2013 'A' Level
H2 Biology
Mark Scheme

PAPER 1 (MCQ)

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

PAPER 2 (CORE)

QUESTION 1

(a) Describe the processes that occur in the golgi body. [3]

1 Receive proteins and lipids from the ER (rER and sER) and chemically modifies them / **Further** chemically modifies glycoproteins and glycolipids;

e.g. short carbohydrate chains or oligosaccharides are added to the proteins and lipids to form glycoproteins and glycolipids / enzymes of the Golgi body cleave a sugar from a chain or modify one or more of the sugars;

2 Various molecules are sorted and packaged;
Then, they are targeted to various cellular locations; or secreted out of the cell;

3 Hydrolytic enzymes of the lysosome will be sorted and packaged together;
To form lysosomes;

4 Synthesis of carbohydrates e.g. non-cellulose polysaccharides such as pectin;
For formation of plant cell walls;

Examiner's comment: The majority of candidates correctly described protein modification and most went on to provide further details.

(b) Describe the roles of the vesicles that fuse with the forming face of the golgi body. [3]

1 The vesicles are made up of membranes;
which buds off from the endoplasmic reticulum;
2 These vesicles transport proteins and lipids synthesized in rER and sER ;
To be further chemically modified in the Golgi body ;
3 The vesicles has proteins which recognize and bind to specific receptors on the forming face of golgi body;
Before membranes of vesicles fuse with membrane of the forming face of golgi body, emptying their contents into the lumen of the Golgi body;

Examiner's comment: Most candidates were able to provide full responses. However, some candidates omitted to mention the involvement of proteins.

(c) Outline two roles of the vesicles that are formed from the maturing face of the golgi body. [4]

1 Secretory vesicles **migrate** toward the plasma membrane ;
Membrane of the vesicles **fuse** with the plasma membrane ;
2 Substances (e.g. hormones, digestive enzymes of the gut) **released** to the outside of the cell;
via exocytosis;

3 Lysosomes contain hydrolytic enzymes;
which are introduced to food vesicles produced by phagocytosis or autophagosomes containing worn-out organelles upon fusion of both membranes;
4 Hydrolytic enzymes break down the food molecules / worn-out organelles;
Digested substances are then assimilated back into the cell to be re-used;

Examiner's comment: The majority of candidates provided detailed responses. Some candidates confused the enzyme lysozyme with the hydrolytic enzymes found in lysosomes.

QUESTION 2

(a) State what happens if a cell loses control of the cell cycle. [1]

1 It undergoes uncontrolled cell division and may result in cancer;;

Examiner's comment: A few candidates referred mistakenly to uncontrolled cell growth.

(b) With reference to Fig 2.1, suggest how the dysregulation of checkpoints of cell division may occur. [3]

1 Genes coding for M-cyclin and S-cyclin may be mutated;
resulting in production of hyperactive M-cyclins and S-cyclins/ M-cyclins and S-cyclins which cannot be degraded;
2 These cyclins bind to and activate their respective CDK proteins permanently;;
3 The permanently activated CDK proteins allow the cell (normal or otherwise) to proceed from G2 to M phase, and from G1 to S phases of the cell cycle without any control;;
(This results in continuous DNA replication and mitosis and hence uncontrolled cell divisions)

Examiner's comment: Candidates who considered the information provided in the diagram in the context of loss of control of the cell cycle were able to provide full responses. Not all considered mutations as a possible cause of changes. A significant minority of candidates described changes that would have arrested the cell cycle completely, such as cyclins being unable to bind with CDK.

(c) (i) Name one causative agent of cancer. [1]

1 UV irradiation / exposure to carcinogenic chemicals like Ethidium bromide;;

(ii) Outline the development of cancer, including the effects of this causative agent. [5]

1 Exposure to UV (or carcinogenic chemicals) can cause gene mutations to occur;;
2 Gain-of-function mutation in at least one proto-oncogene can lead to production of abnormal onco-proteins (e.g. hyperactive Ras);
resulting in excessive cell proliferation;
3 Loss-of-function mutations in several tumour suppressor genes can result in production of non-functional gene products;
which are unable to trigger cellular processes involved in cell cycle arrest, DNA repair and apoptosis;
4 Cells will be able to progress through the cell cycle checkpoints unchecked;
allowing further accumulation of mutations to occur;
5 Activation of telomerase gene;
telomerase enzyme prevents the shortening of the chromosome ends / cell can continue to divide indefinitely;
6 angiogenesis / formation of new network of blood vessels to the cancer cells;

blood vessels provide the cancer cells oxygen and nutrients for growth and to remove any waste products;

7 loss of contact inhibition / density-dependence (and cells do not stop dividing), ability to differentiate and anchorage dependence / loss of cell adhesion;;
8 result in metastasis / tumor break loose and may enter the bloodstream / invade other tissues to form secondary tumor which is now malignant;;

QUESTION 3

(a) State the name given to this mechanism of DNA replication. [1]

1 Semi-conservative replication;;

(b) Describe how these results provided evidence for Watson and Crick's proposed mechanism. [3]

1 In generation 0, 1 band is formed near the bottom of the tube (heavy density position);
indicating that both strands in the DNA molecule contains the heavier ^{15}N isotope (^{15}N-^{15}N);

2 In generation 1, 1 band is formed slightly higher in the tube (intermediate density position);
indicating that both DNA strands from generation 0 are templates for the synthesis of new daughter strands, resulting in each DNA molecule containing one ^{15}N and one ^{14}N strand;

3 In generation 2, 1 band corresponds to the band position in generation 1 but half the thickness which indicates that 50% of the DNA is ^{15}N-^{14}N;
while the other band occupies the highest position in the tube which indicates the other 50%of DNA is ^{14}N-^{14}N;

Examiner's comments: Some candidates were unclear in the use of terms between DNA molecules, strands and bands.

(c) List three ways in which transcription is different from DNA replication. [3]

Transcription	Replication
Product formed is **mRNA**	**Product** formed is **DNA**
Only **specific regions** of **DNA molecule** is transcribed	**Entire DNA molecule** is replicated
One DNA strand used as **template**	Both DNA strands used as **template**
Uracil ribonucleotide is used, not thymine	Thymine deoxyribonucleotide is used, not uracil
RNA polymerase does **not** need primers to start synthesis of RNA	DNA polymerase requires RNA primers to synthesize daughter DNA strands

(d) Explain how the information to synthesise polypeptides is coded for by DNA. [3]

1 DNA contains a specific gene sequence of nucleotide bases where the genetic information is transcribed;;

2 The exposed nucleotide bases of the DNA template base pair with the complementary RNA nucleotide bases to form mRNA;
Adenine base pairs with uracil, thymine to adenine, cytosine to guanine, and guanine to cytosine;

3 The genetic information on mRNA is read as triplet bases/codon;
to give rise to an amino acid sequence of the polypeptide in the translation process;

Examiner's comments: Many candidates give detailed descriptions of polypeptide synthesis without explaining how the information is encoded by DNA.

QUESTION 4

4 Fig. 4.1 shows an outline of the first three stages of aerobic respiration.

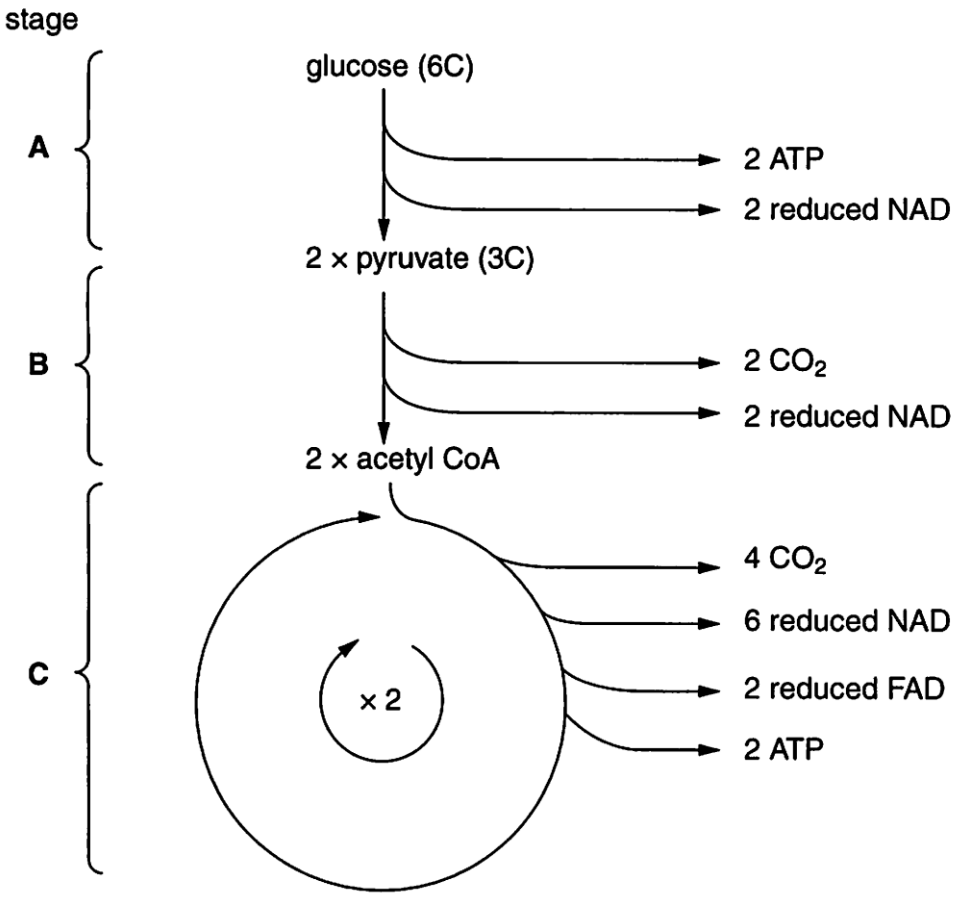

Fig. 4.1

(a) Name the stages A, B and C and state preciously where in a eukaryotic cell these stages occur. [3]

A: Glycolysis; cytoplasm;
B: Link reaction; mitochondrial matrix;
C: Krebs cycle; mitochondrial matrix;

Examiner's comments:
(a) Most candidates were able to identify where the stages occurred. Some candidates were not precise in their responses.

(b) For each glucose molecule, state the total number of molecules of ATP formed as a result of stages A and C, including any ATP produced through oxidative phosphorylation of the products. [2]

A: 2 (from glycolysis) + (2x 3 = 6 from NADH) = 8
C: 2 (from krebs) + (6x 3 = 18 from NADH) + (2x 2 =4 from FADH2) = 24

Note:

The oxidation of one molecule of NADH yields 3 ATP molecules.

The oxidation of one molecule of FAD yields 2 ATP molecules.

OR

A: 2 (from glycolysis) + (2x 2.5 = 6 from NADH) = 7
C: 2 (from krebs) + (6x 2.5 = 15 from NADH) + (2x 1.5 =3 from FADH2) = 20

Note:

The oxidation of one molecule of NADH yields 2.5 ATP molecules.

The oxidation of one molecule of FAD yields 1.5 ATP molecules.

Examiner's comments:
(b) Common errors arose from candidates' lack of care in reading the question and using the information supplied in the diagram. Some candidates did not consider the specific requirement to include any ATP produced through oxidative phosphorylation.

Oxidative phosphorylation regenerates FAD and NAD from the reduced FAD and reduced NAD.

(c) Outline how this results in the production of ATP. [4]

1 NADH or $FADH_2$ transfer its hydrogen atom to a series of electron carriers; in the electron transport chain (ETC) located in the inner mitochondrial membrane
(NADH and FADH2 are oxidised to regenerate NAD+ and FAD for use in glycolysis, link reaction, and Krebs cycle);

2 The hydrogen atoms are split into its constituent hydrogen ions (H+) and electrons;
The electron is transferred to series of electron carriers, while H+ remains in solution in the mitochondrial matrix;

3 Electrons are passed down the series of electron carriers with progressively lower energy levels;
Energy released during the transfer of electrons ;

4 Used to pump H+ ions from the mitochondrial matrix into the intermembrane space;
Proton gradient established;

5 H+ ions diffuse from intermembrane space into the mitochondrial matrix down a concentration gradient through ATP synthase (Ref to chemiosmosis) ;
synthesis of ATP from ADP and Pi;

QUESTION 5

(a) Suggest why the I^A and I^B alleles are dominant over the i allele. [3]

1 In the heterozygote state of I^Ai or I^Bi, the I^A or I^B allele is expressed; while the expression of i allele is masked;
2 Individual with I^Ai genotype results in the production of A antigens/glycoproteins on the surface of red blood cells;
while an individual with I^Bi genotype results in the production of B antigens/glycoproteins;
3 Individual with ii genotype results in no production of antigens/glycoproteins on the surface of red blood cells;;

Examiner's comments: Some candidates did not link the alleles with the production of glycoproteins on the surface of red blood cells.

(b)

(i) Suggest where the Rhesus factor gene may be found. Explain the reason for your answer. [2]

1 Rhesus factor gene is found on another chromosome which is not chromosome 9;;
2 As it was mentioned that the alleles for the two blood typing systems are inherited independently of each other;;
/ If the genes coding for the blood typing system are found on the same chromosome 9, the alleles will not be inherited independently from each other;;

(ii) Use the symbols given above and show all possible genotypes and phenotypes for the offspring of these parents. [5]

	Mother		x		Father	
Parental phenotypes:	Type A Rhesus positive		x		Type B Rhesus positive	
Parental genotype:	$I^Ai\ Rh^+\ Rh^-$				$I^Bi\ Rh^+\ Rh^-$	;;
Parental gametes:	$I^A\ Rh^+$ $I^A\ Rh^-$ $i\ Rh^+$ $i\ Rh^-$				$I^B\ Rh^+$ $I^B\ Rh^-$ $i\ Rh^+$ $i\ Rh^-$	;;

	I^A Rh^+	I^A Rh^-	i Rh^+	i Rh^-
I^B Rh^+	I^AI^B Rh^+ Rh^+ Type AB Rhesus positive	I^AI^B Rh^+ Rh^- Type AB Rhesus positive	I^Bi Rh^+ Rh^+ Type B Rhesus positive	I^Bi Rh^+ Rh^- Type B Rhesus positive
I^B Rh^-	I^AI^B Rh^+ Rh^- Type AB Rhesus positive	I^AI^B Rh^- Rh^- Type AB Rhesus negative	I^Bi Rh^+ Rh^- Type B Rhesus positive	I^Bi Rh^- Rh^- Type B Rhesus negative
i Rh^+	I^Ai Rh^+ Rh^+ Type A Rhesus positive	I^Ai Rh^+ Rh^- Type A Rhesus positive	ii Rh^+ Rh^+ Type O Rhesus positive	ii Rh^+ Rh^- Type O Rhesus positive
i Rh^-	I^Ai Rh^+ Rh^- Type A Rhesus positive	I^Ai Rh^- Rh^- Type A Rhesus negative	ii Rh^+ Rh^- Type O Rhesus positive	ii Rh^- Rh^- Type O Rhesus negative

;;

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Examiner's comments: Some candidates did not match the offspring genotypes with phenotypes.

QUESTION 6

(a) Name structures A, B and C. [3]

1 A – homologous chromosomes;;
2 B – centromere;;
3 C – sister chromatid;;

(b)(i) Explain how structure C is similar to structure D. [2]

C and D both are :
1 Of the same length;;
2 Same position of centromere;;
3 Same gene loci arranged in the same order
/ carry genes for the same characteristic at the same corresponding loci ;;
4 Same staining pattern on a karyogram;;
[Any 2]

Examiner's comments: Most candidates were able to explain the similarities between these structures in terms of genes, loci and other structural properties.

(b)(ii) Explain how structure C is different from structure D. [2]

1 [Diff] C and D carry alternative forms of the gene
/ e.g. C carries dominant allele Y while D carries recessive allele y. Both alleles are involved in determination of the same phenotype;;
2 [Explain] One of the chromosomes is of paternal origin, while the other is of maternal origin;;

Examiner's comments: Fewer candidates were able to describe the differences between these structures. Some correctly explained the reason for these differences in terms of the inheritance from two parents.

(c) Describe what is going on during stage 2 and explain its effects on the products of meiosis. [4]

1 Crossing over during Prophase I ;
between non-sister chromatids of homologous chromosomes ;
2 (Equivalent portions of) chromatids break and re-join at two different chiasmata
/ linked genes separate at two different chiasmata ;;

3 Results in new combinations of alleles on chromosomes of all 4 gametes after meiosis ;;
4 (Genetically variable gametes) contributes to variation in the population;;

QUESTION 7

(a) Describe the properties of the phospholipid bilayer and the aquaporin channels in relation to the movement of water across the cell surface membrane. [4]

1 The phospholipid bilayer comprises two layers of amphipathic phospholipid molecules
/ polar phosphate heads facing the aqueous extracellular and cytoplasm of the cell and the hydrophobic hydrocarbon tails are oriented inward;
the non-polar hydrocarbon tails form the hydrophobic core ;

2 The hydrophobic core restricts the free movement of the polar water molecules across the cell membrane ;
However, the fluidity of the membrane allows the small sized, polar water molecule to pass through ;

3 The aquaporin is a trans-membrane channel;
consisting of a pore lined with hydrophilic amino acids with polar or charged R groups ;

4 This allows easy passage of the polar water molecules ;
down their water potential gradient;

(b) Explain what has happened to the treated cells after 3 minutes. [4]

1 [What happened] The treated cells burst / lyse ;
The cell contents are released / leaked out into the surroundings;

2 [Explain]The treated cells have many aquaporin channels in their cell surface membranes ;
This is due to the expression of the injected aquaporin genes ;

3 The cells have a lower water potential compared to the surrounding;
Water molecules enter into the cell through the aquaporin channels by <u>facilitated diffusion</u> ;

4 With time, this passage of water into the cell increased the cell volume ;
The plasma membrane breaks down (as frog cell does not have a cell wall) ;

Examiner's comment: The majority of candidates were able to use appropriate terminology to develop full responses.

(c) Outline the differences between osmosis and facilitated diffusion. [2]

1 Osmosis refers to the net movement of <u>water molecules</u> across a selectively permeable membrane from a region of higher water potential to a region of lower water potential<u>;</u>
facilitated diffusion refers to the net movement of <u>any charged / polar molecules</u> from a region of higher concentration to a region of lower concentration;

2 Osmosis can occur without requiring the presence of a trans-membrane protein such as a channel / carrier protein ;

Facilitated diffusion requires the presence of a transmembrane protein such as a channel / carrier protein;

Examiner's comment: Most candidates correctly defined osmosis but many did not draw out differences between osmosis and facilitated diffusion.

QUESTION 8

(a) Explain the significance to the alpha and beta cells of the blood supply. [3]

1 Alpha and beta cells are in close proximity to the blood supply;
which transport and provide necessary nutrients to the cells and carries away metabolic wastes and secretion from cells, if any;

2 Glucose level in the blood supply is detected by alpha and beta cells which have glucose receptors;
determining the secretion of hormones, either glucagon or insulin respectively, into the blood supply to be transported to their target cells to effect the appropriate cellular responses;

3 When blood glucose level exceeds the normal set point of 90mg/100ml, the beta cells will release insulin into the blood supply to trigger target cells' uptake of glucose from the blood;
When blood glucose level falls below the normal set point, the alpha cells will release glucagon into the blood supply to promote release of glucose from target cells into blood supply;

(b) With reference to Fig. 8.2, outline the advantages of such a cell signalling pathway. [3]

Any three:

1 Conformation (shape) of the receptor and the glucagon are complementary;
and their specific interaction ensures that the pathway is responsive only to the appropriate signal molecule;

2 Pathway allows the transmission of signal (that cannot traverse the hydrophobic phospholipid bilayer) across membrane barrier;
as binding of glucagon to receptor occurs outside the cell and glucagon remains outside the cell;

3 Only one glucagon is required for the production of many cyclic AMP by the activated adenyl cyclase;
thus the pathway allows for signal amplification;

4 Binding of glucagon to the receptor results in G-protein exchanging GDP for GTP to become activated G-protein which dissociates into two polypeptide chains and moves along the cell suface membrane. One of the G-protein polypeptide chain binds to and activates the adenyl cyclase which catalyses the formation of cAMP;
Hence, single glucagon molecule can activate multiple molecules, e.g G-protein and adenyl cyclase, in a sequential manner allowing for regulation of synthesis of cAMP / the final amount of cAMP can be adjusted depending on the level of GTP and number of adenyl cyclase in the cell;

(c) Describe how cAMP increases blood glucose concentration. [3]

1 cAMP, as a second messenger, activates protein kinase A;;
2 Each activated protein kinase A activates a large number of phosphorylase kinase in a phosphorylation cascade;;
3 Phosphorylase kinase activate a large number of glycogen phosphorylase; which catalyses the conversion of a large number of glycogen to glucose-1-phosphate and eventually to glucose which is then released into the blood;

(d) State how the cell signalling pathway for insulin differs from that for glucagon as shown in Fig. 8.2. [1]

1 Insulin initiates cell signalling pathway by binding to the dimerised receptor tyrosine kinase;;
2 It does not involve G-protein in the signalling pathway;;
[Any 1]

QUESTION 9

(a) Describe the binomial nomenclature of a species and the basis of hierarchical classification of species into taxonomic groups. [7]

Binomial nomenclature of a species

1 Binomial classification is a naming system to organise living things into easily understood scientific groupings; ;
2 Each species of organism is assigned a unique; two-part name;
3 first part of the name identifies the genus to which the species belongs; the second part identifies the species within the genus;

Hierarchical classification of species into taxonomic groups

4 Organisms are grouped into 7 taxonomic categories – from kingdom to species;;
5 Categories are based on shared physical characteristics, or phenotypes, within each group;;
6 Evolutionary relationships are not considered;;
7 Species that have many characteristics in common (i.e.closely related) are grouped into a genus. Closely related genera that share combinations of traits are grouped into a single family. Related families are grouped into orders. Related orders are grouped into classes. Related classes are grouped into phyla. Related phyla are grouped into kingdoms. Kingdoms are grouped into domains;;

(b) Explain the biological concept of species. [7]

1 The biological concept of species defines a species as a group of closely related organisms which are capable of interbreeding in nature ;;
2 to produce viable, fertile offspring;;
3 Members of one species usually cannot produce fertile offspring with members of another species;;
4 [Difficulties] The biological concept of species cannot be used for asexually reproducing species and self-fertilizing species;;
5 biologists assign such organisms to species based mainly on structural and biochemical characteristics;;

6 There is no way to check interbreeding in the extinct forms represented by fossils;;
7 biologists usually classify fossils into species based on differences in morphology;;

Examiner's comment: Effective responses were developed by candidates who limited their answers to a consideration of the biological concept of the species, including difficulties presented by the concept when applied to certain groups of organisms.

(c) Outline the advantages of molecular methods in classifying organisms. [6]

1 Quantifiable and open to statistical analysis;;
2 Large quantities of data are required for statistical analysis; however there is little morphological data available;

3 Unambiguous and objective;;
4 Morphological data may differ depending on the way in which it was classified;;

5 Not affected by convergent evolution / some characteristics may be analogous;;
6 similar morphology may not have been inherited from common ancestor;;

QUESTION 10

(a) Describe how infection by HIV causes disease. [7]

1 Retroviral DNA integrates into the chromosomal DNA with the help of integrase. When the host cell receives a signal to be active, the provirus is used as a template for the synthesis of viral proteins and genomes.
2 Diversion of the cell's energy and resources, hence depriving host cell of it normal metabolism and impeding normal function of T helper cells;;
3 Eg. Make use of host cells' amino acids to synthesise viral proteins;
/ Eg. competition between viral mRNA and host's mRNA for cellular ribosomes
/ Eg. competition of viral promoters for cellular transcriptional factors and RNA polymerases;;

4 New virions are released by budding off from the host cell;
which may destroy T helper cells in the body and results in a decrease in T helper cells to combat other infections;

5 Viral proteins and glycoprotein during viral particle assembly changes the antigenic surface of the host cell membrane;
which could result in it being recognized as foreign and the cell would be destroyed by the immune system;

6 HIV may also induce adjacent host cells to fuse together forming giant multinucleated cells (or syncytia);;
which eventually ruptures;;

7 Loss of T cells leads to a weakened immune system;
cannot defend against opportunistic infections;

8 Insertion of the retroviral DNA into the host genome may also cause cancer;;
9 Due to disruption of tumour suppressor genes ;
and conversion of proto-oncogenes to oncogenes;

(b) Explain why viruses may be regarded as non-living organisms. [7]

1 Viruses cannot reproduce or carry out metabolic activities outside of a host cell;;
2 They lack the raw materials (e.g. amino acids, nucleotides), structures/organelles, transcription and translation machinery found in other cells;;
3 They are acellular, containing no cytoplasm or cellular organelles;;
4 They contain either DNA or RNA, but never both;;
5 They are unable to synthesise ATP;;
6 They are obligate intracellular parasites which need to take over their hosts machinery for its own reproduction;;
7 They have very small genomes compared to even the smallest prokaryotic genomes;;

(c) Outline the structure and function of viral nucleic acid. [6]

1 [Structure] Genetic material of the virus can be either DNA or RNA;;
2 It can be segmented or non-segmented;;
3 Single-stranded of double-stranded;;
4 Positive sense, or negative sense or ambisense;;

5 [Function] Viral nucleic acid function as a template for the replication of more viral nucleic acid;;
6 It contains genes that serve as templates for transcription or translation to code for structures of the virus (capsid proteins) or viral enzymes required for its reproductive cycle;;

PAPER 3 (APPLICATIONS)

QUESTION 1

(a) State two other ways in which RFLP can be used as a biological tool. [2]

1 Identification of individuals through their unique DNA profiles / DNA fingerprinting ;;
2 Detection of individuals with allele for disease through direct or indirect methods / disease detection;;

Examiner's comment: Some candidates misinterpreted the question and described how RFLP analysis could be done or how results could be interpreted. A number of answers were too vague.

(b)(i)

1 <u>EcoRI</u>
2 restriction sites AND 2,3 and 10kb fragments ;;
2 <u>BamHI and EcoRI</u>
3 restriction sites AND 1,2,3 and 9kb fragments;;

(b)(ii)

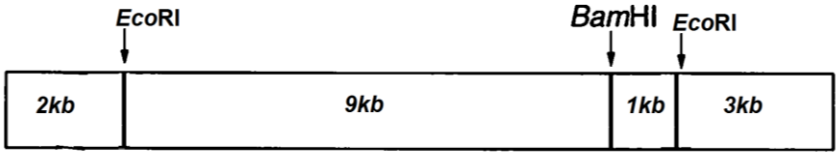


Fig. 1.3

1 Size of restriction fragment correctly indicated ;;
2 Restriction sites correctly ordered;;

Examiner's comments: Most candidates correctly interpreted the results of the double digestion of the length of DNA with BamHI and EcoRI, and digestion with BamHI alone, to establish the order of the restriction sites. Fewer candidates considered the result of digestion with EcoRI to determine the correct sequence of fragment sizes that fitted these data.

(c) Outline why gel electrophoresis separates DNA fragments. [4]

1 DNA is negatively-charged due to negatively-charged sugar-phosphate backbone ;;
2 Migrate towards the positively-charged electrode (in the presence of an electric current) ;;
3 Through an agarose matrix which acts as a molecular sieve ;;
4 DNA fragments separated by size where shorter fragments move faster than longer ones;;

(d) Outline the process of DNA hybridization that allows the RFLP pattern for a particular gene to be visualized. [5]

1 Gel is treated with sodium hydroxide to cause the double-stranded DNA to denature / separate into single strands ;;
2 DNA fragments are transferred onto nitrocellulose membrane;;
3 Fragments baked to permanently crosslink the DNA to the membrane;;

4 Membrane is treated with a single-stranded , radioactive DNA probe which is complementary to the target sequence / marker / restriction fragment of interest;;
5 after hybridisation, excess probe is washed off from the membrane;;
6 pattern of hybridization is visualized on X-ray film / description of X-ray autoradiography;;

QUESTION 2

(a)(i) Cytosine is a pyrimidine. Name one other pyrimidine. [1]

1 Thymine;;

(a)(ii) Suggest how methylation of cytosine nucleotides prevents the DNA of a prokaryote from being cut by its own restriction enzymes. [2]

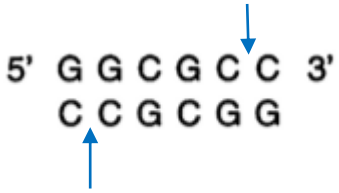
1 Restriction enzymes and DNA methyl-transferases both recognize and act on cytosine nucleotides;;

2 Addition of methyl ($–CH_3$) group to cytosine only in prokaryotic DNA changes the conformation of the DNA molecule;
such that it is no longer complementary to that of the active site of the restriction enzymes;

Examiner's comments: Many candidates were able to consider the effect of methylation of the substrate on the fit to the active site of restriction enzymes. Full responses considered additional details, such as the implication of this methylation only occurring at cytosine nucleotides.

(b)(i) Use arrows to indicate the cut sites. [1]

1



(b)(ii) Compare the ways in which recombinant DNA could be produced from DNA digested with BbeI and DNA digestd by SfoI. [4]

[Differences]

1 BbeI digestion produces DNA fragments with sticky ends;
while SfoI digestion produces fragments with blunt ends;

2 Two DNA molecules (gene of interest and plasmid) cut with the same BbeI enzyme will have 5'-GCGC-3' overhangs at its two ends for each DNA which can hydrogen bond with each other through complementary base pairing. This will hold the DNA fragments together for DNA ligase to catalyse phosphodiester bond to seal the nick between them;;

3 SfoI-digested blunt-end fragments do not bind to each other through complementary base pairing temporarily before DNA ligase carries out its function;;

4 BbeI digested DNA does not require addition of DNA linkers containing KasI or BbeI restriction sites to both ends of the blunt ended fragment before restriction enzyme digestion;
SfoI digested DNA requires addition of DNA linkers;

[Similarity]

5 The method to generate recombinant DNA using BbeI digestion and SfoI digestion involves KasI or BbeI restriction enzyme digestion followed by use of DNA ligase to seal the nick between 2 different DNA fragments with sticky ends;;

(c) Suggest why the scientist made this prediction. [2]

1 BspLI can recognise the same restriction sequence and cut it to form blunt end as SfoI which can make 20 cuts in the standard DNA;;

2 However, the N in the restriction site of BspLI can be any of the four DNA nucleotides beside C and G compared to that of SfoI which is limited to C and G. As such, BspLI should be able to make more than 20 cuts in the standard DNA;;

QUESTION 3

The CFTR gene is located on chromosome 7 in humans. It has at least 200 000 base pairs coding for a protein of 1480 amino acids. This protein forms a transmembrane channel which allows chloride ions to leave cells.

In the autosomal recessive genetic condition cystic fibrosis, the most common cause is a mutation in the CFTR gene resulting in the deletion of 3 nucleotides. This results in the loss of a phenylalanine molecule from the transmembrane protein. The CFTR gene can be mutated in at least 1500 other ways, resulting in the same symptoms of cystic fibrosis varying in extent between individuals.

(a)(i) Explain why the gene has at least 200 000 base pairs, but the protein only has 1480 amino acids. [3]

1. Genetic code states that every codon consists of 3 nucleotides, which code for an amino acid;
   A protein of 1480 amino acids will be the result of translation of 4440 nucleotides, which are the coding sequences / exons;

   DNA level:
2. Some of the 5' and 3' regions of the gene may be transcribed to form the 5' and 3' untranslated regions of the mRNA;;

   mRNA level:
3. The remaining nucleotides are non-coding sequences of the gene;
   e.g. introns which are removed during mRNA splicing;

Examiner's comments

(a) (i) Most candidates were able to describe some of the reasons for the difference between the number of base pairs in a gene and the number of amino acids in a protein. Candidates giving full responses were able to draw these together to produce a detailed and coherent answer.

(ii) When the CFTR transmembrane protein loses a phenylalanine, fewer chloride ions are transported out of the cell.

Describe the effects of cystic fibrosis on a person and how these are linked to the reduction in removal of chloride ions. [3]

Effects of CF:

1. Trapping of bacteria that leads to inflammation and infection of lungs and poor rate of gaseous exchange;
2. Blockage in pancreas, inadequate secretion of pancreatic enzymes, malnutrition due to inability to absorb essential nutrients;
3. Blocked sperm ducts & fallopian tubes (reduced infertility);
4. reduces life expectancy;

Any 2

Linkage to reduction in chloride ion removal:

(Non-functional CTFR channels ➔ no longer able to transport chloride ions out of epithelial cells)

5. accumulation of Na^+ ions in the cell to neutralise Cl^- ions, resulting in high ionic concentration (i.e. water potential in cell drops);

6. water is drawn into the cell / cannot leave the cell;
   causing a thick mucus

(resulting in stated problem in, lungs / pancreas / reproductive system);;

Examiner's comments
(ii) Most candidates were able to describe the mucus produced by sufferers of cystic fibrosis and many were able to explain the reason for this. Some candidates described the effect without relating this to the underlying cause.

(iii) Suggest, with reasons, why other mutations of the CFTR gene vary in the extent to which they cause symptoms of cystic fibrosis. [3]

1. Different gene mutations give rise to different mature mRNA sequences and subsequently, different amino acid sequences/ primary structure of the polypeptides are produced;
   3D conformation of the protein affected to varying extent;
2. Ref. nonsense mutation ➔ creation of a stop codon which terminates translation prematurely ➔ truncated protein which will not be functional.
   / Ref. addition/deletion of nucleotides ➔ frameshift mutation ➔ different codon sequence ➔ different amino acid sequence ➔ different 3D conformation of protein ➔ loss of function of protein;;
3. If mutations affect important amino acids involved in the specific conformation of the protein, the symptoms are severe as the protein is no longer functional.
   / If the mutations affect other amino acids which are less crucial to the specific conformation of the protein, the symptoms are less severe.

Examiner's comments
(iii) Many candidates were able to describe a suitable mutation. Candidates who performed well described a frameshift mutation and explained its significance.

(b)(i) Outline how liposomes can be used as part of a gene therapy treatment for cystic fibrosis. [3]

1. Normal CFTR allele is packaged into liposomes *in vitro*;
   which are incorporated into a <u>nasal spray</u>, and sprayed into the nose and mouth of CF patients;
2. Liposomes <u>fuse</u> with the cell membrane of tracheal cells, releasing the normal CF allele into the cell cytoplasm;
   CFTR allele is then transported to the <u>nucleus</u> and transcribed into <u>mRNA</u> and the normal CFTR protein is subsequently produced;
3. The normal CFTR protein embeds itself into the cell membrane and begins to transport chloride ions out of cells;
   thereby thinning the mucus (water moves out of the cell and dilute the thick and sticky mucus) and alleviating the symptoms of CF;

Examiner's comments
(b) (i) Effective responses considered the mechanism and site of delivery and provided an outline as to how a functional allele can be introduced into a cell.

(ii) Describe and explain the factors that prevent this method of gene therapy for cystic fibrosis from becoming an effective treatment. [3]

Difficult to get DNA to integrate into target cell genome

1 difficult to get DNA to integrate into target cell genome
2 introduced gene may not segregate equally to daughter cells if the cells are actively dividing

Short-lived nature of gene therapy

3 results can be short-lived / transient expression of the transgene if not integrated into host genome
4 due to rapidly dividing nature of many cells
5 patients will have to undergo multiple rounds of gene therapy;

Low efficiency of gene transfer

6 low efficiency of gene transfer using liposomes as delivery system

Epithelial cells have low dividing capacity.

7 epithelial cells has low dividing capacity
8 if the modified target cell (with the normal functional allele) does not divide and multiply, the introduced gene is lost when the cell dies after a while

Any 3 sets of ideas

Examiner's comments

(ii) The majority of candidates gave full responses that explained the limitations of using liposomes for gene therapy.

QUESTION 4 (SPA PLANNING)

Proteins can be denatured by heavy metals including lead and copper.

Beetroots are plants that have storage roots that are 5 to 10 cm in diameter. The storage tissues of these plants have cells that contain betacyanin (red pigment) in the cell vacuole. If the membrane proteins of the cell are denatured, the pigment will leak out.

Physical damage to the storage roots of beetroot, for example by cutting, causes large loss of pigment.

Using this information and your own knowledge, design an experiment to discover the lowest concentration of copper sulfate that has an effect on the leakage of pigment from these cells.

You must use:

- 0.3% copper sulfate solution,
- distilled water,
- beetroot.

You may select from the following apparatus and use appropriate additional apparatus:

- normal laboratory glassware e.g. test-tubes, beakers, measuring cylinders, graduated pipettes, glass rods etc.,
- syringes,
- pipette fillers,
- white card,
- white tile,
- knife, scalpel or cork borers,
- blunt forceps,
- Bunsen burner with tripod, gauze and bench mat,
- timer e.g. stopwatch or stop clock,
- thermometer.

Theoretical considerations:
Cell membrane is made up of mainly phospholipid molecules arranged in a bilayer. Trans-membrane proteins are embedded within the bilayer.
Such proteins can be denatured by copper sulfate, resulting in a loss of 3-D conformation through the breakage of bonds such as ionic bonds, hydrogen bonds etc and thus allowing the leakage of betacyanin into the surrounding solution, staining it red.

Cells can be mixed with different concentrations of copper sulfate for a fixed amount of time, and leakage of betacyanin can be detected by visual comparison of the surrounding solution against a white background (white card provided).

Note: hypothesis setting is not related to this question set.

Variables:
Independent variable:
Copper sulfate concentration /%
5 different concentrations (0.06, 0.12, 0.18, 0.24, 0.30%), prepared by stock dilution from 0.30% copper sulfate solution.

Dependent variable:
Presence of red coloration in surrounding solution containing the copper sulfate-treated beetroots.

Variables to be kept constant:
Temperature, to be kept constant at 37^0C by adding cold water or heating of water bath, monitored with a thermometer.

Incubation time, to be fixed by a stopwatch at 60 minutes.

Cutting dimensions of beetroot samples, to be fixed at 3mm thickness, measured by a ruler.

Control:
Replace copper sulfate solution with equal volume of distilled water. This is to prove that any leakage of betacyanin due to denaturation of membrane proteins is due to copper sulfate.

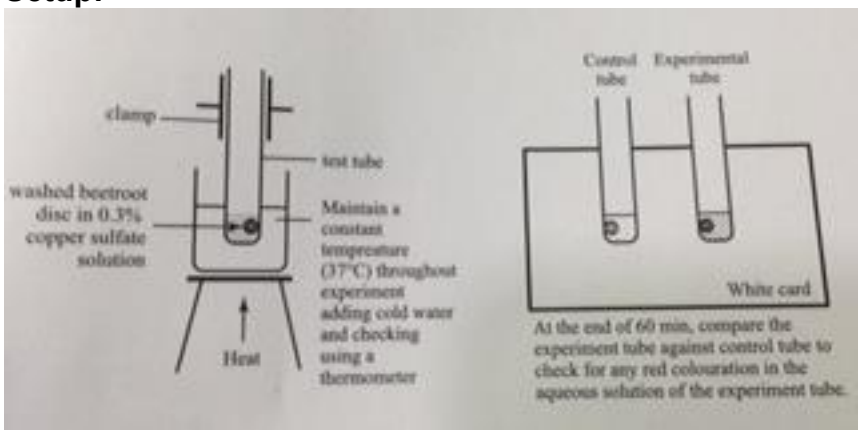
Procedure:
1. Obtain a cylinder of beetroot using a cork-borer.
2. Using a ruler and scalpel and white tile, prepare discs of 3mm thickness.
3. Wash the discs with distilled water to remove the pigments which were leaked during cutting.
4. Transfer the washed discs to a beaker of distilled water. After 60min, check that surrounding solution has same clarity as another beaker of distilled water.
5. Prepare 5 concentrations of copper sulfate using stock dilution from 0.3% copper sulfate solution.

Final concentration of copper sulfate solution /%	Final volume of copper sulfate solution /cm^3	Volume of stock copper sulfate solution needed /cm^3	Volume of distililed water / cm^3
0.06	10.0	2.0	8.0
0.12	10.0	4.0	6.0
0.18	10.0	6.0	4.0
0.24	10.0	8.0	2.0
0.30	10.0	10.0	0.0

Formula: M1V1 = M2V2

6. Transfer 5cm^3 of each concentration into a labeled test tube. Place tube in a 37 ^{0}C water-bath for 5min to allow solution to attain accurate temperature.
7. After 5min, use a blunt forceps to transfer one beetroot disc to each test tube. Be careful not to damage the disc.
8. Start stopwatch and allow the disc to incubate for 60 min. Keep the temperature at 37 ^{0}C using a thermometer, and adding cold water or heat the water bath if necessary.
9. After 60min, check for red coloration in the surrounding solution of each test tube by placing it against a white card.
10. Compare against a reference tube containing a washed disc in 5cm^3 of distilled water.
11. Record results in a table.
12. For each copper sulfate concentration, perform 2 more sets.
13. Repeat entire experiment for 2 more times to ensure reliability.

Setup:



Results:

Final concentration of copper sulfate solution /%	Detection of red coloration in surrounding solution after 60 min		
	Replicate 1	**Replicate 2**	**Replicate 3**
0.06			
0.12			
0.18			
0.24			

0.30			

Legend:
+ : Presence of red coloration
- : Absence of red coloration

Risks and Safety:
Scalpel and cork-borer are sharp and may cause cuts easily. Handle them with care. Broken glassware may cause cuts. handle and dispose broken pieces carefully.

QUESTION 5

(a) Describe features of zygotic stem cells and embryonic stem cells that distinguish them from each other. [5]

Zygotic stem cells	Embryonic stem cells
Isolated from morula / zygote;	Isolated from inner cell mass of blastocyst;
Totipotent, pluripotent and multipotent;	Pluripotent, not totipotent but are multipotent;
able to differentiate into any cell type to form any organ or type of cells, including **extra-embryonic membranes**;	able to differentiate into almost any cell type to form any organ or type of cells, **except** those of the extra-embryonic membranes;
ability to differentiate into any cell type to form **whole organisms**;	give rise to various **organs** in organism / multiple specialized cell types that make up the heart, lung, skin and other tissues in the developing foetus

Examiner's comments:
Most candidates could describe a number of features that distinguished between zygotic and embryonic stem cells.

(b) Describe the features of blood stem cells and explain their normal functions. [8]

Blood stem cells

Features

1 Undifferentiated ;;
2 unspecialized cells found in a specialized tissue, which can renew itself for a lifetime. Eg. in the bone marrow;;
3 have self-renewal ability; divide continuously by mitosis to produce new stem cells;;
4 Multipotent;;
5 Able to differentiate into a limited range of cell type / produce only cells of a specific lineage ;;
6 Are not pluripotent or totipotent;;

Functions

7 Can differentiate into red blood cells, white blood cells, platelets;;
8 Function = replacement of worn out blood cells from daily blood turnover and fighting infections, as well as through normal wear and tear, disease, injury;;

Examiner's comments:
The majority of candidates were confident in describing the properties of blood stem cells and in giving some examples of types of blood cells produced.

(c) Therapeutic genes can be introduced into stem cells. Discuss why the genes used are more likely to be obtained from a cDNA library, than a genomic DNA library. [7]

1. Genes obtained from cDNA library are made from the reverse transcription of the mature mRNA; ➔ cDNA only contain coding sequences and are **smaller in size;**
2. Genes obtained from genomic DNA library contain non-coding sequences (e.g. introns, promoters and regulatory sequences) and are larger; ➔ The size of the eukaryotic genes is **too large** to be packaged into most gene delivery systems (e.g. viral and liposomal vectors);

3. Genes obtained from cDNA library have no non-coding sequences and need not undergo mRNA splicing compared to genomic DNA;;

4. Genes obtained from genomic DNA library may be fragmented due to restriction enzyme digestion;
   Genes obtained from cDNA library are not fragmented as there is no restriction enzyme digestion;
5. Specific information on time and tissue-specific expression is already available;
   Thus, can easily isolate more copies of mRNA of heavily expressed therapeutic genes from specific cDNA libraries;

Examiner's comments:
Most candidates were able to give a reasoned response as to why cDNA libraries may be a better source of therapeutic genes than a genomic DNA library. Many clearly stated the origin of cDNA libraries.

Some candidates did not note the wording of the question sufficiently and discussed the problems of prokaryotic cells expressing genomic DNA. This was not relevant.