical structure
- compact for storage function
- Both bonds can be broken enzymatically to release α glucose for respiration [1/2]
- Hydrolysis of α 1,6 glycosidic bonds breaks up amylopectin into many branches for more efficient breakdown [1/2]

(c) Phosphofructokinase is an allosteric enzyme. Explain how the presence of an allosteric inhibitor affects the enzymatic activity of an allosteric enzyme. [2]

- Allosteric inhibitor binds to allosteric site [1/2]
- Causes the enzyme conformation to change to inactive state [1/2]
- Active site not complementary to substrate [1/2]
- Prevent effective collision and formation of enzyme-substrate complex [1/2]

2. The diagram below shows an enzyme involved in the activation of tRNA for translation in prokaryotes.



(a) (i) Explain the mode of action of this enzyme [3]

- amino-acyl tRNA synthetase;
- has a specific active site that is
- complementary to specific tRNA anticodons and a specific amino acid
- Ref. to induced fit theory
- catalyses the attachment of a specific amino acid to the 3' stem of the tRNA in the formation of the amino-acyl tRNA complex
- by lowering the activation energy of the reaction
- through the formation of an enzyme structure complex

(ii) Explain the significance of having more than one type of the enzyme named in (ai) in the cell [2]

- There are 20 different amino acids and hence 20 different amino-acy-tRNA synthethases are needed
- This ensures that each of the 20 amino acids are correctly linked to their tRNAs/ ref to specificity of enzyme for substrate
- As the anticodons of the tRNA bind by complementary base pairing to the condons in the P and A site of the ribosome
- When an amino acid has been linked to a tRNA, it will be incorporated into a growing polypeptide chain at a position dictated by the codon of the mRNA.
- Allowing the primary structure of the polypeptide to be synthesised correctly according to the codons of the mRNA that is being translated

(b) How does the order of nucleotides in a gene encode the information that specifies the primary structure of a polypeptide? Include two features of the genetic code in your answer.[3]

- Transcription of the gene by RNA polymerase produces a complementary sequence of mRNA;
- Three consecutive nucleotides on mRNA make one codon;
- One codon codes for one amino acid;
- Although more than one codon can code for the same amino acid due to the degenerate nature of the genetic code;
- The ribosome read the codons one after another with no space between codons as the genetic code is non-overlapping.
- The ribosome thus joins the amino acids in the correct sequence as coded for by the codon sequence to form the polypeptide's primary structure/ the codon sequences hence determine the number, type and sequence of amino acids of the polypeptide synthesized by the ribosome
- As the genetic code is punctuated where 3 codons do not code for amino acids but function as stop codons that mark the end of translation. The ribosome stops polypeptide synthesis when a stop codon is located in the ribosome A site as a release factor enters the site to release the completed polypeptide.

(c) Explain how different polypeptides can be synthesised simultaneously from a single mRNA in prokaryotes. [2]

- A prokaryotic mRNA is a polycistronic mRNA;
- And contains the coding sequence for more than one polypeptide/ structural gene product involved in a related metabolic pathway;
- Each coding sequence has its own start and stop codon;
- Allows more than one ribosome to bind to the polycistronic mRNA and start simultaneous translation beginning at the start codon
- more than one translation initiation complex can be formed at a time;
- Translation of each polypeptide stops when the ribosomes read the stop codon for the coding sequence

3

(a) Using Fig 3.1, explain the mode of control of the Arg operon. [2]

- Negative control of arg operon;
- as the repressor (activated by arginine) is required to switch / turn off gene expression;

(b) Explain why it is useful for a bacterial cell to decrease expression of the structural genes when arginine is present. [2]

- Trp genes code for enzymes (involved in / necessary for) (anabolism / synthesis) of tryptophan
- Decreased expression helps to conserve resources that could be diverted for other uses /preventing wastage of resources

(c) Name and describe the process which can result in a population of bacteria acquiring the same allele needed to increase their likelihood of survival.

- **specialised transduction;;**
- Viral DNA **integrates** into a **specific location**;
- When it excises as the cell enters the **lytic cycle**;
- The bacterial DNA removed along with the **excision** of the viral DNA;
- will be those that are **near to the prophage** on the bacterial chromosome;
- DNA transferred will therefore be about the same;

(d)(i) Briefly describe the role of DNAase in this experiment.

- Digest naked DNA fragments

(ii) How does the lack of DNAase in the experiment result in the growth of the hybrid bacterial colonies?

- Without the DNAase, the naked DNA fragments from bacteria which have died may be taken up by the other strain via transformation
- DNA fragment will be small enough to cross over the filter

4.

With reference to Fig. 4.1

(a) (i) describe the effect of the 2 gene mutations on the occurrence of cancer in Asians. [1]

- 70% of never smokers with lung cancer have mutations in EGFR gene compared to 27% of ever smokers with lung cancer;
- While 4 % of never smokers with lung cancer have mutations in Ras gene compared to 20% of ever smokers with lung cancer;

(ii) suggest how never smokers in Asia developed lung cancer [2]

- never smokers who get lung cancer could have inherited a dominant mutation in the EGFR gene and experienced a loss of heterozygosity for two or more tumour suppressor genes;;
- as seen from the high percentage of never smokers having the mutation in the EGFR gene compared to ever smokers suggesting the never smokers inherited an increased disposition to acquiring lung cancer;
- even in the absence of exposure to chemical carcinogens such as tar in cigarette smoke

(b) Suggest the role of the *EGFR* gene in relation to the development of cancer. [2]

- *EGFR* gene is a proto-oncogene;
- That codes for the production of growth factor receptor involved in the signalling pathway for cell division;
- Gain-of-function mutation converts EGFR gene into an oncogene;
- The hyperactive/ constitutively dimerised EGFR protein/ receptor constantly stimulates cell division/ results in abnormally active signalling to initiate cell division
- The cell is able to proliferate in the absence of growth factors
- The excessive/ uncontrolled cell proliferation leads to formation of cancerous tissue / tumor.

(c) Small molecule inhibitors of the tyrosine kinase enzymatic activity to inhibit autophosphorylation and signalling of the EGFR have been used in clinical treatment in the United States. Results found that the success of treatment of lung cancers is higher for never-smokers than ever-smokers. Based on Fig. 4.111, suggest why this is so. [2]

- Mutations in EGFR is causal to development of cancer in 40% of never-smokers compared to 10% of ever-smokers;
- Hence inhibiting the EGFR is 4 times more likely to lead to success in treatment in never-smokers;
- A higher percentage of ever-smokers (22% compared to 14%) have mutations in Ras;
- Ras acts downstream of the EGFR / involved in another cell signalling pathway;
- A EGFR inhibitor will have no effect as a hyperactive Ras can signal of excessive cell division even in the absence of signalling from the growth factor receptor;

(d) 50% of never smokers with lung cancer also have mutations in the p53 gene. Explain how mutations in the *p53* gene may lead to the development of lung cancer. [3]

- p53 is a tumor suppressor gene that serves to restraint cell division;
- ref. Loss-of-function mutations
- ref. in both alleles of the *p53* gene
- ref. p53 protein is a transcription factor
- When there is DNA damage,the mutated p53 protein is unable to activate:
  - DNA repair genes to repair the DNA damage
  - P21 gene stop the cell cycle to allow time to repair DNA
  - genes controlling apoptosis that cause the (lung epithelial) cell to die when there is excessive DNA damage
- thus allowing accumulation of mutations to occur.

5.

(a) (i) State the genotype and phenotype of the clover plant that was self-pollinated.

Genotype: AaBb (1/2)

Phenotype: Normal petals **(1/2)** [1]

(a) (ii) Use a genetic diagram to illustrate the phenotypic ratio of the offspring from this cross.

Phenotype of parents: normal petals x normal petals

Genotypes of Parents: AaBb x AaBb

F1 Gametes **AB** **Ab** **aB** **ab** ; **1 m for gametes;**

F1 Genotypes and Phenotypes:

Gametes	**AB**	**Ab**	**aB**	**ab**
AB	**AABB normal**	**AABb normal**	**AaBB normal**	**AaBb normal**
Ab	**AABb normal**	**AAbb fused**	**AaBb normal**	**Aabb fused**
aB	**AaBB normal**	**AaBb normal**	**aaBB small**	**aaBb small**

ab	AaBb normal	Aabb fused	aaBb small	aabb fused

1 m for all correct F1 genotypes;

1 m for all correct

F1 Phenotypic Ratios: normal : small : fused

9 : 3 : 4 ; **1 m for correct ratio;** [3]

(b) (i) State the <u>expected</u> phenotypic ratio of the offspring in Table 5.1.

1:1:1:1 [1]

(ii) A X^2 (chi-squared) test was conducted on the results. Using the formula and table of probabilities given below, calculate the X^2 value and give the conclusion that may be drawn from it.

$$\chi^2 = \sum \frac{(\text{Observed Value} - \text{Expected Value})^2}{(\text{Expected Value})}$$

Calculated X^2 value = 67.36 **[1/2]**

Degree of freedom = 3

Probability = < 0.001

Distribution of X^2

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

Conclusion:
Since p<0.001

Difference between expected and observed phenotypic ratio is significant and not due to chance;
Observed ratio does not follow expected ratio of 1:1:1: [2]

(b) (iii) Explain the observed phenotypic ratio in Table 5.1.

- Linked genes
- Allele **T** is linked to allele **Y** and allele **t** is linked to allele **y**;
- If crossing over occurs, linkage between alleles is broken / new allele linkages will be formed;
- Such that recombinant gametes/chromosomes (**Ty** and **tY**) are formed ;
- Large no. of tall, yellow-flowered and dwarf, white-flowered / offspring with parental phenotypes OR small no. of dwarf, yellow-flowered and tall, white-flowered / offspring with recombinants phenotypes; [Max 2]

6 **(a)** Myasthenia gravis is a disease of the neuromuscular junctions which causes muscular weakness. It develops because the muscle's response to repeated nerve signals declines with time, and the muscles become weak and tired.

(i) State one similarity in the structure of a normal and myasthenic neuromuscular junction as seen in Fig. 6.1a and 6.1b and explain how it aids in synaptic transmission. [2]

Any one:

- Pre-synaptic neurones are able to secrete acetylcholine. [1/2]
- Acetylcholine diffuses across the synaptic cleft [1/2] and bind to the acetylcholine receptors [1/2] present on the post-synaptic membrane [1/2] / muscle cell → membrane depolarisation [1/2]

Or

- Acetylcholine receptors [1/2] present on the post-synaptic membrane [1/2] / muscle cell.
- Upon binding with acetylcholine, ligand / chemical / Na^+-gated channel opens [1/2] → influx of Na^+ [1/2]→ membrane depolarisation [1/2]

(ii) State one difference in the structure as seen in Fig. 6.1a and 6.1b and explain how it affects synaptic transmission. [2]

Any one:

- Unlike normal muscle cell which is deeply folded [1/2], Myasthenic neuromuscular junction has less shallow in-folding [1/2] of the post-synaptic membrane / muscle cell
- Affects the number of acetylcholine receptor [1/2] (or AChR) embedded on the membrane available to bind to acetylcholine

- Abnormal acetylcholine receptor (or fewer normal acetylcholine receptors present) [1/2] present on the post-synaptic membrane / muscle cell
- Cannot bind to acetylcholine [1/2] / bind to auto-antibodies

- Hence, acetylcholine cannot bind [1/2]
- No post-synaptic depolarisation possible [1/2] → no action potential

(b) Describe the mechanism that ensures unidirectional movement of nerve impulses along the axon of a neurone and no overstimulation of the neurone. [2]

- Refractory period [1/2] – short time immediately after an action potential in which the neurone cannot respond to another stimulus [1/2]

<u>Absolute refractory period</u>

- Voltage-gated Na^+ channels are either already opened (during depolarisation phase) or are inactivated (during repolarisation phase) [1/2]
- Cannot initiate an action potential no matter how strong is the stimulus [1/2]

<u>Relative refractory period</u>

- Voltage-gated K^+ channels are open and membrane is hyperpolarised / during hyperpolarisation phase) [1/2]
- An action potential can only be initiated if the stimulus is stronger than usual [1/2]

- Correct mention of both absolute and relative refractory period [1/2]

(c) The diagram shows the action potentials generated over a fixed time, X.

In the space provided, draw the action potentials generated over the same time period if the stimulus is more intense. [1]

- more AP within period X

7

(a) State the type of receptor that glucagon binds to and explain how this receptor is the fully activated. [2]

- **G-protein linked/coupled receptor**
- Binding of glucagon to complementary binding site on receptor cause a **change in conformation** of the receptor which can now bind G-protein

(b) With reference to fig 7.1, briefly explain how cAMP can lead to an increase in blood glucose concentration. [3]

- cAMP is a **second messenger** in the **signal transduction pathway** of glucagon
- cAMP triggers different signal pathways leading to **different cellular responses** such as glycogenolysis and gluconeogenesis
- cAMP activates the **protein kinase A** which phosphorylates and activates the enzymes needed for the glycogenolysis/the breakdown of glycogen to glucose-1-phosphate and glucose
- Gluconeogenesis which is stimulated results in the generation of glucose from non-carbohydrate carbon substrates such as pyruvate and lactate in the liver.
- Inhibition of the enzymes (e.g. glycogen synthase) needed for the formation of glycogen from glucose
- The glucose is then released by facilitated diffusion through glucose carriers on the surface of the liver cells to raise blood glucose concentration

8 (i) Explain why the evolution of *Jadera haematoloma* after the introduction of a non-native soapberry plant in the 1920s is not considered a form of divergent evolution. [1]

- It did not involve the an inherited characteristics / homologous structure (e.g. sharp beak) undergoing modification to perform different functions [1/2]
- nor did it involve speciation to become two or more different species [1/2]
  or
- There is actually a decrease in phenotypic variation [1/2]
- Q.V [1/2]

(ii) With reference to the information and Fig. 7.2, account for the beak lengths over time. [4]

<u>Description of trend</u>

- Variation of beak lengths prior 1920 / before introduction of the non-native soapberry plant / prior 1920 [1/2]
- QV: 5.5 to 9.1mm [1/2]
- Reduction of beak lengths from 1920 onwards / after introduction of non-native plant [1/2]
- QV: 5.5 to 6.5mm [1/2]

Explanation

- Thinner skin of the non-native fruit provides a selective pressure favouring shorter beak / shorter beak bugs are selected for [1/2]

- Possible reason – same access to nutrients / food as the longer beak bugs but able to survive longer than the longer beak bugs since they do not need to channel additional resources to develop a longer beak [1/2]

- Higher reproductive success [1/2]
- pass favourable alleles to the offspring [1/2]
- Higher proportional of alleles coding for shorter beaks in the gene pool over time [1/2]

(ci) Suggest a likely explanation for the results seen in island Y.

- Ref to genetic drift affected the outcome [1/2]

- Since only 40 individuals (or idea of small population) [1/2] were initially taken to the laboratory, by random chance none of the individual carried the alleles coding for the longer beak. [1/2]

Also accept: By random chance, the individuals carrying the alleles coding for the longer beak in the initial population failed to reproduce successfully after one or few generations in the laboratory and so the alleles were removed in the gene pool [1/2]

- Ref to alleles coding for short beak were fixed in the gene pool [1/2]

(ii) Explain the results in Fig 8.4. [2]

- Bug population in island Y suffered from a low variation of alleles for beak length / only have alleles for short beak length [1/2]
- No natural selection [1/2]
- When only thick-skinned fruits were available, the short beaks could not penetrate the skin to obtain the nutrients so entire population became extinct [1/2]

- Bug population in island X had a greater variety of alleles for beak length [1/2]
- When only thick-skinned fruits were available, the bugs with longer beaks could penetrate the skin to obtain the nutrients and are favoured by natural selection and so increase in number over time [1/2]

(c) Analysis of the DNA sequences of the soapberry bugs in both islands before the viral invasion revealed differences that could not be explained by the theory of natural selection alone. How may the neutral theory of molecular evolution and the genetic code account for the differences? [2]

- Neutral mutations [1/2] occurred but did not affect the fitness / reproductive success of the individuals [1/2]

- Mutations occurred in the non-coding regions which comprise a large proportion of the genome did not affect the phenotype [1/2]

- Mutations occurred in the coding regions could still give rise to the same phenotype because:

  - different codons may code for the same amino acid / degenerate nature of the code [1/2]
  - different codon codes for a different amino acid but retained similar property as the amino acid coded by the original codon [1/2]

2016 H2 BIOLOGY PRELIM PAPER 2 ESQ answers

9 (a) Explain how molecular and anatomical homology supports Darwin's theory of natural selection. [6]

Darwin's theory of natural selection

- It is based on **descent with modifications** [1/2] where different species are **related by descent / share common ancestors** [1/2]

- Heritable traits underwent modifications which resulted in the **differences between present species and the ancestral species** [1/2]

Anatomical homology

- Different but related species would share a basic anatomical plan which they inherited from their common ancestor.

- Named example 1: **Pentadactyl limb** [1/2]
- The **limbs** of all mammals / air-breathing vertebrates share a **basic bone arrangement plan** [1/2]
- Modified differently amongst the descendent species → **locomotion** [1/2]
- As adaptation to the **particular environment / selective pressure** [1/2]
- At least **2 examples** [1/2]: Human forelimbs – manipulation; whales – swimming; bats – flying; AVP
- Named example 2: **Vestigial structure** [1/2]
- **Reduced and may be non-functional** in some species but **functional in other species** [1/2]
- Degeneration of structure due to the **absence of selective pressure** which used to be present [1/2]
- At least **1 example** [1/2]: Limbs in snakes – no longer needed / beneficial for locomotion; Hind-limbs in whales – not needed for swimming / ancestor was a land mammal; AVP

Molecular homology

- Similarities in **genetic language of DNA and RNA** [1/2]
- **Universal genetic code** [1/2] among all species

- **Similarities in DNA / RNA / amino acid sequences** in **homologous genes or proteins** [1/2]
- Known **closely related species or members of the same species** sharing a more recent common ancestor have **greater similarity** than **less related species** [1/2]

10b) Explain the significance of mutations and genetic drift in the neutral theory of molecular evolution [6]

- Evolutionary change at the molecular level occurs primarily through neutral mutations [1/2] which do not affect the phenotype of the organism [1/2]
- According to the theory, most of the genetic variation in populations is the result of mutation and genetic drift and not selection [1/2]
- Mutations result in the creation of new alleles [1/2]
- May or may not affect the biological fitness of the individual [1/2]
- However, many mutations which are neutral
- Reasons:
    - Mutations occurred in the non-coding region of the genome [1/2]
- since non-coding sequences make up the majority of the eukaryotic genome [1/2]
    - Mutations in the coding region may not affect the phenotype
- due to the degenerate code where one amino acid may be coded by more than one codon [1/2]
- If mutations in the coding region result in a slightly different protein, it may also not affect the fitness of the individual (i.e. neither detrimental or adaptative) [1/2]

- In subsequent generations, the frequency of the neutral alleles changes due to genetic drift [1/2] which is the random change of allele frequencies in the gene pool of a (especially small) population from one generation to the next [1/2] Through genetic drift, some of the neutral alleles may be over or under represented [1/2] or lost or become fixed [1/2] in the gene pool. The evolutionary change in the population or species is more pronounced if it is small [1/2]
- Ref to small size due to Founder Effect or Bottleneck Effect [1/2]
- Founder effect – when a few individuals become isolated from a larger population and establishes a new population [1/2]
- Bottleneck effect – when sudden change in environment (e.g. any named natural disaster or over-hunting/over-predation) led to drastic reduction in population size [1/2]
- Hence, only neutral mutations and genetic drift are significant to the theory [1/2]

9b)Describe how the response of muscle cells to insulin with respect to cell signalling. [8]

- Insulin is released from the **β cells of the islets of Langerhans** in the pancreas;

- in response to a rise in blood glucose level **above the norm** of 100mg /100cm^3 blood;
- Before the insulin (ligand) binds, the insulin receptors exist as two individual polypeptides **subunits**;
- The insulin is carried by the blood and **binds to insulin receptor** on the target cell to form a hormone-receptor complex;
- The ligand binding causes two receptor subunits to associate closely with each other forming a dimer (ref . to **dimerization**);
- Dimerization **activates the tyrosine kinase** region of each polypeptide;
- Each tyrosine kinase adds a phosphate from an ATP molecule to a tyrosine on the tail of the other polypeptide (ref. to **cross phosphorylation**);
- The fully-activated receptor protein is now recognized by specific **relay proteins** inside the cell;
- Each relay protein will bind to specific phosphorylated tyrosine residues and will undergo a resultant conformation change that activates it;
- Each activated relay proteins triggers a **specific transduction pathway**;
- leading to a **cellular response**;
- As a result of the activation of **different** relay molecules by **one** activated RTK, ligand binding to a RTK may results in **multiple transduction pathways and cellular responses** (see examples below);
- Ref. to **phosphorylation cascade**, in which a series of different proteins in a pathway are phosphorylated sequentially;
- Ref. to **signal amplification** as one ligand can result in many different downstream proteins being activated
- These cellular responses help bring blood glucose levels back to the norm of 100 mg/100 ml blood. Once the norm level is reached, the **negative feedback mechanism** prevents the further release of insulin from the β cells.

Examples of cellular responses:

- Insulin facilitates the transport of glucose into cells by **increasing the number** of **glucose carriers** at the membranes of the cells;
- Upon activation of the insulin receptor, a signal transduction pathway is activated that causes vesicles in the cytoplasm that contain glucose carrier proteins to **move and fuse to the cell membrane**;
- Increase in glucose carrier proteins, **increase in facilitated diffusion of glucose** into cells;
- Stimulate **glycogenesis** - Activates enzymes involved in glycogen synthesis e.g. glycogen synthase which polymerises glucose-1- phosphate (formed from G6P) to glycogen;

9c) Define control elements and explain how they interact with other factors to influence transcription. [6]

Control elements

- Non-coding regions of the genome that function as binding sites of transcription factors [1/2]
- To control the rate of transcription of genes [1/2]
- Include promoters, enhancers and silencers [1/2]

Promoter

- Proximally upstream of the gene it controls [1/2]
- Contains TATA box [1/2]
- Recognised and bound by general transcription factors [1/2]
- Which recruit RNA polymerase to form transcription initiation complex in order to turn on transcription [1/2]

Enhancer

- Recognised and bound by activators [1/2]
- DNA bending protein causes DNA to bend, bringing the bound activator close to the promoter [1/2]
- Bound activator interacts with the transcription initiation complex to increase transcription [1/2]

Silencer

- Recognised and bound by repressors [1/2]
- Bound repressor interacts with the transcription initiation complex to decrease transcription, prevent activator binding or function, as well as causing DNA to be tightly coiled [1/2]

- Ref. to enhancers and silencers being distal to genes they control [1/2]
- Ref. to transcription factors being able to bind to the control elements when DNA is less tightly coiled / in euchromatin form [1/2]
- Due to histone acetylation and lack of DNA methylation [1/2]

10 (a) Explain how recessive alleles may be preserved in a natural population. [6]

D1. Diploidy – eukaryotes are diploid / have two copies of alleles present for each locus; (each gene can have more than 2 alleles but at any one time, a diploid organism can only have 2.)

D2. Recessive alleles carried by heterozygotes; not subjected to natural selection (hidden from selective effect);

D3. Heterozygotes express dominant trait;

D4. Through masking of recessive allele by the dominant allele of the gene;

D5. (Ref. to effect of natural selection) dominant trait selected for;

D6. Variation only exposed to selection in rare occasion when both parents carry recessive allele, and both copies ending in the same zygote (recessive trait selected against);

D7. Recessive alleles being passed on to the offspring when heterozygotes propagate;

B1. Balancing selection;

B2. When natural selection maintains 2 or more forms in population; (Note: each form is a result of a particular genotype i.e. combination of alleles)

B3. Heterozygote advantage;

B4. When heterozygotes have greater fitness / selective advantage over both kinds of homozygotes;

B5. The recessive allele (in heterozygotes) will be maintained by natural selection / natural selection favours recessive alleles;

B6. Frequency-dependent selection;

B7. When fitness of a phenotype declines if it becomes too common in the population;

B8. The different alleles (including recessive alleles) can be maintained within the population; or frequency of different alleles oscillates over time;

N1. Neutral variation;

N2. Mutation resulting in recessive allele ultimately has no effect on survival or reproductive fitness of individual; not selected against/ selectively neutral;

10 (b) Explain the advantages and significance of having a cell signalling system. [6]

- Cell signalling system comprise of 3 stages: signal reception, signal transduction and cellular response;
- Helps ensure crucial activities/reactions occur in the right cells, at the right time and in proper coordination with other cells of the organism;;
- (At signal reception stage) Specific cells detect specific ligands/signalling molecules; (e.g. hormone glucagon binds to receptor on alpha-cells of islets of Langerhans)

- Ligand shape is complementary to receptor found on/within target cell;
- (Signal transduction stage) Ligand binding causes conformation of receptor protein to change; triggering transduction/signal transduction/multi-step pathway; that involves a sequence of changes in a series of different molecules; such as relay proteins and second messengers (e.g. glucagon biding to GCPR causes it to underdo conformational change, in turn activating G protein, which in turn activates adenylyl cyclase, causing increased production of cAMP (second messenger), which in turn activates protein kinase, and triggers phosphorylation cascade)
- (Cellular response stage) Specific cellular response elicited;

Advantages of multistep pathway:

- Signal amplification; some of the molecules in the pathway transmit the signal to numerous molecules at the next step of the series resulting in a large number of activated molecules at the end of the pathway; (e.g. phosphorylation cascade mentioned above)
- Regulation allows for fine tuning and control of cellular response;
- Numerous cellular responses can be elicited from a single ligand molecule; (e.g. activation of numerous transcription factors and consequently genes, like glycogen phosphorylase and glycogen synthase, which catalyse various reactions that ultimately bring blood glucose levels up to the norm)

10c) Describe binary fission and explain how it differs from bacterial conjugation [8]

- First the circular DNA attaches itself to the cell membrane;
- Duplication starts at the origin of replication; and occurs bidirectionally;
- DNA replicates semi-conservatively;
- When the cell divides, the duplicated DNA is separated and the cell membrane folds inwards to form a double layer across the long axis of the cell.
- New cell wall layers are secreted within the membrane layers.
- This divides the cell into two smaller, identical cells;

	Binary fission	Bacterial conjugation
Purpose of process	Form of asexual reproduction to form genetically identical offspring	Way in which bacteria acquire new genetic material
Number of bacterium involved	Involves one parent bacterium only	Involves 2 bacteria (one F+ /donor cell and one F- /recipient cell)
Type of genetic material replicated	The bacterial chromosomes (and plasmids) of the cell is	The F plasmid is copied and transferred to the recipient

and how it is transferred to recipient (conjugation) or progeny (binary fission)	copied and the cell divides to allow each cell to have one copy.	using a cytoplasmic bridge.
Change in size of bacteria/Fate of bacteria after the process	The parent cell enlarges before dividing equally into two/ Parent cell becomes 2 daughter cells	No change in size of the bacteria involved
Length of time needed for process	Takes a longer time as all genetic material and cellular structures like ribosomes have to be duplicated, as well as the synthesis of a new cell wall to divide the bacterium into two	Takes a shorter time as only time needed for the construction of a cytoplasmic bridge and the transferring of the small F plasmid
What is synthesised in the process	Involves duplication of all genetic material, bacterial ribosomes and new cell wall layers	Involves the synthesis of a cytoplasmic bridge for transfer of the F factor from donor to recipient

BIOLOGY DEPARTMENT
JC2 PRELIMINARY EXAMINATIONS
2016 Higher 2

CANDIDATE NAME

CLASS | 1 | 5 | S | | |

INDEX NUMBER | | |

BIOLOGY 9648/03

Paper 3 **19 September 2016**

Additional Materials: Answer Booklet/ Paper **2 Hours**

READ THESE INSTRUCTIONS FIRST

Write your CT GP/ INDEX NO. and name on all the work you hand in.
Write in dark blue or blue pen.
You may use a soft pencil for any diagrams, graphs or rough working.
Do not use any staples, paper clips, highlighters, glue or correction fluid.

Answer **all** questions.

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

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

For Examiner's Use	
Section A	
1	
2	
3	
Planning Question	
5	
Total	

Answer **all** questions

Question 1

(a) The bacterial plasmid, pBR322, was used as a vector for Gene X as shown in Fig. 1.1 below.

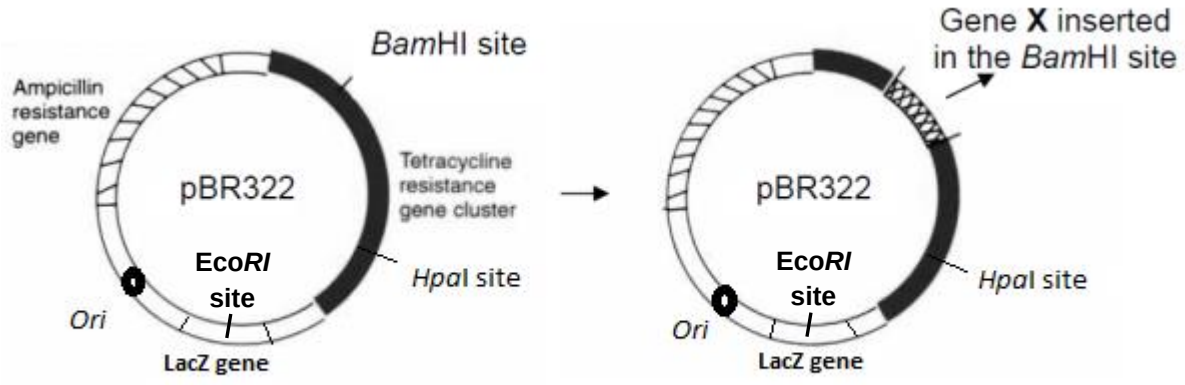


Fig. 1.1

Gene X was inserted in the *Bam*HI restriction site. pBR322 also contain the *Eco*RI and *Hpa*I restriction sites. The target sites for these restriction enzymes are shown in the table below. The lines drawn in each sequence show where the enzyme cuts the DNA molecule.

restriction enzyme	specific target base sequence of DNA
EcoRI	G | A A T T C C T T A A | G
BamHI	G | G A T C C C C T A G | G
HpaI	G T T | A A C C A A | T T G

(i) With reference to Fig. 1.1, explain how two properties of plasmid pBR322 allow it to be used as a vector.

……..

……..

……..

…… [2]

(ii) Outline the steps taken to produce the recombinant plasmid shown in Fig. 1.1.

……..

……..

……..

…… [2]

(iii) Explain the disadvantage that would arise if gene X was to be inserted into the *Hpa*I restriction site instead of the *Bam*HI site.

……..

……..

……..

…… [2]

(b) Calcium chloride heat shock treatment was then used to introduce the recombinant plasmid into *Escherichia coli*. However, the process of creating recombinant plasmids is typically not 100% efficient. Often, a mixture of re-annealed plasmid and re-annealed DNA is produced along with the recombinant plasmid. These may be taken up by the bacteria as well. This necessitates the process of selecting for the bacteria that have successfully taken up the recombinant plasmid. As such, the bacteria was first plated onto a nutrient agar plate containing ampicillin. Replica plating was subsequently carried out onto a nutrient agar plate containing tetracycline. Bacterial growth on both plates is shown in Fig. 1.2.

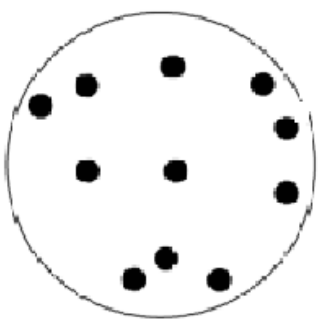


Plate A
Master plate containing ampicillin

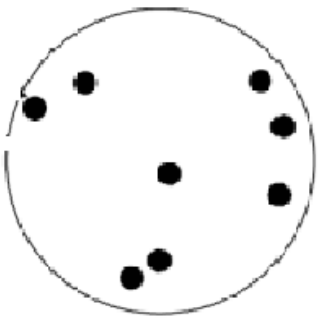


Plate B
Replica plate containing tetracycline

Fig. 1.2

With reference to Fig 1.2,

(i) Circle the colonies that were successfully transformed with the recombinant plasmid.

[1]

(ii) Account for the difference in colony numbers in Plate A and B.

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…… [3]

(c) In a separate cloning experiment, the same plasmid pBR322 was used to introduce another gene, Gene Y, into a batch of E.coli cells. Fig. 1.3 shows the results of plating the transformed E.coli cells onto an agar plate with the appropriate substances.

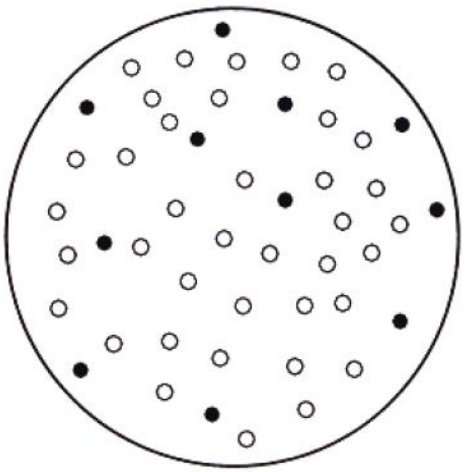

Fig. 1.3

(i) Explain why the colonies differ in colours in Fig. 1.3.

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……..

…… [4]

(ii) Suggest why replica plating was not necessary in this experiment.

……..

…… [1]

(d) In another cloning experiment, another plasmid pBR33 was used to introduce Gene Z into a different strain of *E.coli* bacteria. Gene Z was inserted into one of the three selection markers found in pBR33 – neomycin resistance gene, kanamycin resistance gene and streptomycin resistance gene.

The bacteria were then plated onto nutrient agar plate containing neomycin. Replica plating was subsequently carried out onto nutrient agar plate containing streptomycin. Bacterial growth on the two plates is shown in Fig. 1.4 below.

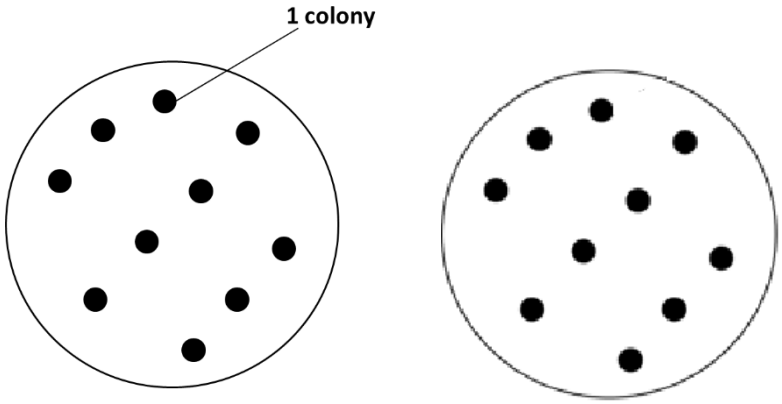

Fig. 1.4

Account for the results obtained in Fig. 1.4.

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……..

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…… [3]

[Total 18]

Question 2

Explain why two primers are used for polymerase chain reaction.

……...

……...

……...

…… [2]

Duchenne muscular dystrophy is a genetic disease in which there is a progressive loss of muscle mass, leading to physical weakness, difficulty in standing and walking, and eventually paralysis and death. Early symptoms of the disease can only be observed between the ages of 2 and 3 in most patients. A group of doctors and medical biologists discovered a RFLP marker, found on the same chromosome as the disease gene, which can be used in the screening of the disease during pregnancy.

To investigate the effective of the RFLP marker in disease screening, samples of DNA were obtained from a family known to have the disease. The RFLP locus was isolated and amplified using polymerase chain reaction, which was then mixed with *Bam*HI restriction enzymes. The pedigree tree of the family and results of gel electrophoresis are shown in Fig. 2.1.

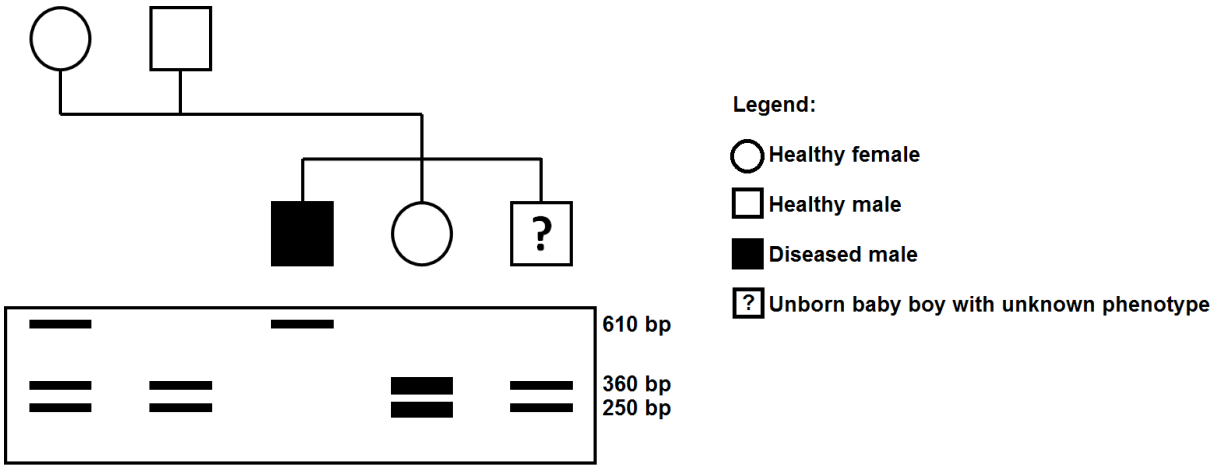

Fig. 2.1

(a) With reference to Fig. 2.1,

(i) state the mode of inheritance of the Duchenne muscular dystrophy.

…… [1]

(ii) explain why there are different fragment lengths after restriction digest.

……...

……...

……...

……...

…… [3]

(iii) Explain the difference in the band patterns between the father and the daughter.

……...

……...

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……...

……...

……...

…… [3]

(b) Some years later, the baby boy with unknown phenotype is born and has reached two years of age. Clinical diagnosis reveals that he too suffers from Duchenne muscular dystrophy.

(i) Explain why the band pattern of the baby boy is different from that of his older brother even though both are with the disease. Assume no new mutations occurred in the disease gene or RFLP locus.

……...

……...

……...

…… [2]

(ii) Suggest an ethical implication that may arise due to the use of this RFLP marker to screen for Duchenne muscular dystrophy.

……...

……. [1]

[Total: 12]

Question 3

(a) Patients with severe combined immunodeficiency disorder (SCID) are vulnerable to serious infections and death. There are two main types of SCID, X-SCID and ADA-SCID. Besides the location and difference in their modes of inheritance, give two differences between the two types of SCID.

Difference	X-SCID	ADA-SCID
1		
2		

[2]

(b) Gene therapy can be used to treat SCID by introducing retrovirus containing the normal gene into hematopoietic stem cells (HSCs). The HSCs are then infused into the patient.

Explain how this choice of vector and target cells may theoretically lead to long term treatment of the disease.

……...

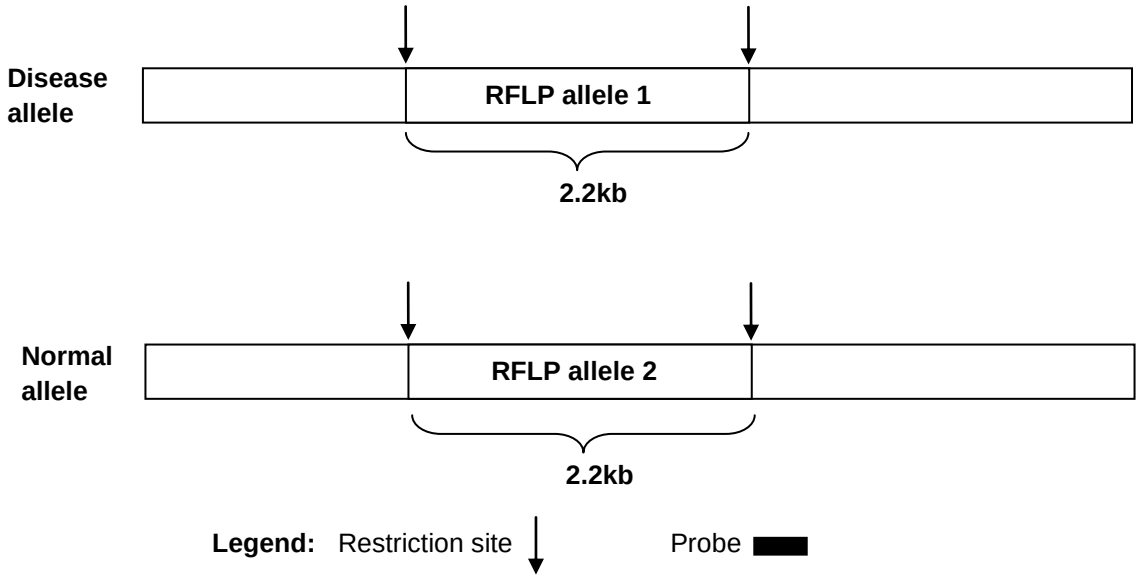
……...

……. [2]

(c) In one attempt to treat ADA-SCID, hematopoietic stem cells (HSCs) from the bone marrow of baby X patient were collected and treated with retrovirus containing the ADA gene before infusing them back.

Genomic DNA from the T lymphocyte cells of baby X was later extracted. The extracted DNA was digested with a restriction enzyme and subjected to Southern blot analysis using a specific probe that binds to a known RFLP marker found within the gene associated with the disease.

The RFLP allele 1 associated with the disease allele gives rise to a 0.8kb band while the RFLP allele 2 associated with the normal allele gives rise to a 2.2kb band.



(i) With the information provided, draw to indicate the position of another restriction site and the position where the probe binds to. You should also indicate the length of the restriction fragments that would be produced. [1]

The RFLP band patterns from baby X's normal brothers, Tom and John, are shown in Fig. 3.1.

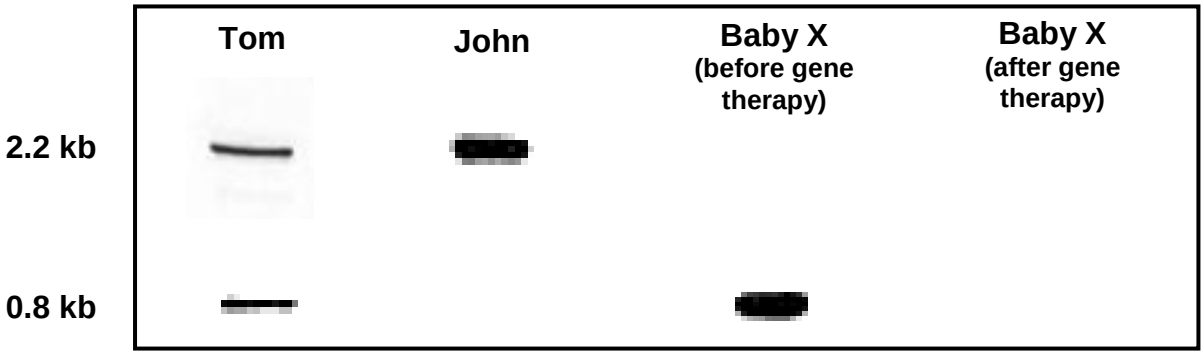


Fig 3.1: Results of Southern Blot analysis of DNA extracted from T lymphocytes

(ii) With reference to Fig 3.1, explain the difference in the band pattern of Tom and John.

……...

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……...

…… [2]

(iii) Draw the expected band pattern of baby X after gene therapy treatment. [1]

Over the next 360 days after infusion, the total number of various blood cells (e.g. red blood cells and white blood cells) of baby X were recorded at regular intervals.

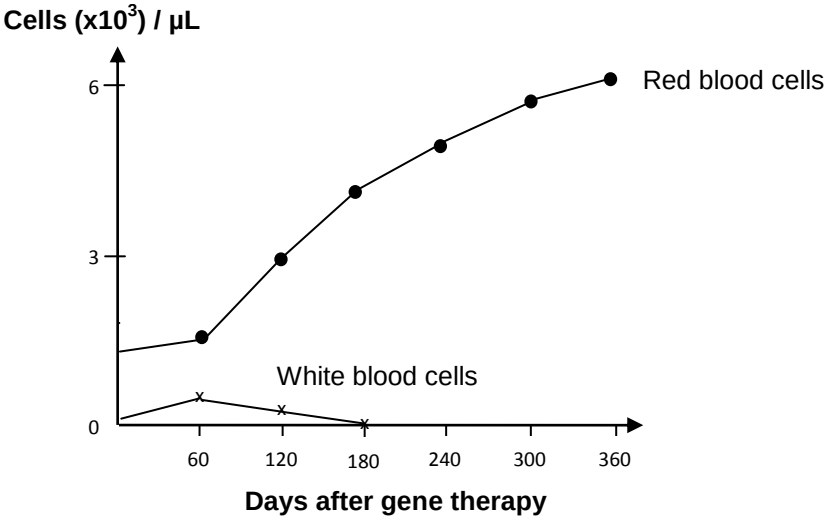

Fig 3.2

(iv) With reference to Fig 3.2, describe the effectiveness of the treatment in baby X.

……...

…… [1]

(v) Suggest a reason for your answer. [1]

……...

…… [1]

[Total 10]

Planning Question

4. An orange plantation owner wants to find out the amount of ascorbic acid (vitamin C) that his breed of oranges produces. He believes that his oranges produce the most vitamin c compared to the standard orange breeds which typically contain 0.8 to 1.6 $mmolL^{-1}$.

The amount of ascorbic acid present in a sample can be determined using a bioassay method. At pH 8, ascorbic acid reduces solutions of the dye dichlorophenol indophenol (DCPIP) from blue to colourless. For the bioassay to work, the pH of the samples must be adjusted to pH 8. Ascorbic acid does not chemically change when neutralised by sodium hydroxide, or when boiled.

Using this information and your own knowledge, design an experiment to determine the validity of the plantation owner's claim that orange juice from his plantation contains higher concentrations of ascorbic acid.

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

- 100 cm^3 of 5.0 $mmolL^{-1}$ stock solution of ascorbic acid, adjusted to pH 7
- 100 cm^3 distilled water
- 100 cm^3 molten agar containing DCPIP
- Sterile petri dishes
- 1ml syringe
- plastic straw to create wells in the agar plate
- Ruler / 2mm graph paper
- Labels
- Timer, e.g. stopwatch
- Bunsen burner
- Normal laboratory glassware e.g. test tubes, beakers, graduated pipettes, droppers, glass rods etc
- 10 cm^3 orange juice, supplied by the plantation owner
- 10% sodium hydroxide solution
- pH indicator paper to indicate alkaline pH

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

[Total: 12]

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Free-response question

Write your answers to this question on the separate paper provided.

Your answer:

- should be illustrated by large, clearly labelled diagrams, where appropriate.
- must be in continuous prose, where appropriate.
- must be set out in section (a), (b), etc., as indicated in the question.

5 **(a)** Outline the procedure for the production of human growth hormone by genetic engineering techniques. [8]

(b) Describe the benefits of the Human Genome Project. [8]

(c) Discuss the ethical concerns that have arisen from genetically modified organisms. [4]

[Total: 20]

ANSWERS

Question 1

(a) The bacterial plasmid, pBR322, was used as a vector for Gene X as shown in Fig. 1.1 below.

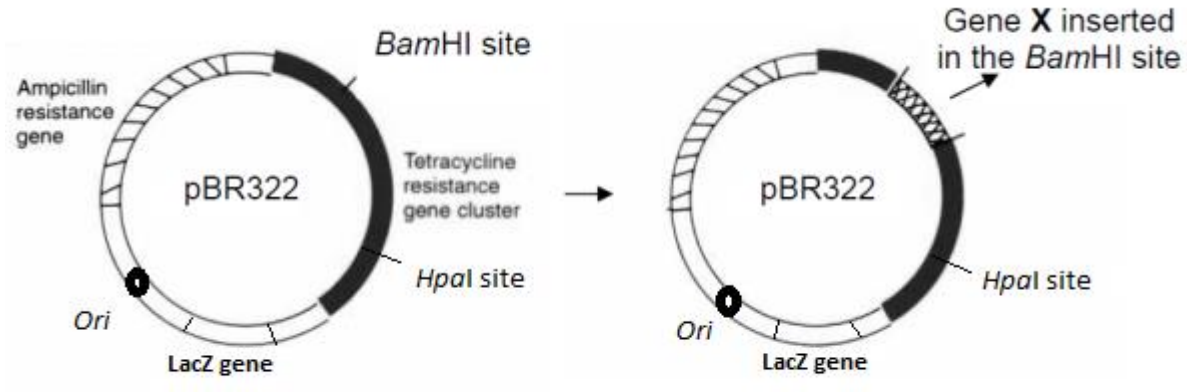


Fig. 1.1

Gene X was inserted in the *Bam*HI restriction site. pBR322 also contain the *Eco*RI and *Hpa*I restriction sites. The target sites for these restriction enzymes are shown in the table below. The lines drawn in each sequence show where the enzyme cuts the DNA molecule.

restriction enzyme	specific target base sequence of DNA
EcoRI	G | A A T T C C T T A A | G
BamHI	G | G A T C C C C T A G | G
HpaI	G T T | A A C C A A | T T G

(i) With reference to Fig. 1.1, explain how two properties of plasmid pBR322 allow it to be used as a vector. [2]

- Presence of origin of replication; so inserted gene can be replicated;
- Has ampicillin and tetracycline resistance genes; as selection markers / allow identification of host cells that have successfully taken up the recombinant plasmid

(ii) Outline the steps taken to produce the recombinant plasmid shown in Fig. 1.1. [2]

- Cut plasmid and gene X with BamHI restriction enzyme;
- "Sticky ends" generated;
- Allow plasmid and gene to anneal with ref. complementary base pairing;

- Add ligase to seal the nicks in the sugar-phosphate backbone / form phosphodiester bonds between the ends of the cut plasmid and gene;

(iii) Explain the disadvantage that would arise if gene X was to be inserted into the *Hpa*I restriction site instead of the *Bam*HI site. [2]

- HpaI generates "blunt ends";
- No hydrogen bonds to hold the cut plasmid and gene together;
- Ref. extra step that will require linker;
- To generate "sticky ends"

(b) Calcium chloride heat shock treatment was then used to introduce the recombinant plasmid into *Escherichia coli*. However, the process of creating recombinant plasmids is typically not 100% efficient. Often, a mixture of re-annealed plasmid and re-annealed DNA is produced along with the recombinant plasmid. These may be taken up by the bacteria as well. This necessitates the process of selecting for the bacteria that have successfully taken up the recombinant plasmid. As such, the bacteria was first plated onto a nutrient agar plate containing ampicillin. Replica plating was subsequently carried out onto a nutrient agar plate containing tetracycline. Bacterial growth on both plates is shown in **Fig. 1.2**.

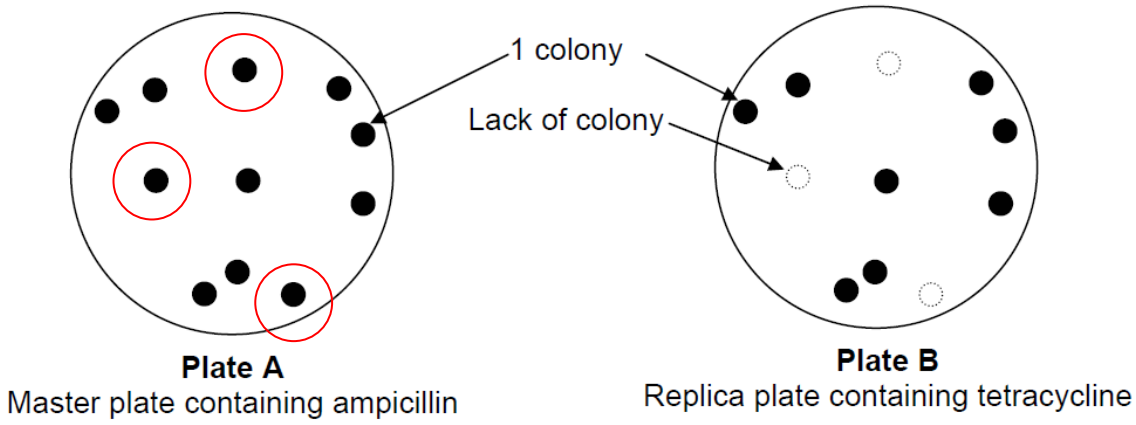


Fig. 1.2

With reference to Fig 1.2,

(i) Circle the colonies that were successfully transformed with the recombinant plasmid. [1]

(ii) Account for the difference in colony numbers in Plate A and B. [3]

- Plate A selects for all successfully transformed cells/ taken up plasmid with ampicillin gene; (maybe recombinant plasmid or re-annealed plasmid);
- Selected against / killed off cells which took up re-annealed DNA;
- Plate B contain 3 fewer colonies compared to Plate A;
- Missing colonies had successfully taken up the recombinant plasmid;
- where gene X had been inserted into tetracycline gene and disrupted it (ref. to insertional inactivation) / colonies lost tetracycline resistance and died;

- Therefore comparing the difference in colony numbers between Plate A and B will allow us to identify recombinant cells/ colonies;

(c) In a separate cloning experiment, *E.coli* cells were transformed with another type of plasmid carrying a different selectable marker. Fig. 1.3 shows the results of plating the transformed *E.coli* cells onto an agar plate with the appropriate substances.

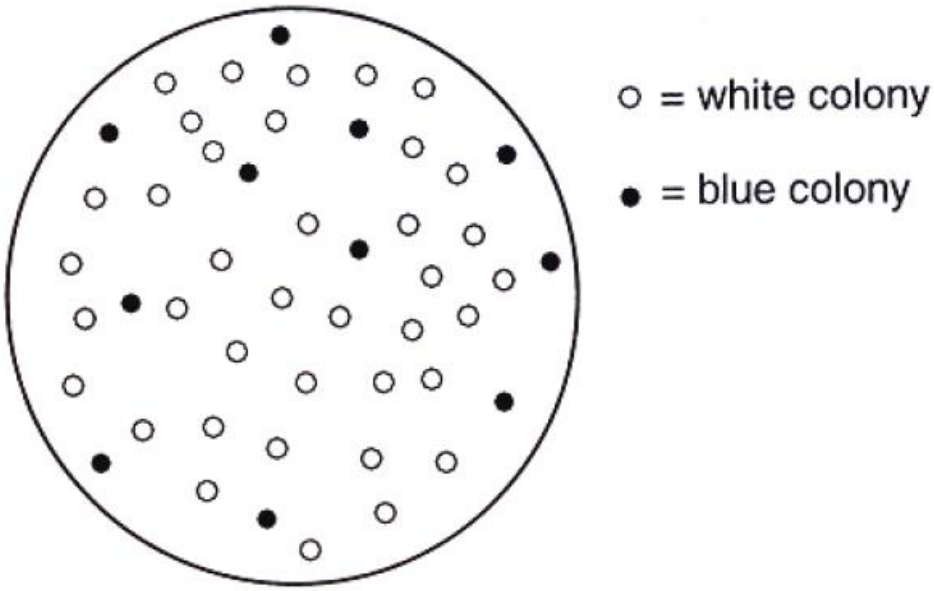


Fig. 1.3

(i) Explain why some colonies appeared white while others appeared blue. [4]

- The cells must have been transformed by plasmids containing intact lacZ gene as the selectable marker;
- The agar plate must have contained X-gal and IPTG;

- lacZ gene codes for β-galactosidase enzyme;
- β-galactosidase enzyme catalyses the conversion of X-gal in the agar from colourless to blue;
- Hence, colonies that contain the reannealed / non-recombinant plasmids appeared blue.

- Should the lacZ gene in the plasmid be disrupted as a result of insertion of foreign DNA / gene, ref. to insertional inactivation of lacZ gene;
- no functional β-galactosidase enzyme will be produced and thus no blue product is formed;

- Hence, colonies that contain the recombinant plasmids appeared white.

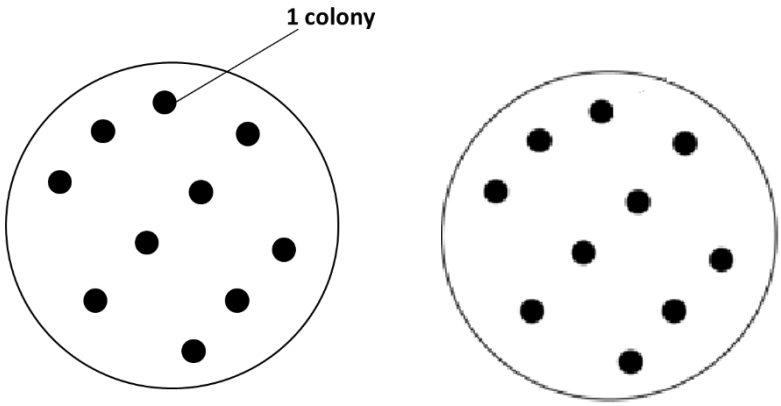
(ii) Suggest why replica plating was not necessary in this experiment. [1]

- By their colours, colonies that contain the recombinant plasmids (appeared white) can be differentiated from those that do not (appeared blue);
- Replica plating is necessary only when the selection process kills off the desired colonies.

- In the case of using two antibiotic resistance genes as the selection markers, the insertion of foreign DNA / gene disrupts only one of the two antibiotic resistance genes in the plasmid.
- To identify colonies that contain the recombinant plasmids, the colonies must be treated with two types of antibiotics.
- Those that survived one antibiotic treatment but not the other would be the desired colonies.
- However, adding both antibiotics to the same agar plate would kill off the desired colonies.
- To get living cells that contain the recombinant plasmids, replica plating must be done.)

d) In a separate cloning experiment, another plasmid pBR33 was used to introduce Gene Z into a different strain of *E.coli* bacteria. Gene Z was inserted into one of the three genetic markers found in pBR33 – neomycin resistance gene, kanamycin resistance gene and streptomycin resistance gene.

The bacteria were then plated onto nutrient agar plate containing neomycin. Replica plating was subsequently carried out onto nutrient agar plate containing streptomycin. Bacterial growth on the two plates is shown in Fig. 1.4 below.



Account for the results obtained in Fig. 1.4. [3]

- Gene Z inserted in kanamycin-resistant gene;
- Insertional inactivation;
- 3 functional genes in re-annealed/non-recombinant plasmids vs 2 functional genes is recombinant;

- Bacteria with recombinant plasmid can survive in presence of neomycin and streptomycin
- Bacteria with re-annealed plasmid can survive in all 3 antibiotics
- Both plates have same number; and position; of colonies; QV: 10 colonies
- Both plates do not contain any non-transformed bacteria; consists of transformed cells with recombinant and non-recombinant plasmid

Alternative:

- Gene Z inserted neomycin-resistant gene;
- So plate N has killed off all bacteria with recombinant plasmids and non-transformed bacteria
- So when is done on from Plate N to plate S, only the bacteria that survive (i.e. the recombinant bacteria) are picked up by the nitrocellulose membrane and transported to plate S;

[Total: 18]

Question 2

Explain why two primers are used for polymerase chain reaction. [2]

- Ref. to the 2 primers as **forward and reverse primers** [1/2]
- **Flank the targeted sequence** to be amplified [1/2]
- **Amplify large quantities of a specific sequence** of DNA in a short period of time [1/2]
- **1 primer anneals to 1 of the separated DNA strands** after denaturation [1/2]
- **Provide free 3' OH for *Taq* polymerase** to elongate the complementary strands of **both templates** to produce 2 DNA molecules [1/2]

Duchenne muscular dystrophy is a genetic disease in which there is a progressive loss of muscle mass, leading to physical weakness, difficulty in standing and walking, and eventually paralysis and death. Early symptoms of the disease can only be observed between the ages of 2 and 3 in most patients. A group of doctors and medical biologists discovered a RFLP marker, found on the same chromosome as the disease gene, which can be used in the screening of the disease during pregnancy.

To investigate the effective of the RFLP marker in disease screening, samples of DNA were obtained from a family known to have the disease. The RFLP locus was isolated and amplified using polymerase chain reaction, which was then mixed with *Bam*HI restriction enzymes. The pedigree tree of the family and results of gel electrophoresis are shown in Fig. 2.1.

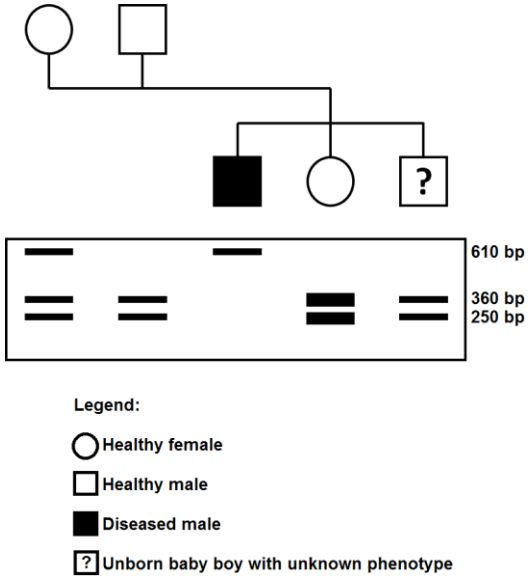


Fig. 2.1

(a) With reference to Fig. 2.1,

(i) state the mode of inheritance of the Duchenne muscular dystrophy. [1]

- Sex-linked recessive [1]

(ii) explain why there are different fragment lengths after restriction digest. [3]

- Ref. to the RFLP marker having **2 alleles** [1/2]
- 1 allele contains a ***Bam*HI restriction site** while the other does not [1/2]
  - Variation due to a **mutation** [1/2]
- Allele with restriction site produces 2 RFLP fragments of 360 bp and 250 bp [1/2]
  - Due to digestion by *Bam*HI restriction enzyme [1/2]
- Allele without restriction site produces 1 RFLP fragment of 610 bp [1/2]
  - *Bam*HI restriction enzyme **cannot recognise the mutated sequence** resulting in no restriction digest [1/2]

(iii) Explain the difference in the band patterns between the father and the daughter. [3]

- The daughter has **thicker bands** than the father corresponding to the same sizes [1/2]
  - 360 and 250 bp [1/2]
  - **Twice** as thick [1/2]
- The RFLP marker is **found on the X chromosome** [1/2]
  - Father has only 1 X chromosome thus 1 copy of the allele of the RFLP marker [1/2]
  - Daughter has 2 X chromosomes thus 2 copies of the allele of the RFLP marker [1/2]
- Ref. to thickness of the bands due to amount of RFLP fragments [1/2]

(b) Some years later, the baby boy with unknown phenotype is born and has reached 2 years of age. Clinical diagnosis reveals that he too suffers from Duchenne muscular dystrophy.

(i) Explain why the band pattern of the baby boy is different from that of his older brother even though both are with the disease. Assume no new mutations occurred in the disease gene or RFLP locus. [2]

- **Crossing over** occurred during gamete formation in the mother [1/2]
- Between the disease gene and RFLP locus [1/2]
- Giving rise to gametes with a X chromosome with **different combinations of alleles** [1/2]
  - X chromosome inherited by older brother has RFLP allele producing 610 bp fragment linked to the disease allele [1/2]
  - X chromosome inherited by baby boy has RFLP allele producing 360 bp and 250 bp fragments linked to the disease allele [1/2]

(ii) Suggest an ethical implication that may arise due to the use of this RFLP marker to screen for Duchenne muscular dystrophy. [1]

Any 1:

- **Stigmatisation** of the parents and child even **before the onset** of the disease
- Parents may want to **terminate the pregnancy** if the unborn baby is diagnosed with the disease
- Parents may have to pay a **higher premium for child insurance** even **before the onset** of the disease
- AVP

[Total: 12]

Question 3

(a) Patients with severe combined immunodeficiency disorder (SCID) are vulnerable to serious infections and death. There are two main types of SCID, X-SCID and ADA-SCID. Besides the location and difference in their modes of inheritance, give two differences between the two types of SCID.

Difference	X-SCID	ADA-SCID
1 (Name of gene; Idea of what the gene codes for / function of normal protein)	Mutation of **IL2RG (interleukin-2 receptor gamma) gene** that codes for **gamma chain** on **lymphocyte receptor**	Mutation of gene coding for **adenosine deaminase (ADA)** involved in **purine metabolism / breakdown of deoxyadenosine**
2 (Consequence)	**Defective receptor** unable to activate **cell signal pathways** leading to **normal development of specific white blood**	Hence, **ADA deficiency** leads to the **accumulation of deoxyadenosine** which **kill developing white blood cells**

[2]

(b) Gene therapy can be used to treat SCID by introducing retrovirus containing the normal gene into hematopoietic stem cells (HSCs). The HSCs are then infused into the patient. Explain how this choice of vector and target cells may theoretically lead to long term treatment of the disease. [2]

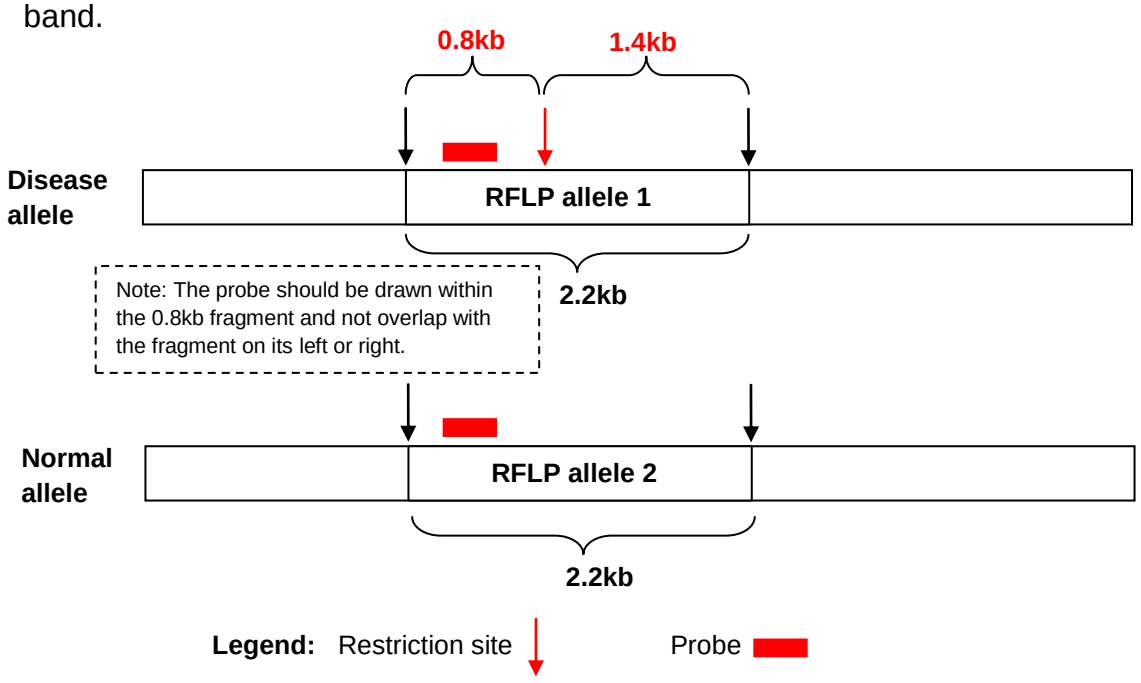
Retroviral vectors are able to **integrate** the **normal gene into the chromosome** [1/2]. The HSCs are able to undergo **long term self-renewal** by **mitotic cell divisions** [1/2]

Hence, patient will have the normal gene in his/her white blood cells since the **normal gene would be replicated and passed to the daughter cells** [1/2] When the HSCs **differentiate into white blood cells** [1/2], the normal **gene can be expressed** and produce the **normal enzyme** [1/2]

(c) In one attempt to treat ADA-SCID, hematopoietic stem cells (HSCs) from the bone marrow of baby X patient were collected and treated with retrovirus containing the ADA gene before infusing them back.

Genomic DNA from the T lymphocyte cells of baby X was later extracted. The extracted DNA was digested with a restriction enzyme and subjected to Southern blot analysis using a specific probe that binds to a known RFLP marker found within the gene associated with the disease.

The RFLP allele 1 associated with the disease allele gives rise to a 0.8kb band while the RFLP allele 2 associated with the normal allele gives rise to a 2.2kb band.



(i) With the information provided, draw to indicate the position of another restriction site and the position where the probe binds to. You should also indicate the length of the restriction fragments that would be produced. [1]

The RFLP band patterns from baby X's normal brothers, Tom and John, are shown in Fig. 3.1.

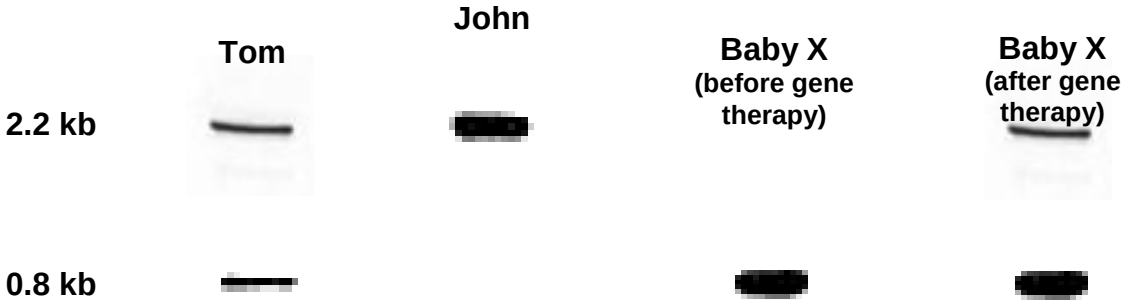


Fig 3.1: Results of Southern Blot analysis of DNA extracted from T lymphocytes

(ii) With reference to Fig 3.1, explain the difference in the band pattern of Tom and John. [2]

ADA-SCID is an **autosomal recessive condition**. [1/2]
Tom has **one copy of the recessive and one copy of the dominant allele / heterozygous**. [1/2]
Ref to **two bands** of **2.2kb and 0.8kb** [1/2]

John has **two copies of the normal dominant allele / homozygous dominant** [1/2]
Ref to **one thicker band of 2.2kb** [1/2]

(iii) Draw the expected band pattern of baby X after gene therapy treatment. [1]

Over the next 360 days after infusion, the total number of various blood cells (e.g. red blood cells and white blood cells) of baby X were recorded at regular intervals.

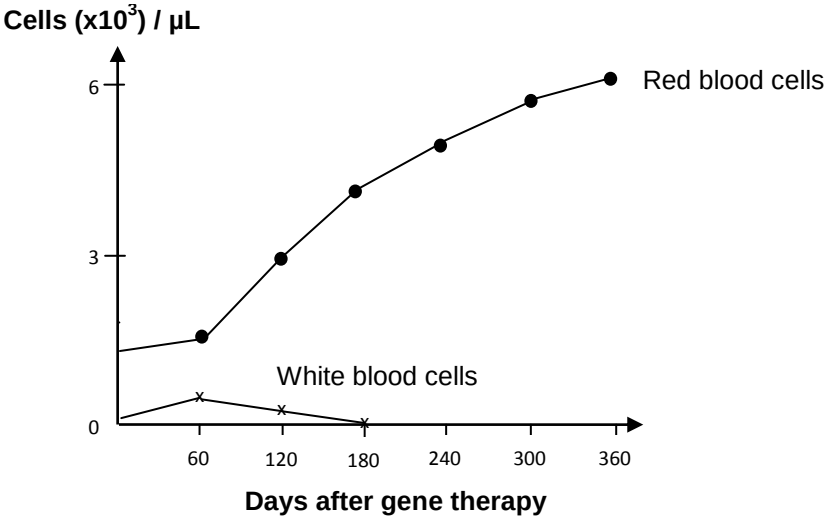


Fig 3.2

(iv) With reference to Fig 3.2, describe the effectiveness of the treatment in Baby X. [1]

The treatment was **ineffective / effective only for a short term** [1/2]
Decline of the white blood cells / QV: from about **0.5 x10^3 /µL in day 60 to 0 by day 180** [1/2]

(v) Suggest a reason for your answer. [1]
Any one:
The gene inserted into the chromosome **could not be expressed**
The inserted gene was **mutated** and so **could not produce a functional enzyme**

[Total: 10]

(4) An orange plantation owner wants to find out the amount of ascorbic acid (vitamin C) that his breed of oranges produces. He believes that his oranges produce the most vitamin c compared to the standard orange breeds which typically contain 0.8 to 1.6 $mmolL^{-1}$.

The amount of ascorbic acid present in a sample can be determined using a bioassay method. At pH 7 and above, ascorbic acid reduces solutions of the dye dichlorophenol indophenol (DCPIP) from blue to colourless. For the bioassay to work, the pH of the samples must be adjusted to pH7 - 9. Ascorbic acid does not chemically change when neutralised by sodium hydroxide or when boiled.

Using this information and your own knowledge, design an experiment to determine the validity of the plantation owner's claim that orange juice from his plantation contains higher concentrations of ascorbic acid.

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

- 100 cm^3 of 5.0 $mmolL^{-1}$ stock solution of ascorbic acid, adjusted to pH 7
- 100 cm^3 distilled water
- 100 cm^3 molten agar containing DCPIP
- Sterile petri dishes
- 1ml syringe
- plastic straw to create wells in the agar plate
- Ruler / 2mm graph paper
- Labels
- Timer , e.g. stopwatch
- Forceps
- Bunsen burner
- Normal laboratory glassware e.g. test tubes, beakers, graduated pipettes, droppers, glass rods etc
- 10 cm^3 orange juice, supplied by the plantation owner
- 10% sodium hydroxide solution
- pH indicator paper to indicate alkaline pH

Your plan should:

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

[Total: 12]

Proposed answer

Introduction

- Ascorbic acid reduces blue DCPIP to colourless. .
- Increase in concentration of ascorbic acid will increase the rate of decolourisation of DCPIP
- Different concentrations of ascorbic acid can be created from the stock solution. A standard curve of the amount of decolourisation of DCPIP by the different concentrations of ascorbic acid can be created. The amount of ascorbic acid in orange can be determined by reading off the standard curve

Explain how to determine concentration of ascorbic acid in oranges using the standard curve [1m]

Procedure

1. Obtain 10cm^3 of different concentrations of ascorbic acid solution by dilution.

Describe how to obtain different concentrations of ascorbic acid [1m]

Concentration of ascorbic acid solution / mmolL-1	Volume of 5.0 mmolL-1 ascorbic acid solution / cm^3	Volume of distilled water / cm^3
5	10	0
4	8	2
3	6	4
2	4	6
1	2	8
0	0	10

1. Pour the molten agar containing DCPIP into the petri dishes and allow the agar to cool.
2. Once the agar is cooled, use the plastic straw to make eight equal sized wells in the agar gel plate. Ensure that the wells are well-spaced.

Describe the settling up of the DCPIP agar plates and wells in the plates[1m]

3. Prepare a control experiment using boiled and cooled orange juice, following the same experimental procedures and conditions, to show that the decolourisation of DCPIP is due to the action of ascorbic acid and not due to the action of any enzymes in the juice.

Describe control to prove reaction is due to ascorbic acid in the orange juice and is not enzyme catalysed [1m]

4. Add 10% sodium hydroxide solution to the boiled and cooled orange juice, drop by drop with a dropper, until the pH is between 7 to 9. Check the pH by removing a drop of solution with a clean glass rod and placing it on indicator paper.
5. Neutralise the fresh orange juice in the same manner as described in step 4.

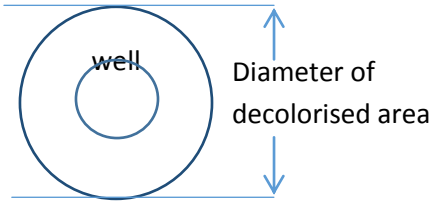
Describe neutralisation of fresh orange juice.[1m]

6. Using the 1 ml syringe, place 0.2 ml of each of the ascorbic acid solutions prepared according to the dilution table, 0.2 ml of orange juice and 0.2 ml of boiled and neutralised orange juice into one well each. Label the wells.

State appropriate volumes scorbic acid [1m]

7. Replace the lid of the petri dish and leave the plates on the table for one hour.
8. After one hour, place the dish on the graph paper and measure the diameter of each of the rings where the blue DCPIP has been decolourised
9. Repeat step 1 to 7 three times.

Describe the measurement of ring of decolourisation [1m]
Describe repeats [1m]

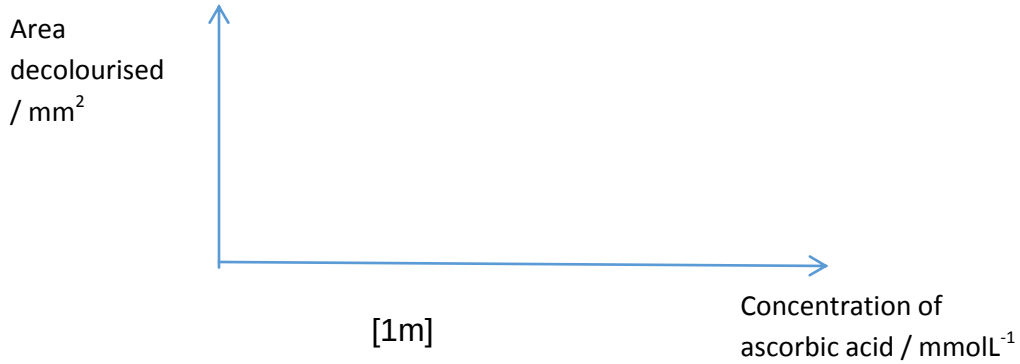


Draw a labelled diagram [1m]

10. Record the results in the table below and calculate the area of decolorisation

Concentration of ascorbic acid	Diameter of ring of decolourisation / mm				Area decolourised/ mm^2
	Experiment 1	Experiment 2	Experiment 3	Average	
5					
4					
3					
2					
1					
0					
Sample of neutralised orange juice					
Sample of boiled and cooled orange juice which has been neutralised					

[1m]



[1m]

From the standard curve drawn, calculate the concentration of ascorbic acid found in the sample of orange juice from the area of decolorisation obtained from the experiment

with the sample of orange juice. If the concentration is higher than 0.8 to 1.6 mmolL^{-1}, the plantation owner's claim of his breed producing a higher concentration of Vitamin C than standard orange breeds is valid. [1m]

Safety
Sodium hydroxide and ascorbic acid may cause irritation when in contact with skin. Wear gloves when handling these reagents. [1m]

Question 1

(a) Outline the procedure for the production of human growth hormone by genetic engineering techniques. [8]

(b) Describe the benefits of the Human Genome Project. [8]

(c) Discuss the ethical concerns that have arisen from genetically modified organisms. [4]

Question 5

(a) Outline the procedure for the production of human growth hormone by genetic engineering techniques. [8]

Isolation of human growth hormone - max 2
- **Human growth hormone mRNA** (1/2)
- extracted from **anterior pituitary gland** is used (1/2)
- as template to synthesize **complementary DNA** (**cDNA**) (1/2)
- Using the enzyme **reverse transcriptase** (1/2)
- Reason: ***E.coli*****,** being **prokaryotes** → **lack mRNA processing machinery** (1/2)
- **Introns** are **not excised** and **exons spliced** (1/2)
- Therefore, **no mature mRNA** can be formed from the **eukaryotic gene** (1/2)
- Ref to use of **DNA polymerase** → double stranded DNA (1/2)

Formation of recombinant DNA – max 2
- Both **plasmid vector and cDNA** are cut with **restriction enzyme** that produces **blunt ends** [1]
- (In separate reactions) **terminal transferase** (1/2)
- is used to add **extra guanines to vector** and **extra cytosines to the cDNA** *(or vice-versa*) [1]
- To create **complementary sticky ends** (1/2)

Or
- Both **plasmid vector and cDNA** are cut with **same** restriction enzyme (1/2)
- Any e.g. of appropriate restriction enzyme (**HindIII, BamHI**, etc) (1/2)
- To create **complementary sticky ends** (1/2)

- **DNA ligase** is used to facilitate the joining of cDNA to vector to form **recombinant DNA** (1/2)
- By forming **phosphodiester bond** between the sugar and the phosphate group / nucleotides (1/2)

Transfer of recombinant DNA to bacteria host followed by screening – max 3

- **Bacteria** (e.g. *E.coli*) is **transformed** with recombinant DNA (1/2)
- By **$CaCl_2$ heat-shock** method (1/2)
- **Transformed bacteria** with the recombinant DNA are **selected** (1/2)
- In the presence selection markers E.g. **antibiotics resistance genes** – transformed cells survive in the presence of antibiotics (1/2)

- Identification of **correct transformed colonies** with **recombinant plasmid** from transformed colonies with re-annealed vector only by *(either one)* ***b*lue-white screening** or **replica plating** (1/2)
- Elaboration of either method:

1. **Replica plating**
   - Bacterial cells are plated on a nutrient plate with **one antibiotics** and then replica plated on **another plate with another antibiotics** or idea of 2 plates (each with an antibiotics) are used (1/2)
   - Colonies with re-annealed vector only are **resistant to both antibiotics** because both antibiotic resistant genes are intact. (1/2)
   - **Colonies with correct recombinant DNA** are **resistant to one antibiotics** but **susceptible to another** because the corresponding **antibiotic resistant gene is disrupted** during **insertion of the gene** (or ref to **insertional inactivation**) (1/2)
   - Hence **comparing the position of the colonies on both plates** help to identify correct colonies (1/2)

Or

2. **Blue-white screening**
   - Bacteria cells are plated on a nutrient plate containing an **antibiotics** and the substrate **X-gal** (1/2)
   - Colonies with re-annealed vector only are resistant to **antibiotics** / contain antibiotics resistance gene and **intact / functional β-galactosidase / Lac Z gene**. (1/2)
   - **β-galactosidase enzyme** that act on X-gal resulting in **blue** colonies (1/2)
   - Colonies with correct recombinant DNA will appear **white** because the β-galactosidase / Lac Z gene is **disrupted** during **insertion of the gene** (or ref to **insertional inactivation**) (1/2)

Culture of correct transformed cells and extraction and purification of insulin - max 1

- And **cultured** in a **nutrient / growth medium / fermenter** (1/2)
- Ref to **prokaryotic promoter** inserted next to eukaryotic gene (1/2)
- The eukaryotic **gene** is **expressed** in the bacteria (1/2)
- The protein is **extracted** and **purified** for use (1/2)

(b) Discuss the benefits of the Human Genome Project. [8]

A. Molecular medicine (no marks for heading; max 2 mks, @ 1 mk)
1 Earlier diagnosis/detection of genetic diseases;
2 Gene therapy;
3 Rational drug design/control systems for drugs/rational drug design/pharmacogenomics & custom drugs;

B. Energy and Environmental Applications (max 1 mk, @ 1 mk)
4 Use microbial genomics research to create new energy sources (biofuels);
5 Use microbial genomics research to develop environmental monitoring techniques to detect pollutants ;
6 Use microbial genomics research for safe, efficient environmental remediation;

C. DNA Forensics (max 3 mk, @ 1 mk)
7 Identify potential suspects whose DNA may match evidence left at crime scenes;
8 Exonerate persons wrongly accused of crimes;
9 Identify crime and catastrophe victims;
10 Establish paternity and other family relationships;
11 Identify endangered and protected species as an aid to wildlife officials (could be used for prosecuting poachers);
12 Detect bacteria and other organisms that may pollute air, water, soil, and food;
13 Match organ donors with recipients in transplant programs;
14 Determine pedigree for seed or livestock breeds;
15 Authenticate consumables such as caviar and wine;

D. Agriculture, Livestock Breeding, and Bioprocessing (max 1 mk, @ 1 mk)
16 Healthier, more productive, disease-resistant crops/ farm animals / higher yield;
17 More nutritious produce ;
18 Edible vaccines incorporated into food products;
19 New environmental cleanup uses for plants like tobacco;

E. Bioarchaeology, anthropology, evolution and human migration (max 1 mk, @ 1 mk)
20 Study human evolution (through germline mutations in lineages);
21 Study of migration of diff pop groups based on female genetic inheritance/lineage and migration of males via Y chromosomes;
22 Compare breakpoints in the evolution of mutations with ages of populations and historical events;

F. Risk assessment (@ 1 mk)
23 Assess health damage and risks caused by radiation exposure/mutagenic chemicals/ cancer-causing toxins;

Total max: 8 mk

(c) Discuss the ethical concerns that have arisen from genetically modified organisms. [4]

Ethical (deals with right or wrong; equity; fairness)

- Genetic manipulation of plants may not be acceptable by some as it involves altering the genetic makeup of the plants which can be seen as tampering with nature;;
- Religious groups with strong dietary restrictions may not be informed about the genetic content of the food they are eating and may unknowingly consumed GM food with genes from unacceptable sources;; (example to illustrate)
- Patenting of the transgenic crops is viewed as unethical as it promotes the treatment of living things as mere objects or commodities to be owned and redesigned at will;
- Patenting of the transgenic crops by companies in order to ensure they profit from the technique may end up causing farmers to be dependent on them;; (Use example of the patent for the genetically engineered seeds)
- Companies with the patents become very rich at the expense of the farmers or consumers who have to pay for the high cost of the seeds or plants;;
- World food production may be controlled/ dominated by a small number of large biotechnology companies with the technical know-how;;