2012 'A' Level
H2 Biology
Mark Scheme

PAPER 1 (MCQ)

1	**D**
2	**A**
3	**D**
4	**D**
5	**A**

6	**B**
7	**B**
8	**C**
9	**B**
10	**D**

11	**D**
12	**D**
13	**A**
14	**D**
15	**B**

16	**A**
17	**C**
18	**C**
19	**D**
20	**C**

21	**B**
22	**A**
23	**A**
24	**B**
25	**C**

26	**D**
27	**C**
28	**A**
29	**A**
30	**D**

31	**A**
32	**B**
33	**C**
34	**B**
35	**B**

36	**B**
37	**C**
38	**A**
39	**C**
40	**C**

PAPER 2 (CORE)

QUESTION 1

(a)

1 Metaphase.

(b)

1 Centromeres divide during anaphase ;
2 Sister chromatids separate to form independent chromosomes ;
3 Chromosomes pulled to opposite poles of the cell by the shortening of kinetochore microtubules.

(c)

1 Centrioles are found in pairs at each pole and their position is important in determining the polarity of the cells ;
2 Centrioles play a role in organising the assembly of the spindle fibres, which aid in the separation of sister chromatids during nuclear division.

Examiner's comment: Most candidates correctly outlined the role of the centrioles in organising the synthesis of the spindle fibres, which then led to the separation of the chromatids. Fewer candidates mentioned that there was a pair of centrioles at each pole and that their position was important in determining the polarity of the cells.

(d)

1 During prophase, the nuclear envelope disintegrates and disappears ;
2 This allows free movement of chromosomes to align at the metaphase plate ;

3 During telophase, the nuclear envelope reappears and encloses the separated chromosomes at opposite poles of the cell ;
4 This prevents entanglement of the de-condensing chromosomes and prepares the cell for cytokinesis.

Examiner's comment: While the majority of the candidates correctly described what happens to the nuclear envelope during mitosis, very few provided a full response to the question by explaining the changes that they had described.

(e)

Structures in region A: chromosomes (consisting of replicated sister chromatids) ;
Structures in region B: spindle microtubules.

QUESTION 2

(a)

1 Chloride ions are charged ; **REJECT** polar / hydrophilic
2 Unable to diffuse through the hydrophobic core of the phospholipid bilayer of the membrane ;

3 Transmembrane CFTR protein has a water-filled pore lined with hydrophilic amino acids which allows the diffusion of the chloride ions down their concentration gradient ;

Examiner's comments: Some candidates incorrectly referred to chloride ions as polar.

(b)

1 Hydrophobic interactions between the non-polar tails of the phospholipids and the hydrophobic R-groups of the non-polar amino acids in contact with the phospholipids ;
2 Hydrophilic interactions between the polar phosphate heads and the hydrophilic R-groups of the polar amino acids on the exterior of the protein.

(c)

1 ATP binds to the ATP binding domain of the CFTR protein ;
2 This changes the 3D conformation of the protein (and hence opens the channel).

Examiner's comments: Many candidates did not make use of the information provided in the introduction to the question and the diagram, giving accounts that incorrectly referred to active transport and pumping of ions.

(d)

1 Deletion mutation.

(e)

1 Loss of amino acid results in a change in the primary structure of the protein ;
2 Since site A is near the ATP binding domain, the change in primary structure results in changes in the 3D conformation in this domain such that it is no longer complementary to ATP and is unable to bind to it ;
3 The pore remains closed and prevents the facilitated diffusion of chloride ions out of the epithelial cells.

(f)

1 When one copy of the normal dominant allele is present, functional CFTR protein will still be expressed (cystic fibrosis is a recessive disease where two copies of the alleles must be mutated for the disease to manifest) ;
2 Facilitated diffusion of chloride ions out of the epithelial cells can still occur, hence no accumulation of chloride and sodium ions in the cell that will cause water to be drawn in and result in thick mucus formation on the surface of the epithelial cell.

QUESTION 3

(a)

A capsid (icosahedral) head ;
B tail sheath.
C tail fiber.
D base plate

(b)

1 (Inside the bacterial cell), viral DNA is used as a template for replication of more copies of the viral DNA genome ;

2 Host RNA polymerase binds to the promoter of the viral DNA template and synthesize messenger RNA (mRNA) of viral genes ;
3 Viral mRNA is translated by host ribosomes to produce viral proteins and enzymes (to direct the assembly of new phages).

(c)

1 (viral DNA incorporated into the bacterium's DNA to become pro-phage), shield virus from unfavourable environmental conditions
(potential survival strategy to maintain phage population when host abundance is too low for the population to be maintained by lytic infection);
2 (integrated viral DNA replicates along with host chromosome), increase number of copies of viral DNA / more pro-phage in bacteria population, increases the chance of propagation and hence survival of viruses.

QUESTION 4

(a)

1 Transcription factors contain a DNA-binding domain which recognize and bind to specific DNA sequence in the promoter / TATA box ;
2 Nucleotide sequence of TATA box has complementary shape to DNA-binding domain ;

Examiner's comments: A number of candidates referred to complementary base pairing between transcription factors and DNA indicating uncertainty about the difference between complementary base pairing (only between nucleic acids) and the complementary shape of the binding site of specific proteins and the molecules that they bind to.

(b)

1 unwinding of the double helix of DNA exposes the promoter sequence
2 for general transcription factors and RNA polymerase II to bind to, forming the transcription initiation complex for the subsequent initiation of transcription

Examiner's comments: Candidates engaging effectively with this question recognized the significance of the process in exposing bases for RNA polymerase attachment and subsequent transcription.

(c)

1 eukaryotic genes contain both coding and non-coding sequences / exons and introns, which are transcribed ;
2 Introns are excised and exons ligated together ;
3 to make the coding sequences continuous in the mature mRNA for translation ;

Examiner's comments: Candidates were familiar with the processing of pre-mRNA molecules and its significance.

QUESTION 5

(a)

1 Epistasis refers to gene interaction where the expression of one gene masks the expression of another gene.
2 (Given the 9:7 ratio observed in the F_2 generation) expression of a pair of recessive alleles of the first gene locus (i.e. aa) masks the expression of the alleles of the second gene locus, B.
3 The expression of a pair of recessive alleles at the second gene locus (i.e. bb) also masks the expression of the alleles at the first gene locus, A.

(b)

F_1 phenotype: purple x purple
F_1 genotype: AaBb x AaBb ;

Parental gametes: (AB) (Ab) (aB) (ab) x (AB) (Ab) (aB) (ab) ;

F2 genotypes in Punnett square ;
F2 genotypes correspond to phenotype ;

	(AB)	(Ab)	(aB)	(ab)
(AB)	AABB Purple	AABb Purple	AaBB Purple	AaBb Purple
(Ab)	AABb Purple	AAbb White	AaBb Purple	Aabb White
(aB)	AaBB Purple	AaBb Purple	aaBB White	aaBb White
(ab)	AaBb Purple	Aabb White	aaBb White	aabb White

F_2 phenotypes: purple flowers white flowers
F_2 phenotypic ratio: 9 : 7

(c)

1 The critical χ^2 value at p=0.05 and degrees of freedom of 1 is 3.841 ;
2 As the calculated χ^2 value of 1.60 is smaller than 3.841, p value is larger than 0.05
/ More than 5% probability that any difference between observed and expected results is due to chance
3 No significant difference between observed and expected results, therefore the observed results fit the expected ratio of 9: 7, as predicted by epistasis.

(d)

1 Pea plants have short life cycles and produce many offspring in one cross. This allows statistical analysis of the results ;
2 They also have observable traits with contrasting forms (e.g. purple vs white) which are easy to score ;
3 They are easy to manipulate for pollination, allowing researchers to perform cross-pollinations or selfing easily.

QUESTION 6

(a)

1 Anaerobic respiration produces 2 ATP per glucose molecule as compared to aerobic respiration which produces 36-38 ATP per glucose ;
2 Under anaerobic conditions, oxygen no longer available as the final electron acceptor to accept electrons from the electron transport chain ;
3 The conversion of pyruvate to acetyl CoA (link reaction), the Krebs cycle, and oxidative phosphorylation (where the bulk of ATP are made) cannot take place ;

4 Pyruvate is reduced by reduced NAD (NADH) at the end of glycolysis via substrate level phosphorylation with a net formation of 2 molecules of ATP
5 To regenerate oxidized NAD (NAD^+) for glycolysis to continue, pyruvate is converted to ethanol with the aid of pyruvate decarboxylase and dehydrogenase
6 Incomplete breakdown of glucose meant that energy was still trapped in pyruvate or ethanol.

(b)

1 Yield of ATP per glucose during anaerobic respiration = 2
2 Yield of ATP per glucose during aerobic respiration = 38
3 Number of ties glycolysis must proceed to produce same number of ATP molecules as aerobic respiration in the same time = 38/2 = 19

(c)

1 At low ATP concentration, PFK activity increases to its maximum at a faster rate than at high ATP concentration when substrate concentration increases ;
2 ATP is an allosteric inhibitor of PFK, binding to the allosteric site and inducing changes to the 3D conformation of PFK ;
3 The active site of PFK is altered and hence unable to bind to its substrate F6P to form enzyme-substrate complex ;
4 This negative feedback inhibition by ATP reduces PFK activity and hence reduces rate of glycolysis.

(d)

1 Passive transport of polar molecules or ions from a region of higher concentration to a region of lower concentration ;
2 across a partially permeable membrane, down a concentration gradient ;
3 Requires the presence of **protein channels specific** to the molecules or ions it transports ;
/ Glucose transporters specific to glucose transports glucose into the yeast cells

Examiner's comments: A few candidates correctly mentioned the specificity of the protein channels.

QUESTION 7

(a)

1 Populations on different islands are geographically isolated due to the surrounding sea acting as barriers, no interbreeding between species on different islands, disruption to gene flow in the ancestral population ;
2 Founder effect (e.g. migration of mainland species to different islands) a few individuals become isolated from a larger population and establishes a new population ;
3 Different selective pressures on different islands, eg. food availability/different predators ;

4 Genetic variations exist within each population;
5 individuals with a selective advantage in a particular environment survived till reproductive age and pass on their alleles to offspring;
6 change in allele frequency of gene pool of population over time;
7 accumulation of genetic differences over time;
8 Speciation: different populations ultimately cannot interbreed to produce viable, fertile offspring;

9 Allopatric speciation: Population is divided into two or more sub-populations as a result of physical barrier
10 Sympatric speciation can also occur: Different species arise from ancestral species which occupy different parts of the same island

(b)

1 mtDNA is common in all species thus allows for valid basis for comparison between distantly related species
Eg. highly conserved sequences like cytochrome b /c genes allow comparison of sequences even between distantly related species
/Eg. poorly conserved sequences like hypervariable regions (HVR I and HVR II) allow comparison of sequences of closely related individuals

2 inherited only from the mother which allows tracing of a direct genetic line / maternal ancestry of a population
/no genetic recombination between the mother and the father unlike nuclear DNA which will creating an unclear genetic history

3 average somatic cell has hundreds to thousands of copies of mtDNA, compared to just 2 copies of any given nuclear gene so fewer samples is required, easier to obtain mtDNA for analysis

(c)

1 Nucleotide data is quantifiable and open to statistical analysis ;
2 Nucleotide data is unambiguous and objective as it is based strictly on heritable material ;
3 Nucleotide data is not affected by convergent evolution ;

4 Neutral mutations do not confer any selective advantage or disadvantage and are accumulated at a relatively constant rate for any particular gene ;
5 Nucleotide data can be used as a molecular clock to date evolutionary event , by comparing nucleotide sequence of a particular common gene (in this case, a mtDNA gene can ND5 gene) between different species, the number of mutations in nucleotide sequence is used to calculate the length of time since divergence.

(d)

1 The first 3 protein complexes are electron carriers that transport electrons, energy released during electron transport is used to pump H^+ ions from mitochondrial matrix, across the inner mitochondrial membrane and into the intermembrane space ;

2 The 4^{th} protein complex plays the role of a hydrophilic protein channel for the facilitated diffusion of H^+ ions down its concentration gradient from the intermembrane space back into the matrix ;
3 This complex also consist of an ATP synthase that catalyses the synthesis of ATP from ADP and P_i as H^+ ions diffuse down its concentration gradient.

QUESTION 8

(a) Outline the molecular structure of phospholipids in relation to their function in cell membranes. [7]

1 Phospholipids are made up of a polar phosphate head and two hydrophobic hydrocarbon chains, all of which are joined covalently to a glycerol molecule ;
2 This makes phospholipids amphipathic in nature, allowing them to arrange themselves into a phospholipid bilayer when in an aqueous medium to form membranes ;

3 Membranes allow <u>compartmentalization</u> within the cell, which provides optimal conditions (e.g. pH, localised concentration of substrates and enzymes) for specialized biochemical reactions to occur in each organelle / allows separate biochemical pathways to proceed simultaneously without affecting each other ;

4 The phospholipids also play a role in intracellular transport. They have weak interactions between them (hydrophobic interactions between hydrocarbon chains and hydrogen bonds between polar heads), which give rise to the fluid property of the membrane ;
5 This allows organelles such as the endoplasmic reticulum and golgi apparatus to form transport/secretory vesicles which bud off from these organelles ;

6 Phospholipids also constitute the hydrophobic core of the cell membranes which do not allow polar molecules to pass through ;
7 This allows cell membranes to regulate the movement of substances in and out of the cell/organelles
OR This allows organelles to generate concentration gradients of solutes across their membranes.

(b) Explain the role of the Golgi body and its link to the rough endoplasmic reticulum. [7]

1 cis face of Golgi body receives proteins from the rER;
2 via transport vesicle containing the protein that buds off from the rER ;

3 Proteins are further post-translationally modified in the lumen of Golgi body;
4 ref glycosylation / addition of short carbohydrate chains to the proteins to form glycoproteins
ref proteolysis
ref phosphorylation
ref folding

5 Golgi body sorts and packages proteins and targets them to various cellular locations ;
6 vesicles carrying the secretory proteins buds off from the trans face of Golgi body, fuses with the plasma membrane and release protein by exocytosis ;
7 packaging of hydrolytic enzymes during the formation of lysosomes ;
8 formation of golgi vesicles for the cell walls (in plants only).

(c) Describe the structure of ribosomes [6]

1 made up the large and small ribosomal subunits
/ 30S and 50S in prokaryotes **AND** 40S and 60S in eukaryotes ;
2 large and small subunits assemble to form a functional ribosome only when they attach to an mRNA ;

3 small ribosomal subunit contains the binding site for mRNA

4 there are three sites for tRNAs binding ;
5 A (Aminoacyl-tRNA binding) site which holds the tRNA carrying the next amino acid to be added to the chain
6 P (peptidyl-tRNA binding) site which holds the tRNA carrying the growing polypeptide chain
7 E (Exit) site for the discharged tRNAs to leave the ribosome

QUESTION 9

(a) Outline the light dependent reactions of photosynthesis. [7]

Step 1: Light harvesting at PS II

1 A photon of light strikes a pigment molecule in a light harvesting complex.
2 The pigment molecule absorbs light energy, and its electron is excited to a higher energy level.
3 When the "excited" chlorophyll returns to the "ground" state (unexcited state), energy released is passed on to a neighboring chlorophyll molecule.
4 The energy is transferred from one pigment molecule to another, and is finally channelled to P680 chlorophyll a molecules in the PS II reaction centre.
5 An electron of chlorophyll a molecule is boosted to a very high energy level and captured by PS II's primary electron acceptor.

Step 2: Photolysis of water (Hill reaction)

6 The electron displaced from the PS II reaction centre is replaced via the Hill reaction.
7 A water molecule is split by an enzyme during photolysis, leading to production of H+ ions and oxygen molecule, and the removal of electrons
8 The electrons are then donated to the P680 chlorophyll a molecules in the PS II reaction centre.

Step 3: Electron transport between PS II to PS I

9 Photoexcited electrons are passed from PS II's primary electron acceptor to PS I, via a series of electron carriers in an electron transport chain

Step 4: ATP synthesis via chemiosmosis

10 As the electrons are transported along the series of electron carriers of progressively lower energy levels, the energy released from the electron transport is used to pump H^+ from the stroma, across the thylakoid membrane and into the thylakoid space.
11 H^+ ions in the thylakoid space are also generated by photolysis of water.
12 A proton gradient is generated, where H^+ ion concentration is much greater in the thylakoid space than in the stroma.
13 H^+ ions diffuse from the thylakoid space into the stroma of the chloroplast, through the ATP synthase complex/stalked particle. This process is referred to as chemiosmosis[1].
14 ATP synthase enzyme of the complex catalyses the synthesis of ATP from ADP and P_i.

Step 5: Light harvesting at PS I

15 Meanwhile, light is harvested by the accessory pigments in the light harvesting system of PS I and the energy is channelled to P700 chlorophyll *a* molecules.
16 An electron of chlorophyll *a* molecule is boosted to a very high energy level and captured by PS I's primary electron acceptor.

Step 6: Electron transport between PS I and $NADP^+$

17 Photoexcited electrons are passed from PS I's primary electron acceptor down a second electron transport chain to $NADP^+$.

Step 7: NADPH synthesis

18 H^+ ions from the stroma and the electrons from PS I reduce $NADP^+$ to NADPH.

19 The removal of H^+ ions from the stroma when it is taken up by $NADP^+$ also contributes to the proton gradient.

20 In cyclic photophosphorylation, Electrons displaced from PS I are transferred to PS I's primary electron acceptor and then on to the ETC between PS II and PS I.

(b) Explain the role of membranes in chloroplast. [7]

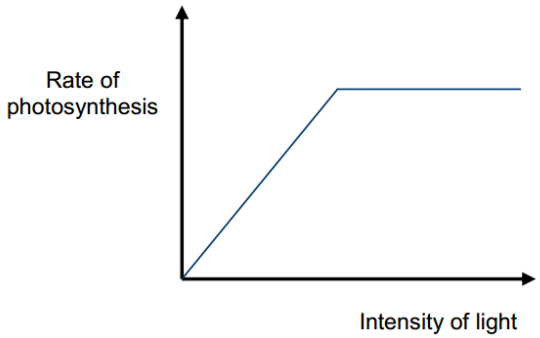
1 light-dependent stage of photosynthesis takes place on thylakoid membranes in chloroplasts
2 thylakoid membranes surround the thylakoid space and are arranged into stacks known as grana
3 Large surface area of thylakoid membranes, for more photosystems consisting of photosynthetic pigments for absorbing light
/ for more electron carriers of the electron transport chain and ATP synthase complexes
4 As the electrons are transported along the series of electron carriers of progressively lower energy levels, the energy released from the electron transport is used to pump H^+ from the stroma, across the thylakoid membrane and into the thylakoid space

5 Thylakoid membrane is impermeable to H^+, allows for proton gradient to be generated, where H^+ ion concentration is much greater in the thylakoid space than in the stroma.

6 H^+ ions diffuse from the thylakoid space into the stroma of the chloroplast, through the ATP synthase complex, for synthesis of ATP from ADP and P_i
7 chloroplast membranes allow compartmentalisation of enzymes and substrates involved in photosynthesis, increasing the efficiency of the light independent reactions
/ Eg. Rubisco enzyme is concentrated in the stroma of chloroplast for carbon fixation during Calvin cycle

(c) Describe the effects of increasing light intensity on the rate of photosynthesis. [8]

At low light intensities



1 As light intensity increases, rate of the light-dependent reaction increases;
2 Due to increased light energy absorbed by the photosynthetic pigments;
3 Results in more ATP and NADPH produced in light dependent reactions;
4 Which are used in the Calvin cycle for reduction of glycerate-3-phosphate to triose phosphate;
5 ATP is also required for regeneration of ribulose bisphosphate (RuBP);
6 Therefore, rate of photosynthesis increases proportionately;
7 Light intensity is the limiting factor;

As light intensity is increased further

8 the rate of photosynthesis is eventually limited by some other factor (e.g. CO_2 concentration), rate of photosynthesis plateaus / levels off ;

At very high light intensity

9 chlorophyll may be damaged and the rate of photosynthesis drops steeply (not shown in graph);

10 Use of labelled diagram in the explanation;

Examiner's comments:

Candidates were able to state the broad relationship between light intensity and the rate of photosynthesis, but descriptions often lacked further details, such as consideration of how the relationship changes at higher light intensities. References to the results of increased light intensity on the light dependent and light independent stages of photosynthesis would also have been relevant.

PAPER 3 – Applications Paper and Planning Question

QUESTION 1

(a)

1 Collection of total genomic DNA from a single organism ;
2 Stored in a population of bacteria, each containing a vector with a different insert of DNA from the genome ;
3 Amplification and retrieval of specific clones from the library can be made for analysis.

Examiner's comments: Those candidates who carefully read the question gave clear answers. The question did not ask how a genomic DNA library was constructed or what it was used for.

(b)

1 Reverse transcriptase synthesises single-stranded complementary DNA from mRNA templates extracted from target cell;
2 RNAase degrades the mRNA template ;
3 DNA polymerase uses the single-stranded complementary DNA strand as a template to synthesise a second complementary DNA strand.

(c)

1 The template used for synthesis of cDNA is mRNA extracted from tissues ;
2 Since the expression of different genes varies at different times in the life of the cell, the expression of mRNA also varies ;
/ for example, certain genes transcribed at a particular time to produce mRNA while other genes are turned off ;

3 For genomic library, the set of DNA remains the same throughout the life of the cell.

(d)(i)

1 Annealing stage at temperatures between 50 – 65^0C ;
2 Forward and reverse primers bind to complementary sequences flanking the target sequence to be amplified at the 3' ends of single (DNA) strands ;

(ii)

1 Free DNA nucleotides and DNA primers ;
2 More than half of the total number of the Taq DNA polymerases are denatured and non-functional, hence they are unable to bind to and elongate DNA ;
3 Double-stranded DNA template with regions bound to DNA primers non-specifically ;

(e)

A

1 Denaturation of double-stranded DNA template through the breaking of hydrogen bonds between complementary bases of the two DNA strands.

B

1 Binding of single-stranded DNA probe to its complementary region on the single-stranded DNA template.

QUESTION 2

(a)

1 Multipotent.

(b)

1 The donor cells are foreign to the patient;
2 Presence of glycoproteins and glycolipids on the donor cell surface serve as antigens, which will be recognized by white blood cells;
3 that would launch an attack on the donor cells / there will be stimulation of an immune response; leading to rejection of foreign cells/tissue rejection/ destruction of donor trachea;
4 so that the donor trachea can serve its function in the patient;

Examiner's comments: Few explained the basis of this rejection or its significance for the continued care of the patient.

(c)

1 Only the stem cells were removed from patient's body ;
2 Thus absence of growth factors to stimulate cell division;
3 Chemicals must be added *in vitro* to induce cells to undergo mitosis and cell division;
4 Binding of growth factor to receptors on the stem cells will result in cell signaling processes, leading to switching on and off of certain genes for cell division to occur;

Examiner's comments: Most candidates made reference to mitosis, but many did not go on to explain the underlying changes to the cells.

(d)

1 Stem cells have activated telomerase gene, active telomerase enzyme produced which extends the telomeres at the ends of chromosomes;
2 Thus stem cells able to undergo long term mitotic cell division/ self-renewal ability;

3 Other cells do not have activated telomerase genes
/ no production of telomerase enzyme to extend telomeres at ends of chromosomes;
4 End-replication problem occurs, where daughter DNA strands become shorter at the 5' end after every round of DNA replication;
5 Once the telomeres are shortened to a critical length, it will signal for cell division to stop and in some cases, apoptosis occurs/ ref. Hayflick limit;

Examiner's comments: In some cases, candidates described the process that enables stem cells to continue to divide but did not go on to explain why, without this process, mitosis is limited to a set number of divisions.

QUESTION 3

(a)

(i)

1 Multiple cloning site containing many different but unique restriction sites ;
2 Allows insertion of DNA fragment isolated from any species of organism as long as both the insert and MCS are cut by the same restriction enzyme.

(ii)

1 Enables independent replication of the plasmid and foreign gene inside the host cell ;
2 Sequence of DNA recognized and bound by certain enzymes and used in order to start the replication of DNA ;
3 This results in multiple copies of the plasmid and foreign gene within one bacterium to be formed.

(b)

(i)

1 Plasmid and gene of interest have complementary sticky ends which can base pair with each other via hydrogen bonding

(ii)

1 Plasmid re-annealed without the gene of interest ;
2 Gene of interest re-annealed ;
3 Two or more plasmids annealed with each other ;
4 Two or more genes of interest annealed with each other.

(c)

1 Bacteria are made competent through the addition of ice-cold calcium chloride solution and chilled on ice ;
2 followed by a brief heat shock at 42°C for 45 seconds to create transient pores created in the cell membranes for recombinant plasmid to enter the bacteria.

(d)

1 Using 2 antibiotic-resistance genes (eg. Tet^R and Kan^R genes) as genetic markers for the identification and election of bacteria transformed with recombinant plasmid, gene of interest inserted into one of the antibiotic-resistance gene (eg. Kan^R gene) in the plasmid ;

2 grow bacteria on LB plate containing first antibiotic (Tet) ;
3 transformed cells can survive, as they contain plasmid with intact antibiotic-resistance gene (intact Tet^R gene) for the first antibiotic;

4 Replica plate on LB plate containing a second antibiotic /transfer a small amount of each colony to an identified spot on LB plate containing a second antibiotic (eg. Kan) ;

5 bacterial colonies that cannot survive on LB plate with the second antibiotic have the recombinant plasmid, as gene of interest has disrupted the sequence of this particular antibiotic-resistance gene (disrupted Kan^R gene);

(e)

1 Only Plasmid P and Q have <u>both</u> *Pst*I and *Fok*I restriction enzyme recognition sites for the insertion of Genes S and T, respectively

2 <u>Plasmid Q</u> should be used as it has a medium copy number compared to Plasmid P which has a low copy number, hence greater amount of Genes S and T can be cloned as there are more copies of plasmids within a bacterium

QUESTION 4

Relationship between independent variable (sucrose concentration) and dependent variable (rate of respiration)

- Yeast takes in sucrose is broken down to form fructose and glucose.
- Yeast uses glucose in cellular respiration
- $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$
- Rate of respiration increases proportionally with an increase in sucrose concentration initially and gradually reaches a constant rate at maximum sucrose concentration.

- There are respiratory enzymes in yeast; so at maximum sucrose concentration, any increase in sucrose concentration will not cause an increase in rate of respiration since glucose concentration is no longer a limiting factor, but some other factor such as temperature or oxygen is limiting.

- CO_2 is produced during oxidative decarboxylation during the link reaction and the Krebs cycle
- Frequency of successful collision between the enzyme molecules present in yeast and substrate molecules increases proportionally.
- Then the enzyme active sites become saturated.
- Increasing number of substrate (sucrose) molecules has to wait for the dissociation of enzyme-substrate complexes before new enzyme-substrate complexes can be formed.
- Hence further increase in substrate (sucrose) concentration will not further increase the rate of CO_2 production.

- Since CO_2 reacts with barium hydroxide to form insoluble barium carbonate, an increased rate of respiration therefore causes a decrease in concentration of barium hydroxide solution, hence less hydrochloric acid is needed to neutralise barium hydroxide solution.

- The end-point of the titration is used to determine the remaining concentration of barium hydroxide after respiration.

Independent variable

- Concentration of sucrose
- 5 different sucrose concentrations produced by serial dilution
- 0.2 mol dm^{-3}, 0.4 mol dm^{-3}, 0.6 mol dm^{-3}, 0.8 mol dm^{-3} and 1.0 mol dm^{-3}

Various concentrations of sucrose are prepared from the 1.0 mol dm-3 stock solution as follows

Concentration sucrose solution / mol dm^{-3}	**Total volume of solution / cm^3**	**Volume of 1.0 mol dm^{-3} sucrose stock solution used / cm^3**	**Volume of distilled water/ cm^3**
0.2	10.0	2.0	8.0
0.4	10.0	4.0	6.0
0.6	10.0	6.0	4.0
0.8	10.0	8.0	2.0
1.0	10.0	10.0	0.0

Dependent variable

- Rate of respiration in yeast cells
- Mass of CO_2 produced per minute (rate of CO_2 production)
- Calculated using the **decrease** in volume of hydrochloric acid required to turn mixture of barium hydroxide solution and phenolphthalein indicator from pink to colourless.

Constant variables

- Volumes of yeast and sucrose solutions in the boiling tubes, using distilled water to make up the same volume of solution in the boiling tubes
- Volume of barium hydroxide, using syringe
- Concentration of yeast solution, using the same stock solution and stirring suspension with a glass rod before measuring out desired volume of yeast suspension

Control

- Set up a control by replacing the sucrose with distilled water. The control is subjected to the same environmental factors as that for the experiment.
- This is to show that the CO_2 evolved is due to yeast cells undergoing aerobic respiration in the presence of sucrose.

Method

1. Label 5 boiling tubes with each of the different concentrations of sucrose solution.
2. Prepare another beaker of about 100cm^3 of yeast suspension.
3. Place this beaker with the boiling tubes of sucrose solution in a thermostatically-controlled water bath at 30˚C. Allow the yeast and sucrose to equilibrate to the temperature for 10 mins.
4. Use a glass rod to stir the active yeast suspension before using a 10 cm^3 syringe to transfer 5.0 cm^3 of yeast suspension into 10.0 cm^3 of 1.0 mol dm^{-3} sucrose solution in a boiling tube.
5. Equilibrate for 10 s before the start of the experiment.
6. Seals the end of the boiling tube with a rubber bung to the boiling tube to allow bubbling of CO_2 into the test tube containing 10.0 cm^3 of fresh 0.025 mol dm^{-3} barium hydroxide solution.
7. Start the stopwatch immediately and allow bubbling for 1 min.
8. Remove the test tube from the delivery tube after 1 min.
9. Use a 1 cm^3 syringe to add 0.5 cm^3 of 1.0% phenolphthalein indicator into the test tube. The mixture will appear pink.
10. Hold the test tube of pink mixture against a white tile.
11. Using a 10.0 cm^3 syringe, draw out 10.0cm^3 of 0.1 mol dm^{-1} hydrochloric acid.
12. Add the acid dropwise, into the test tube of pink mixture, swirling the mixture after very drop.
13. Record the volume of hydrochloric acid used for the colour of the mixture to change from pink to colourless.
14. Perform Steps 1 to 13 using the other concentrations of sucrose solutions.

Replicates and repeats

- Perform 3 replicates for each sucrose concentration to calculate the average volume of hydrochloric acid required;
- Repeat the entire experiment 2 more times using fresh yeast and sucrose solutions, and barium hydroxide solution; to ensure reproducibility of results.

Results table with clear headings and correct units

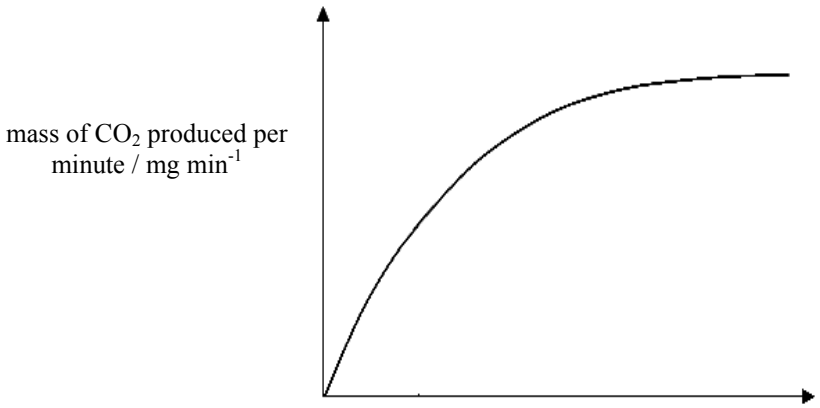
- Substrate the average volume of HCl from 5.0cm^3 to obtain the decrease in volume of HCl required to neutralise barium hydroxide.
- Using the conversion of a decrease of 1.0cm^3 of 0.1 mol dm^{-3} HCl equivalent to 2.2mg of CO_2, calculate the amount of CO_2 produced by 1 min to obtain the rate of CO_2 production per min.

Sucrose concentration / mol dm^{-3}	Volume of hydrochloric acid required to neutralise remaining barium hydroxide solution / cm^3				Decrease in volume of HCl required / cm^3	Mass of CO_2 produced	Mass of CO_2 produced per minute / mg min^{-1}
	Reading 1	Reading 2	Reading 3	Average			
0							
0.2							
0.4							
0.6							
0.8							
1.0							

Mass of CO_2 produced per minute
= Average decrease in volume of hydrochloric acid required to neutralise remaining barium hydroxide solution x 2.2

Graph with labels with correct units

- Plot a graph of mass of CO_2 produced per minute / mg min^{-1} against sucrose concentration / mol dm^{-3}



Risk and safety measures

- Hydrochloric acid is corrosive. Barium hydroxide may also irritate the skin. Wear gloves to protect skin and goggles to protect eyes from splashes of barium hydroxide.
- Yeast cells may cause infections if they replicate on mucous membranes. Wear gloves to protect skin and googles to protect eyes from splashes of yeast solution.

QUESTION 5

(a)

1 explant of meristematic/ totipotent / pluripotent cells or tissues obtained which are normally free from infections (e.g. virus) ;
2 surface sterilised with sodium hypochlorite solution to remove micro-organisms which might proliferate in nutrient culture and kill plant cells ;
3 placed on nutrient medium containing nutrients sugars (e.g. sucrose), amino acids, vitamins, minerals (e.g. K^+) and plant growth regulators (e.g. auxin and cytokinin) ;
4 grown under aseptic conditions using a laminar flowhood to prevent contamination of the nutrients by bacteria and fungi from the surrounding air ;
5 Intermediate ratio of auxin and cytokinin results in the explant developing into a mass of undifferentiated cells called callus ;
6 The presence of nutrients induce the cells to proliferate/to stimulate mitosis/ division and callus to increase in size until a certain size is reached ;
7 subcultured in medium containing appropriate growth regulators such as auxins and cytokinins at varying concentrations to stimulate differentiation ;
8 Higher cytokinin to auxin ratio induces shoots formation ;
9 Shoots are then transferred to another nutrient agar with lower cytokinin to auxin ratio to induce roots formation ;
10 plantlets first transferred into a greenhouse for acclimatization before transferring to soil in the field to allow further growth.

Examiners' comment:
Not all candidates went on to provide an explanation for the steps that they had described, highlighting the importance of carefully reading questions to identify what is required.

(b)

Negative aspects

Pesticide resistant plants

1 Pests may eventually become resistant to the pesticide (e.g. Bt toxin).
2 farmers are required to plant at least 20% of non-Bt crops alongside Bt crops in refugia to support insect populations that are not under selection pressure and so slow development of resistance.

Unintentional harm to other organisms

3 GM crops that kill pests could also cause unintentional harm to other organisms
4 Eg. Pollen from Bt corn could harm larvae from the Monarch butterfly.

Superweeds

5 Seeds from GM crops might be carried to other places and establish themselves as weeds
6 Cross-pollination between the GM crops and their wild relatives may spread the resistance to weeds
7 Emergence of vigorous weeds with herbicide-resistant genes

Disruption of ecological balance

8 Ecological balance is disrupted
9 Accidental release of transgenic organisms into the environment might upset the balance of the ecosystem.
10 Fast-growing salmon may outcompete the wild salmon population and affect the food chain.
11 Larger transgenic salmon may be preferably selected as mates over smaller wild types.
12 Danger that the active growth hormone gene is transferred to other fish.

Health and safety

13 Introduction of foreign gene may result in production of secondary metabolites
14 which may be toxic to animals themselves and/or livestock/humans that consume them.
15 New proteins in GM plants may be potentially allergenic to humans that consume them.
16 Vectors used in GE contain genes for antibiotic resistance. When these transgenic crops are eaten, these genes may pass from the plant to the E. coli in the gut, making them resistant to antibiotics.

Positive aspects

Increase yield

17 Pesticides specifically kills insect pests, reduce crop losses (leading increased profits in agriculture)

18 can spray herbicides to kill weeds without affecting crops, reduce crop losses (leading increased profits in agriculture)

Increase quality

19 E.g. Golden rice enriched with beta-carotene
20 produced by transplanting genes from daffodil and bacteria

21 help prevent Vitamin A deficiency, which leads to blindness and susceptibility to disease

Examiners' comment:
Beneficial aspects with social implications (for the better) were dealt with in less detail. For example, the inclusion of pest-resistance genes in crop plants were mentioned, but often without referring to the benefit of this.

(c)

For statement

1 Evolutionary theory describes all life descended from a common ancestor
2 The best evidence for evolution and common descent is the DNA sequence shared among organisms since it is the hereditary material.
3 Closely related organisms which share the most recent common ancestor would have the highest DNA similarity compared to distantly related organisms

4 Since genetic modified organisms acquire genes from another species,
5 their genomic sequences would be different from the unmodified organisms.
6 Therefore, if extensive modification occurred, the differences in DNA sequences would justify them being classified as new species.

7 Also, GM organisms may acquire new morphological structures not found in unmodified organisms.
8 According to morphological species concept, where organisms are classified based on their body shape and other structural features, this also supports classifying them as new species.

Against statement

9 According to the biological species concept, however, the concept of species is based on reproductive compatibility.
10 Sexually mature GM organisms are still able to cross with the unmodified organisms and produce viable and fertile offspring.
11 There is still presence of gene flow between the two populations of GM organisms and unmodified organisms.