2014 'A' Level
H2 Biology
Mark Scheme

PAPER 1 (MCQ)

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

PAPER 2 (CORE)

QUESTION 1

(a) With reference to Fig. 1.1, describe the curve between points **A** and **B.** **[2]**

1 Initially, rate of reaction increases gradually e.g. from 0 to 8 arbitrary unit (a.u) as temperature increases from 35 ^{o}C (point A) to 50^{o}C.

2 Subsequently, the rate of reaction increases rapidly e.g. from 18 to 65 a.u as temperature increases from 55 ^{o}C to 65^{o}C (point B).

Examiner's comment: The majority of candidates correctly described the change in the rate of the reaction and supported their answers by quoting figures from the graph from both axes. Candidates who considered explanation of the shape of the graph had not recognised the significance of the instruction to 'describe' the curve.

(b) Explain why the reaction rate changes from:

(i) **A** to **B**, **[3]**

1 (As temperature increases,) heat increases and increases the kinetic energy of enzymes and substrates.

2 Increasing frequency of effective collision between enzyme and substrates.

3 More enzyme-substrate complexes formed per unit time, hence the rate of reaction increases.

(ii) **C** to **D**. **[3]**

1 At the optimum temperature at point C, the rate of reaction is at its highest. As temperature increases beyond the optimum temperature, the rate of reaction decreases drastically.

2 Increased heat increases the thermal agitation of the enzyme molecule and put strain on the bonds holding the tertiary structure of the enzyme. Eventually high enough temperature leads to the disruption of weak bonds e.g hydrogen bonds, ionic bonds and hydrophobic interaction that stabilised the conformation of the enzyme and its active site.

3 The three dimensional (3D) conformation of enzyme is eventually broken permanently. Enzyme is denatured and binding of the substrate is affected.

(c) Suggest how the structural features of Taq polymerase make it thermostable. **[2]**

1 (Taq polymerase is a protein whose structural features are influenced by) the number, types and sequence of the different amino acids in the primary structure which determines how the polypeptide chain coils and folds into tertiary structure. Tertiary structure is maintained by bonds formed by chemical interactions between the different R groups of the amino acids.

2 (To be thermostable, Taq polymerase) would have more cysteine amino acids (to form disulfide bonds) and polar or acidic / basic amino acids with polar or charged R groups, respectively (to form ionic bonds). More disulfide bonds and ionic bonds would require higher temperature to break these bonds to denature the protein.

Examiner's comment: Candidates needed to consider features of proteins that are likely to make them more thermostable. This proved challenging for many.

QUESTION 2

Fig.2.1 shows the molecular structure of maltose, which is formed from two glucose molecules.

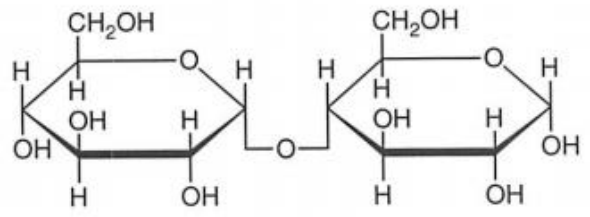


Fig. 2.1

(a) Give the full name of the bond holding the two glucose molecules together in the way shown. [2]

1 α(1→4) glycosidic bond ;

Examiner's comment: The majority of candidates were able to correctly give the full name of the bond shown in Fig.2.1.

(b) Describe how this bond may be broken to release two molecules of glucose. [2]

1 Hydrolysis reaction with the addition of one water molecule.
2 Bond broken between –OH group on carbon atom 1 of one α-glucose molecule and carbon atom 4 of **adjacent** α-glucose molecule ;

Examiner's comment: Most candidates identified the type of reaction responsible for breaking the bond but fewer were able to describe how this could be brought about. A number of candidates incorrectly referred to amylase as the enzyme involved.

(c) Fig. 2.2 represents the molecular structure of a type of phospholipid.

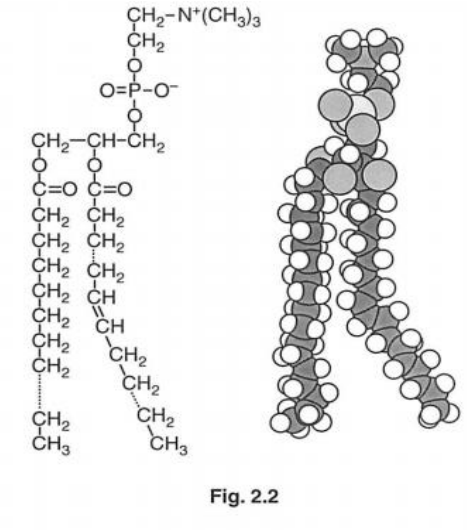


Fig. 2.2

(i) Describe the arrangement of phospholipids in cell membranes. [2]

1 The phospholipid molecules are arranged in two rows to form a bilayer structure of the cell membranes ;
2 The phosphate head of phospholipid molecules faces outwards and interacts with the external environment and aqueous medium of the cytoplasm while the hydrocarbon chains of fatty acids face the interior of the bilayer, forming the hydrophobic core of the cell membrane.

Examiner's comment: Candidates were familiar with the arrangement of phospholipids in cell membranes and many were able to provide full descriptions.

(ii) Explain how the structure of phospholipids is related to this arrangement in cell membranes. [3]

1 The phosphate head is charged (refer to **Fig 2.2**) while the two hydrocarbon chains of the fatty acids are non-polar ;
2 The phosphate heads interact with the water molecules in the aqueous media (e.g. through the formation of hydrogen bonds) ;
3 The non-polar hydrocarbon chains of fatty acids form hydrophobic interactions with each other ;

4 The presence of a carbon-carbon double bond causes a kink in one of the hydrocarbon chains ;
5 This prevents the phospholipid molecules from packing too close to each other which will reduce the fluidity of the cell membrane.

Examiner's comment: Most candidates were able to relate the structure of phospholipids to their particular arrangement in cell membranes.

[Total:9]

QUESTION 3

(a) Describe how bacterial chromosomes differ from eukaryotic chromosomes in terms of structure and organisation. [4]

	Bacterial genome	Eukaryotic genome
Points specified in syllabus		
1. Genome size / Amount of DNA	Smaller genomes, 0.6 to 10Mb./ less total DNA per cell	Large genomes, being less than 10 Mb – 100,000 Mb / more total DNA per cell, about 1000 times more DNA.
2. Gene length	Shorter gene sequences / more compact genetic organisation	Longer gene sequences / presence of more intergenic spaces
3. Chromosome structure	Circular DNA molecule which is closed covalently	Linear chromosomes with 2 ends
4. Packing of DNA	Does not form chromatin; DNA does not package into nucleosomes	Eukaryotic DNA is complexed with histones and other proteins to form chromatin; DNA is packaged into nucleosomes
5. Introns	Coding sequence proceeds from start to finish without interruption by introns (no introns)	Presence of introns within genes.
6. Regulatory sequences	Simple regulatory sequences such as promoters	More complex regulatory sequences such as enhancers and silencers
Points not specified in syllabus		
7. Chromosome number	Single chromosome / Haploid	Many chromosomes / Diploid or polyploid
8. Presence and absence of operons	Two or more genes may be expressed and regulated as a unit (genomes arranged in operons).	Absence of operons.
9. Repetitive sequences	Few repetitive DNA sequences.	Many repetitive DNA sequences
10. Coding and non-coding DNA	Most of DNA are coding sequences (codes for protein, tRNA, or rRNA.	Most of DNA are non-coding.
11. Origins of replication	One origin of replication present	Many origins of replication present
12. Presence of extrachromosomal DNA	Independent small, double stranded, circular DNA called plasmids	Circular, double-stranded DNA in mitochondria / chloroplasts.
13. Telomeres	Absent	Present

(b) Describe what occurs from the end of stage 3 up to stage 4, as shown in Fig. 3.1. [4]

1 One strand of the F plasmid is nicked by an (plasmid-encoded) endonuclease at the origin of transfer, and begins unwinding from another strand;
2 5' end of the nicked strand is transferred through the sex pilus/cytoplasmic mating bridge;
3 RNA primers then bind to the nicked strand (being transferred over to the F- cell) which allows DNA polymerase to form a complementary DNA strand;
4 DNA polymerase adds complementary deoxyribonucleotides to the 3' OH end of the nicked strand, extending the 3' end as the growing point rolls around the circular template via the rolling circle mechanism

(c) Explain the role of F plasmid. [3]

1 It carries a F factor/fertility factor which consists of genes that code for proteins
2 that are needed for replication of the plasmid, and
3 required for the production of sex pilus on the surface of donor bacterium cell which enable the donor cell to transfer DNA to the recipient bacterium cell.

Examiner's comment: Some candidates did not note the requirement of the question to consider the role of F plasmids, and outlined the generic roles of plasmids or specific roles not necessarily related to F plasmids.

QUESTION 4

(a) Suggest the reason for the peak in the rate of DNA synthesis shown in Fig. 4.1. [2]

1 Amplification of ribosomal RNA genes (rRNA) ;
2 Allows the synthesis of many copies of rRNA genes, each serve as a template for the subsequent rapid transcription / synthesis of rRNA ;

(b) Describe and explain the pattern of transcription visible on the part of the DNA coding for ribosomal RNA, labelled X in Fig.4.2 [2]

1 Due to simultaneous transcription of rRNA gene by multiple RNA polymerases, causing RNA transcripts to extend perpendicularly from DNA template strand ;
2 Shorter RNA transcripts seen at the beginning of the DNA template strand, which get longer till the end of the transcription unit, where the transcripts detach from the DNA template after transcription termination ;

(c) Explain why such large amounts of ribosomal RNA are required in frog egg cells. [2]

1 During the early development of a fertilised frog egg, rapid cell division occurs and high levels of protein synthesis (e.g. for the synthesis of DNA replication enzymes, etc) is required ;
2 Large amounts of rRNA allows the rapid assembly of many ribosomes which are sites for the translation of stored mRNAs in the frog eggs ;

(d) Suggest possible roles for the non-transcribed DNA that is found between the ribosomal genes. [2]

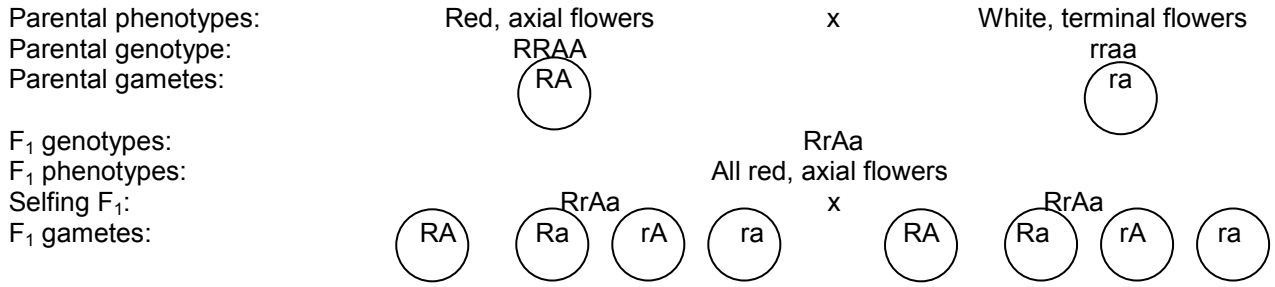
1 Contains promoter sequences which RNA polymerases recognise and bind to for transcription of rRNA genes ;
2 Contains regulatory sequences (e.g. binding sites for specific transcription factors) that control the transcription of rRNA genes ;
3 Contains terminator sequences which signal the termination of transcription of rRNA genes ;

(e) Explain how operons allow for rapid response by bacteria to environmental change. [2]

1 Functionally related genes located adjacent to one another in the genome (structural genes) are transcribed together (under the control of a single promoter and operator) into a polycistronic mRNA ;
2 Allows the rapid switching on and switching off of genes which perform related functions ;
3 Allows the prompt synthesis of proteins or termination of protein synthesis to facilitate the rapid response by bacteria to environmental changes ;

QUESTION 5

(a) Draw a genetic diagram to explain both crosses. [5]



Punnett square:

	RA	Ra	rA	ra
RA	RRAA Red, axial flowers	RRAa Red, axial flowers	RrAA Red, axial flowers	RrAa Red, axial flowers
Ra	RRAa Red, axial flowers	RRaa Red, terminal flowers	RrAa Red, axial flowers	Rraa Red, terminal flowers
rA	RrAA Red, axial flowers	RrAa Red, axial flowers	rrAA White, axial flowers	rrAa White, axial flowers
ra	RrAa Red, axial flowers	Rraa Red, terminal flowers	rrAa White, axial flowers	rraa White, terminal flowers

F_2 phenotypes:	Red, axial flowers	Red, terminal flowers	White, axial flowers	White, terminal flowers
F_2 phenotypic ratio:	9 :	3 :	3 :	1
F2 observed numbers:	261	86	76	28

1 Parental genotypes ;
2 F1 genotypes ;
3 F1 gametes (circled) ;
4 F2 genotypes ;
5 Correspond genotypes to phenotypes ;
6 Expected ratio of phenotypes – 9:3:3:1 ;

(b) Explain how different characteristics can be inherited independently in dihybrid inheritance. [2]

1 2 genes (encoding for flower colour and arrangement on stems) are found on different pairs of homologous chromosomes / are unlinked genes ;
2 Arrangement of the alleles of one gene pair at equatorial plate during metaphase I and II is independent of the alleles of the other gene pair ;
3 Subsequent segregation of alleles of one gene pair is independent of the alleles of the other gene pair during anaphase I and II ;
(allows the production of gametes with different combinations of alleles)

(c) Explain why heterozygous plants for this gene, Tt, have the same phenotype as homozygous dominant plants, TT. [3]

1 Dominant allele T encodes for a functional enzyme for the synthesis of gibberellic acid while the recessive allele t encodes for a non-functional enzyme;

<u>In homozygous plants, TT</u>
2 Contains 2 copies of the dominant allele, allowing the synthesis of gibberellic acid from inactive substrate ;

<u>In heterozygous plants , Tt</u>
3 Presence of 1 dominant allele masks the effect of the recessive allele (t), allows the expression of functional enzyme for minute amounts of gibberellic acid to be synthesised, giving rise to tall plants ;

QUESTION 6

(a) Name the structures labelled **A**, **B**, **C** and **D** on Fig. 6.1. **[4]**

1 A – intergranal lamella

2 B – chloroplast's plasma membrane / chloroplast's membrane

3 C – stroma

4 D – granum / stack of thylakoids

(b) **(i)** Suggest **two** advantages of these large protein complexes being held in the membranes of structures **A** and **D** in Fig. 6.1. **[2]**

1 (These large protein complexes) are arranged adjacent to and in close proximity to each other in the membrane to allow electrons to be transferred sequentially from the photosystem II to photosystem I in non-cyclic photophosphorylation and between electron carriers such as cytochrome.

2 Such membrane bound and close-knit association of protein complexes compartmentalised reactions e.g. proton gradient generated inside the thylakoid lumen allowing protons to diffuse back into the stroma of chloroplast through the ATP synthase to produce ATP via chemiosmosis and also reduces interferences from other reactions to a minimum.

(ii) Outline the role of the ATP synthase that is held in these membranes. **[2]**

1 (ATP synthase) is an enzyme that catalyses the formation of ATP from ADP and inorganic phosphate (P_i) with the coupling exergonic flow of proton (H^+)

2 as protons move down the concentration gradient via facilitated diffusion.

(c) Describe the role of NADP in linking the light dependent reactions to the Calvin cycle. **[2]**

1 $NADP^+$ is reduced to NADPH at the end of the non-cyclic photophosphorylation by H^+ ions from the stroma and the electrons from photosystem I.

2 NADPH is required to reduce an intermediate, 3-phosphoglycerate (PGA), in Calvin cycle to 3-phosphoglyceraldehyde (PGAL).

QUESTION 7

7 Fig. 7.1 shows the reproductive cycle of the influenza virus.

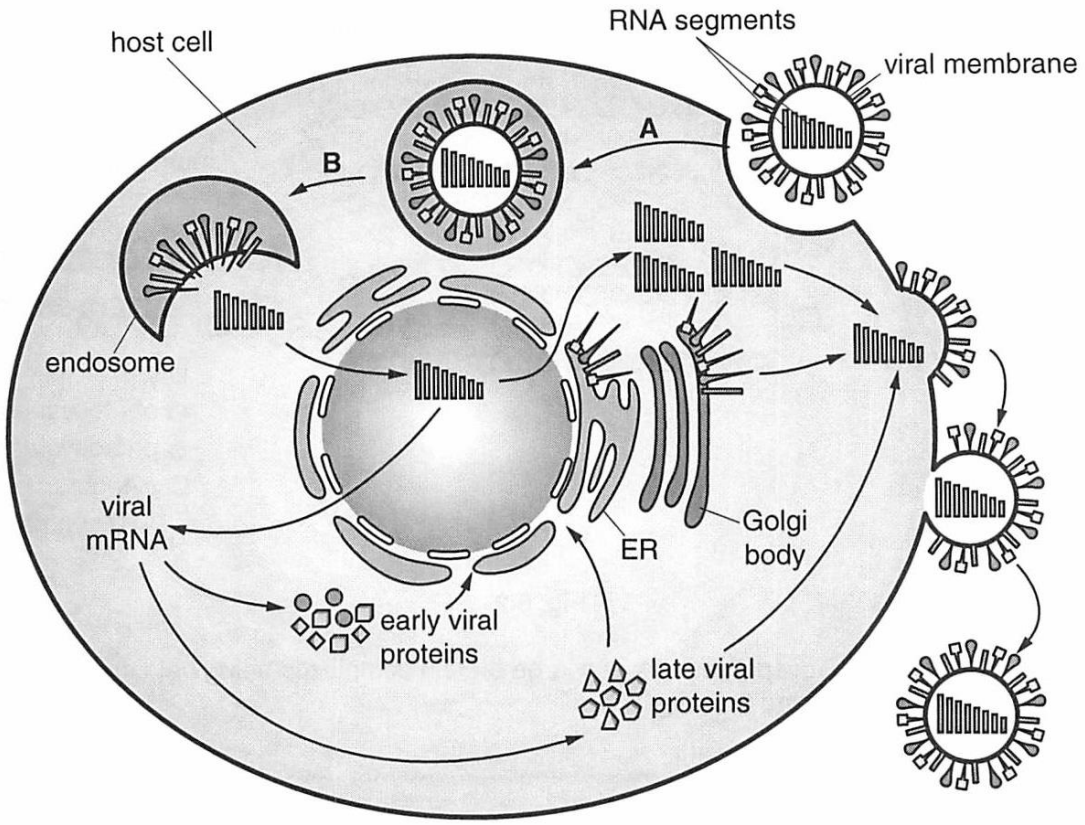


Fig. 7.1

(a) Describe the events occurring in A and B in Fig. 7.1. [4]

(A) Adsorption and entry

1 Haemagglutinin on the viral envelope recognizes and binds to sialic acid (receptors) on host cell

2 Virus enters host cell by receptor-mediated **endocytosis;** host cell cytoplasmic membrane invaginates and pinches off, placing the virus in an endosome (endocytic vesicle).

(B) Uncoating

1 Virus **uncoats** by fusing its envelope with the membrane of the endosome

2 **Viral capsid** is then **enzymatically removed** and the 8 segments of the viral RNA genome is **released**.

(b) Explain why it is necessary for the viral RNA to enter the host cell nucleus. [3]

1 Free RNA ribonucleotides are present in the nucleus for viral (-) sense RNA to be transcribed to (+) sense RNA, with the help of viral RNA-dependent RNA polymerase
2 (+)sense RNA is used as template to synthesize more viral (-) sense RNA genome
3 Viral (-) sense RNA, capsomere and other viral proteins will assemble into new nucleocapsids in the nucleus

(c) Suggest in outline how new strains of influenza virus may arise. [3]

1 During antigenic shift, virus is able to reassort RNA segments ;
2 Occurs when two different strains of virus infect the same cell;
3 Combination of **antigen** (haemagglutinin and neuraminidase) encoded is changed (resulting in a new strain);

QUESTION 8

(a) Suggest why different species of picture-wing fly show different banding. [3]

1 The chromosome structure / arrangement of genes in chromosome between species are different
2 The genes and DNA sequences between species are also different due to mutations
3 Dark banding may correspond to heterochromatin which are highly compacted thus transcriptionally inactive, whereas light banding may correspond to euchromatin which are less compacted and thus transcriptionally active.

(b) Suggest why different species of picture-wing fly evolved on different islands. [4]

1 The sea separating the islands led to geographical isolation, preventing gene flow between different populations of picture-wing fly. This led to reproductive isolation between the different populations.
2 Genetic variations exist among the picture-wing fly. Different selection pressures are present on the different islands
3 Individuals with a selective in a particular environment survived till reproductive age, pass on their alleles to their offsprings, leading to changes in allele frequency within gene pool of each population
4 Accumulation of genetic differences occur over time, and the different populations were cannot interbreed to produce viable, fertile offspring. New species are formed. Ref. allopatric speciation (due to geographical isolation)

(c) Explain the differences between classification and phylogeny. [3]

1 Classification is the organisation of species according to particular characteristics.
2 Classification may not take into consideration evolutionary relationship between the species.
3 Phylogeny is the organisation of species according to particular characteristics which takes into consideration the evolutionary relationship between the species.

QUESTION 9

(a) Describe the main properties and characteristics of an action potential in a neurone. [6]

1 **All-or-nothing principle**, where a given stimulus either triggers a typical action potential or does not produce one at all;
2 If the neurone reaches threshold potential of -50mV, membrane potential increases to +40mV. If not, membrane potential will return to resting potential of -70mV;

3 **Refractory period**, is the period from the time an action potential begins until the normal resting potential has been restored, when the membrane will not respond normally to additional depolarizing stimuli;
4 Absolute refractory period is when no stimulus can initiate another action potential despite its strength, and relative refractory period is when a stronger than usual stimulus can initiate new action potentials;

5 **Regenerative**, where the action potential brings an adjacent region to threshold, which in turn does so for its adjacent downstream region and so on,
6 propagating in a similar form continuously in one direction down the axon to the terminal of the neurone, with the amplitude remaining constant;

Examiner's comment: Candidates often did not adhere to the question asked but instead provided wide-ranging descriptions of nerve impulses. Effective responses targeted the requirements of the question to clearly identify salient points.

(b) Explain the importance of K^+ ions in impulse transmission. [8]

1 At resting potential, sodium-potassium pump transports 2 K^+ ions across the neurone membrane into the neurone, and 3 Na^+ out of the neurone;
2 Also, the presence of more ungated K^+ ion channels than ungated Na^+ ion channel allows more K^+ ions to diffuse out than Na^+ ions diffusing in;
3 These result in a net loss of positive charges from the neurone, causing the inside of neurone to be negative relative to the outside,
4 so that a concentration gradient is established which would allow K+ to diffuse out of the cell and Na+ to diffuse in if the ions could cross the membrane, allowing neurone to respond to a stimulus;

5 During repolarisation phase, all the voltage-gated K^+ ion channels are opened, resulting in an increase in diffusion of K^+ ions out of the neurone,
6 Allowing membrane potential to decrease from +40mV to -70mV, back to resting potential for the generation of the next action potential;

7 During hyperpolarisation phase, voltage-gated K^+ ion channels remain open even when resting potential is reached, resulting in membrane potential more negative than resting potential;
8 This results in refractory period where the neurone is unable to respond to another stimulus, ensuring impulse transmission in one direction;

(c) Outline the effect of the myelination of neurones. [6]

1 Myelination of neurones is due to the wrapping of Schwann cells around the axons (forming myelin sheath);
2 Small unmyelinated gaps between adjacent Schwann cells known as the node of Ranvier, contain high concentration of voltage-gated ion channels;
3 Hence, these are the only regions where the influx of Na^+ ions and efflux of K^+ ions occur and generate action potentials during propagation of an impulse along the myelinated axon ;
4 Lateral diffusion of Na^+ to adjacent areas of the axon results in depolarisation of membrane at the next node of Ranvier;
5 Action potential is repeatedly generated at each node of Ranvier, hence appears to "jump" from one node of Ranvier;
6 This form of propagation called saltatory conduction results in faster impulse transmission;

QUESTION 10

(a) Describe the structure and role of tRNA. [7]

1 tRNA is a polynucleotide of about 80 ribonucleotides linked by phosphodiester bonds where each ribonucleotide contains the ribose sugar, phosphate group and nitrogenous base – adenine, cytosine, guanine or uracil ;
2 tRNA is a single-stranded molecule that folds back upon itself to form a clover-leaf structure formed by complementary base-pairing via hydrogen bonds ;
3 During translation, each amino acid is brought to the ribosome attached to the tRNA ;
4 3'end of tRNA has a 5'-CCA-3' sequence which is the attachment site for an amino acid, whereas the 5'end of the tRNA always ends with guanine ;
5 tRNA has shape complementary to amino acid tRNA synthetase for activation of amino acid (at least 20 different tRNAs – one for each amino acid) ;
6 Sequence of tRNA consists of anticodons which are complementary to the codons on mRNA / the complementary base-pairing between the codons of the mRNA and anticodons of the tRNAs ;
7 For ensuring the correct position and sequence of amino acids on elongating polypeptide chain / allow for the specific amino acid sequence specified by the coding sequence of the mRNA which in turn is determined by the gene sequence.

Examiner's comment: Many candidates gave comprehensive descriptions of the structure and role of tRNA. Some did not consider the specificity of the attachment between different tRNA molecules and amino acids.

(b) Explain how gene mutations may affect the protein coded for by a gene. [7]

1 Gene mutations are a result of a change in the nucleotide sequence of the DNA molecule in a particular region of the chromosome which in turn affects the nucleotide sequence in the mRNA after transcription of the gene ;
2 As the genetic information on the mRNA is read in triplet of bases known as codons, a change in the codon sequence may result in a change in the amino acid sequence / primary structure of the polypeptide chain after translation ;
3 This may in turn lead to the different folding of the polypeptide which may affect its biological function ;

4 Can be due to base pair substitution mutation where one base pair is replaced with another pair in the gene sequence ;
5 This point mutation may result in an altered codon that may encode a different, dissimilar amino acid which drastically affects the structure and properties of the protein ;

OR this point mutation may result in an altered codon that may encode the same amino acid being encoded due to the degeneracy of the genetic code, hence no effect on the structure and properties of protein formed ;

OR this point mutation may result in an altered codon that encode a different amino acid but with similar properties as the original amino acid / a different amino acid found in a region of the protein where the exact sequence of amino acids is not essential to the protein's function ;
OR this point mutation could result in a stop codon (i.e. UAA, UAG, UGA) being encoded, the premature termination of translation forms a truncated protein which most likely would be non-functional.

6 Can be due to base pair insertion or deletion mutation where there is insertion or deletion of one or more base pairs to/from the gene sequence which could drastically affect the reading frame of the genetic information in the mRNA (i.e. frameshift mutation) ;
7 This insertion or deletion mutation could result in drastic change in the amino acid sequence of the polypeptide would adversely affect the biological function of the protein
OR This insertion or deletion mutation could result in a stop codon being encoded (i.e. UAA, UAG, UGA) where the premature termination of translation forms a truncated protein which most likely would be non-functional.

Examiner's comment: The majority of candidates were able to provide comprehensive explanations of how gene mutations may affect the encoded protein.

(c) For a named genetic disease, describe the causal mutation and outline its effect on the phenotype of an organism. [6]

Sickle Cell Anaemia

1 Sickle Cell Anaemia is an inherited homozygous recessive disease of the β-chain of haemoglobin in the red blood cell ;

Causal Mutation

2 The disease is caused by the thymine at the 17th nucleotide of the gene being replaced by an adenine nucleotide ;
3 As such the amino acid glutamic acid at the 6th position is substituted by valine, glutamic acid is hydrophilic whereas valine is hydrophobic, thus due to the different R groups, the tertiary structure of the haemoglobin molecule changes ;
4 At low oxygen concentration, the solubility of deoxygenated HbS decreases, HbS molecules stick to each other via their hydrophobic regions ;
5 HbS molecules polymerise into long fibres inside the red blood cells, deforming the red blood cells into sickle shape ;

Effect on the Phenotype of an Organism

6 Sickle-shaped red blood cells are ineffective in transporting oxygen gas

OR Sickle-shaped red blood cells clump and clog small capillaries, obstructing other cells from moving through the capillaries, leading to other symptoms such as physical weakness, pain or organ damage

OR Sickled-shaped red blood cells have a shorter lifespan compared to normal cells and haemolyse readily in anaemia.

Cystic fibrosis

1 Cystic fibrosis is an inherited autosomal recessive disease of the mucous gland.

Causal mutation

2 The disease is caused by deletion of 3 base pairs in the cystic fibrosis transmembrane conductance regulator gene (CFTR gene) on chromosome 7 which codes for the CFTR protein ;

3 Leading to the loss of the amino acid phenylalanine, resulting in the CFTR protein missing phenylalanine at position 508, this changes the primary structure of the protein, therefore modifying the tertiary/3-D structure ;

4 CFTR is a cAMP-regulated chloride channel important in transporting Cl^- ions **out** of epithelial cells of the airways, lungs, gut and reproductive system, non-functional CTFR channels are unable to transport Cl^- ions out of epithelial cells ;

5 This causes accumulation of Na^+ ions in the cell to neutralise Cl^- ions, resulting in high ionic concentration (i.e. water potential in cell drops), water is drawn into the cell / cannot leave the cell ;

Effect on the Phenotype of an Organism

6 Thick mucus forms on the epithelial surface and blocks passageways (e.g. airways of lungs, pancreatic duct, bile duct, etc.), this thick and sticky mucus will lead to trapping of bacteria that leads to inflammation and infection of lungs and poor rate of gaseous exchange

OR Blockage in pancreas, inadequate secretion of pancreatic enzymes and malnutrition due to inability to absorb essential nutrients

OR Blocked sperm ducts & fallopian tubes (infertility) ;

7 Overall life expectancy of individuals will be reduced considerably due to inflammation of lungs, bronchitis, pneumonia, inability to digest food, malnutrition and development of diabetes etc.

Examiner's comment: Most candidates considered either sickle cell anaemia or cystic fibrosis and were able to provide detailed descriptions of the causal mutation and its effect on the phenotype.

[Total: 20]

PAPER 3 (APPLICATIONS)

QUESTION 1

Scientists are continually studying new techniques for studying various genetic diseases. One approach is to transplant 'healthy 'stem cells into the affected person.

(a)(i) Name one genetic disease which has been treated successfully by stem cell transplantation. [1]

1 Leukemia/ Cancer of the white blood cells

(a)(ii) Explain why stem cells are suitable for this process. [3]

1 blood stem cells are undifferentiated / unspecialized cells;
2 multipotent, no longer totipotent and pluripotent;
able to differentiate into specialized blood cell types / give rise to a limited range of cell type / produce only cells of a specific lineage
e.g. white blood cells

3 capable of self-renewal / dividing rapidly by mitosis;
ref. asymmetric division to generate continuous supply of white blood cells for long-term treatment;

Examiner's comments:
Most candidates recognised properties of stem cells making them suitable for establishment following transplantation. Fewer candidates identified features that would allow them to treat genetic diseases.

(b) Some genetic diseases cannot be treated using stem cells. Therefore, scientists are developing vectors to introduce 'normal' genes into cells that are malfunctioning.

A wide range of vectors have been experimented with, but viral vectors may offer the best rate of success.

Fig 1.1 shows a lentiviral vector, which can bind to cells lining the airways of the lungs. The lentivirus is a form of retrovirus.

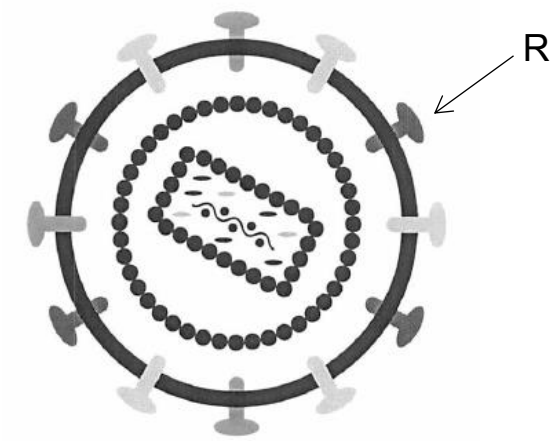


Fig. 1.1

(i) Using the letter R, label Fig 1.1 to identify a feature that allows the virus to bind to cells. [1]

(ii) With reference to your knowledge on retroviruses, explain how expression of an inserted gene (transgene) is brought about following infection of host cells with the lentiviral vector. [3]

1 Viral RNA genome is reverse transcribed into cDNA using viral reverse transcriptase
2 the resulting cDNA is then integrated into host cell DNA using viral integrase
3 cDNA of transgene is expressed using host cell machinery e.g. RNA polymerase and host ribsomes

Examiner's comments:
Most candidates were able to provide detailed responses. Many went unnecessarily beyond the requirements of the question. Not all candidates were aware of the nature of the nucleic acids in a retrovirus or knew the enzyme that incorporates the viral DNA into the host genome.

(iii) This lentiviral vector infects both dividing and non-dividing cells, shows long – term stable expression and only causes a low immune response.

Explain why these features could allow lentivirus to become effective vectors for gene therapy. [3]

1 Infects both dividing and non-dividing cells:
the vector can infect both undifferentiating stem cells as well as the non-dividing specialized cells, thus increasing the choice of target cells used for gene therapy;;

2 Long term stable expression:
cDNA of transgene is integrated into host DNA and reduced the chance of breaking down; thus it can be expressed to form functional proteins over a longer period of time;
In dividing cells, the cDNA of transgene is replicated when the cells divide and each daughter cell will contain the transgene, improving the efficiency of gene therapy

3 Low immune response:
prevents vector from being neutralized by immune system and increases the chance of the vector infector the target cells;
It also prevents severe side effects arising due to the immune response;

4 Overall, there is no need for numerous repeated treatments

Examiner's comments:
There was a tendency for candidates to focus on the meaning of the properties listed in the question, rather than to explain how these features could allow lentiviruses to become effective vectors for gene therapy.

(iv) Describe three ways in which the treatment and management of genetic diseases has benefitted from the human genome project. [3]

1 Detection of genes associated with genetic diseases:

Studying the onset and progression of the disease enables scientists to develop drugs to treat the disease effectively;;

2 Pharmacogenetics:

Allows the creation of drugs based on molecular information / allows the design of "custom drugs" (pharmacogenomics) based on individual genetic profiles;;

3 Genetic testing:

Improve diagnosis of disease by detecting the diseased allele in carriers and in pre-natal testing (testing for diseased allele in the fetus);

We can also detect genetic predispositions to the genetic disease; allows the identification of gene variants that are important for the maintenance of health, particularly in the presence of known environmental risk factors;

Examiner's comments:
The most effective responses identified specific outcomes of the human genome project that have improved the treatment and management of genetic diseases, including carrier screening and prenatal testing. Many candidates instead outlined general benefits of the human genome project.

[Total: 14]

QUESTION 2

(a) (i) Suggest the level of protection (number of ✓) against CE that you would expect for corn Z and explain your answer.

level of protection..... **[2]**

1 level of protection: ✓✓✓

2 V corn containing Bt protein, Cry1a, has a ✓ for protection against CE while X corn containing Bt proteins, Cry1c and Cry1f has ✓✓.
3 Since Z corn contains Bt proteins, Cry1a, Cry1c and Cry1f, the level of protection against CE must be a summation of that for V corn and X corn.

OR

2 The level of protection by Cry1a and that by Cry1b is a ✓ each, deduced from the data given for V corn containing Bt protein, Cry1a, and W corn containing Cry1a and Cry1b. Corn Y containing Cry1a, Cry1b and Cry1f has 4 ✓ for protection against CE. Based on the above, it can be concluded that Cry1f confers 2✓ for protection against CE.
3 As corn X with Cry1c and Cry1f has 2 ✓ for protection, Cry1c must be ineffective and give zero protection against CE. Hence, corn Z with Cry1a, Cry1c and Cry1f must only have 3 ✓ protection.

(ii) Using the information in Table 2.1, state which of the Bt proteins acting individually would provide most protection against CB. **[1]**

1 Cry1a and Cry1c (Bt proteins would provide most protection against CB).

(b) Suggest why none of the individual Bt proteins in Table 2.1 can provide total protection against CB. **[2]**

1 Development of resistance to Bt proteins within populations of corn borers (CB) such that the gene, coding for the enzymes in the insect's digestive tract that convert Bt proteins into an insect-specific toxin causing paralysis and death to the CB, is mutated.
2 All CBs are resistant to Cry1b while Cry1a and Cry1c confer 2 ✓ protection against CB and Cry1f confers 1 ✓ protection.

(c) (i) Suggest why the introduction of genetically modified crops has resulted in an increase in the use of herbicides. **[2]**

1 Less insects among the genetically modified crops would means that weeds are able to grow freely without being eaten by insects.
2 Hence, farmers would need to use more herbicides to kill the weeds without harming their crops which are also made herbicide resistant beside being made protective against insects using Bt proteins.

Examiner's comment: Some candidates appreciated that herbicide resistant crops are designed to be used in association with the herbicide to which they are resistant. Many were not aware of the rationale for the use of herbicide resistant crops and incorrectly considered the possibility of interspecific gene transfer and the development of 'superweeds'.

(d) **(ii)** Describe **three** ethical issues related to an increase in the use of herbicide-tolerant corn. **[3]**

Choose any three:

1 <u>Superweeds</u>

Seeds from GM crops might be carried to other places and establish themselves as weeds

Cross-pollination between the GM crops and their wild relatives may spread the resistance to weeds

Emergence of vigorous weeds with herbicide-resistant genes

2 <u>Human health and safety</u>

Introduction of foreign gene may result in production of secondary metabolites which may be toxic to animals themselves and/or livestock/humans that consume them.

New proteins in GM plants may be potentially allergenic to humans that consume them.

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.

3 <u>Meddling with biological processes [Ethical]</u>

Some groups or individuals see the generation and use of GMO as intolerable meddling with biological states or processes that have naturally evolved over long periods of time

4 <u>Religious issues on GM foods [Ethical]</u>

religious implications in food choice;

especially when GM foods are unlabelled;

Eg. incorporation of pig genes into plants;

Examiner's comment: Most candidates were able to identify a number of ethical issues related to the use of herbicide-tolerant corn. Some candidates made vague references to effects on biodiversity or the environment without explanation.

QUESTION 3

Many of the current advances in biochemistry are due to the use of the polymerase chain reaction (PCR) and gel electrophoresis.

Whilst PCR is able to amplify small quantities of DNA for use in other techniques, the process is not perfect.

(a) Describe the limitations of PCR. [3]

…… [3]

1. Limited size range of DNA sequences (0.1 – 5 kb) cloned by PCR
2. above this length the polymerase tends to fall off
   / the typical heating cycle does not leave enough time for polymerisation to complete

3. High error rate / Low replication fidelity
4. due to lack of proofreading by Taq DNA polymerase
   / Taq polymerase lacks a 3' to 5' exonuclease activity

5. Non-specific binding of primers (leads to non-specific amplification of sequences)
6. due to sequence duplications / partial primer binding

7. Prior sequence information is usually necessary to construct the primers
8. for primers to bind to complementary sequences flanking the target sequence

9. Limited amounts of actual yields of PCR products
10. compared to the amount that can be obtained using cell-based cloning where scale-up of the volumes of cell cultures is possible

Examiner's comments

(a) Most candidates recognized a range of limitations of PCR and were able to describe these accurately. Not all descriptions were sufficiently detailed. For example, many candidates identified the problem of contamination without describing the nature of this contamination.

(b) In order for DNA separation by gel electrophoresis to be successful, a certain protocol has to be followed.

(i) **Describe the role of the buffer solution in the gel electrophoresis protocol.**

…… [2]

1. Provide ions to carry current from negative to positive electrode.
2. Allows negatively charged DNA fragments to move towards the positive electrode when an electric current is applied to the electrophoresis chamber.

Examiner's comments

(b) (i) The majority of candidates were able to describe the role of the buffer in gel electrophoresis and some made correct links to the relevant properties of DNA. A number of candidates included unnecessary detail concerning the migration of DNA fragments.

(ii) Describe the role of the loading or tracking dye in the gel electrophoresis protocol. [3]

…… [3]

1. DNA is low in density and hence will not sink into the well.
2. Glycerol in the loading dye is very dense, hence will help to hold the samples in the well, preventing them from floating up and out of the well.
3. Tracking dye allows visual monitoring of how far the electrophoresis has proceeded.

Examiner's comments

(b) (ii) Many candidates were able to provide full responses but some only considered the role of the tracking dye as a marker of migration.

Ivory obtained from elephants, including the African elephant, *Loxodonta africana*, is a valuable resource in many countries.

African elephants are listed as an endangered species by CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). However, despite a ban on the trade in elephant products, ivory is still sold by poachers.

In June 2002, over 500 ivory tusks and 42 000 other ivory items were seized from a ship arriving in Singapore. Evidence seemed to link this ivory to elephants from Malawi in Africa.

(c)(i) Outline the process of genetic fingerprinting using RFLP that could be used to test this seized ivory.

…… [4]

- Extract nuclear DNA from cells of an ivory sample and cut DNA with just one restriction enzyme.
- Electrophorese the cut DNA to sort the different fragments based on their lengths.
- To visualize a fingerprint pattern for several specific STR sites, the DNA fragments corresponding to the STR is detected by DNA hybridization with several radioactive probes.
- X-ray autoradiography is then performed. The pattern of bands appearing on the resulting autoradiogram is the DNA fingerprint for that individual.

Examiner's comments

(c) (i) Most candidates were able to outline the process of genetic fingerprinting. Some provided accounts that went considerably beyond the requirement to outline the process. Others described how the process could be applied to determine the origin of the seized ivory, which was the focus of the next question. Some candidates mixed up the different methods that can be used to detect and visualize the relevant DNA fragments.

(ii) Explain how the genetic fingerprints of the seized ivory could be used to confirm that it originated from elephants in Malawi.

…… [4]

1 DNA fingerprints make use of polymorphisms that consist of repeated sequences of DNA (eg. STRs).
2 The number of repeating segments varies greatly from individual to individual.
3 Obtain genetic fingerprints of the elephants in Malawi and compare that with the genetic fingerprints of the seized ivory.

4 If the seized ivory originated from elephants in Malawi, their genetic fingerprints will be highly similar with those obtained from Malawi elephants.

Examiner's comments

(ii) Most candidates were able to provide detailed responses. Some repeated material from the previous question about the process rather than its application to confirming the origin of the seized ivory.

[Total: 16]

QUESTION 4 (SPA PLANNING)

The enzyme sucrase, which breaks down sucrose to glucose and fructose, can be obtained from a wide range of sources including potatoes, cucumber and yeast.

P and **Q** are two newly-isolated types of sucrase that have not yet been tested for their activities at a range of temperatures and pH values.

Using your own knowledge, design an experiment to investigate which of the two newly-isolated types of sucrase, **P** or **Q**, has the greatest activity when each is used at its optimal temperature and pH, and at the same concentration.

You must use:

- 2% solution of enzyme **P**,
- 2% solution of enzyme **Q**,

- distilled water,
- 5% sucrose solution,
- thermostatically-controlled water-bath and thermometer,
- Benedict's solution,
- Bunsen burner,
- timer, e.g. stopwatch or stop clock.

You may select from the following apparatus and chemicals:

- normal laboratory glassware, e.g. test-tubes, beakers, measuring cylinders, graduated pipettes, glass rods etc.,
- syringes,
- a range of buffer solutions between pH 2 and pH 9,
- pH probe and digital meter.

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 variables,
- describe the method with the scientific reasoning used to decide the method so that the results are as accurate and reliable as possible,
- show how you will record your results and the proposed layout of results tables and graphs,
- use the correct technical and scientific terms,
- include reference to safety measures to minimise any risks associated with the proposed experiment.

[Total: 12]

Theoretical considerations:

- As temperature increases up to the optimum temperature, rate of enzyme reaction increases. This is due to the increase in kinetic energy of both enzyme and substrate molecules, leading to an increase in the frequency of effective collisions between enzyme and substrate, resulting in formation of more enzyme-substrate complexes per unit time.
- As temperature increases beyond the optimum temperature, there will be an increase in thermal agitation of enzyme molecules, resulting in disruption of hydrogen bonds, ionic bonds and hydrophobic interactions. The specific 3D conformation of enzyme is disrupted and the active site altered, resulting in enzyme denaturation and decreasing the rate of enzyme reaction.
- Changes in pH affect concentration of H^+ (and OH^-) in the medium, which alters ionic charges of acidic and basic R groups of amino acid residues. This disrupts hydrogen bonds and ionic bonds that maintain the specific 3D conformation of enzyme. Enzyme work within narrow range of pH.
- Effects are reversible when pH is altered by a small extent from optimum pH, but enzymes will be denatured when pH is altered by a large extent.

Variables:

<u>Independent variable</u>

- Temperature /°C
  8 different temperatures (25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C), using thermostatically-controlled water bath, monitored using a thermometer

- pH
  8 different pH (pH 2, pH 3, pH 4, pH 5, pH 6, pH 7, pH 8, pH 9) using buffer solutions, monitored using pH probe and digital meter.

<u>Dependent variable:</u>

- Rate of reaction, measured by 1 / Time taken for appearance of brick-red precipitate of Benedict's test (1/T, s^{-1})

<u>Variables to be kept constant</u>

- Concentration and volume of enzymes P and Q used
- Concentration of sucrose solution
- Same time intervals used for sampling the reaction mixtures, using a stopwatch

Control:

- Replace enzymes P and Q with equal volume of distilled water. This is to prove that any hydrolysis of sucrose to form reducing sugars is due to the action of the enzymes and not due to other factors.

Procedure:

1. Take 8 test-tubes, labelled 1 – 8, and add 1 cm^3 of the respective pH buffer and 10 cm^3 of 5% sucrose solution.
2. Place the 8 test-tubes and a separate tube of 2% solution of enzyme P in a thermostatically-controlled water bath at 25°C for 5 min to allow the contents of each tube to equilibrate with the temperature of the water bath.
3. Use a syringe to transfer 1 cm^3 of enzyme P to Test-tube 1, mix well using a glass rod, and start the stopwatch.
4. After 30 s of incubation, remove 1 cm^3 from the reaction mixture and place in another test-tube containing 1 cm^3 of Benedict's reagent.
5. Heat the test-tube in a beaker of boiling water for 2 min.
6. Repeat this procedure every 30 s placing the samples in test tubes containing fresh samples of Benedict's reagent and then heating the tubes in the beaker of boiling water for 2 min.
7. Note the time when the first positive reducing sugar test is obtained indicated by a brick-red precipitate.
8. Perform two more replicates for Steps 1 to 7.

Table showing rate of reaction of enzyme P at 25°C at different pH

Test-tube	pH	Time taken for first appearance of brick-red precipitate / s				Rate of reaction / s^{-1}
		Replicate 1	Replicate 2	Replicate 3	Average	
1	2					
2	3					
3	4					
4	5					
5	6					
6	7					
7	8					
8	9					

9. Repeat Steps 1 to 8 at 30°C, 35°C, 40°C, 45°C, 50°C, 55°C and 60°C
10. Repeat Steps 1 to 9 using 2% solution of enzyme Q instead.
11. Repeat the entire experiment (Steps 1 to 10) thrice using fresh enzyme, sucrose and buffer solutions.

Theoretical graph with correct labels and units (1 mark)

- Rate of reaction / s^{-1} against pH at different temperatures for enzymes P and Q

Risks and Safety:

- Broken glassware is sharp and may cause cuts; handle and dispose broken pieces carefully.
- Enzymes P and Q are irritants; avoid contact with skin and eyes by wearing gloves and goggles when handling them.

QUESTION 5

(a) Describe and explain the properties of plasmids that allow them to be used as DNA cloning vectors. [7]

(b) A eukaryotic gene is isolated with blunt ends. Outline the procedures for cloning this gene in a bacterial plasmid. [6]

(c) Eukaryotic genes cannot be expressed directly in the bacterial plasmid because of differences between prokaryotes and eukaryotes, including the presence of introns.

Outline these problems and explain how they are overcome in order to allow expression of eukaryotic genes in plasmids within E.coli cells. [7]

(a) Describe and explain the properties of plasmids that allow them to be used as DNA cloning vectors. [7]

1 Entire DNA sequence is known
2 Determine specific site where cloned gene inserts / easy to manipulate plasmid

3 One origin of DNA replication
4 Enables independent replication of the plasmid and foreign gene inside the host cell.
/ Sequence of DNA recognised by certain enzymes and used in order to start the replication of DNA, resulting in multiple copies of the plasmid and foreign gene within one bacterium to be formed.

5 Multiple cloning site containing many different but unique restriction sites
6 Wide variety of restriction enzymes can be used to cut plasmid for the insertion of the foreign gene

7 Contains suitable regulatory sequences
8 eg. prokaryotic promoter if bacteria host is used, allows recognition by host cell's RNA polymerase for expression of inserted gene

9 Contains readily genetic / selectable markers which confer well-defined phenotypes on host cell.
10 Antibiotic resistance gene (e.g. amp^r) – allow for selection and identification of transformed cells
11 β-galactosidase gene (lac Z gene) – allow selection and identification of the host cells which have taken up the recombinant DNA molecules.

12 Antibiotic resistance gene
13 Allows selection and identification of transformed bacterial cells

14 Minimal amount of DNA
15 Able to accept inserts of larger sizes / Ease of manipulation as vector is resistant to shearing

16 Relaxed mode of replication (high copy number for plasmid)
17 Large amount of cloned DNA can be produced

Examiner's comments
The majority of candidates were able to provide detailed responses that identified many of the relevant properties of plasmids and how these are significant to their role as DNA cloning vectors. Such responses frequently included examples of the latest research protocols. A small number of candidates were uncertain of some of the properties, such as the timing of plasmid replication. Confusion between replication and transcription of DNA was also apparent in a more limited number of responses.

(b) A eukaryotic gene is isolated with blunt ends. Outline the procedures for cloning this gene in a bacterial plasmid. [6]

1 Linker DNA sequences containing an appropriate restriction site are ligated to both the blunt ends of the blunt ended gene
2 using DNA ligase.

[Formation of a recombinant plasmid]
3 Plasmid and gene insert are cut with same restriction enzyme
4 to **generate complementary sticky ends**.
5 Plasmid and gene insert are mixed, **anneal** with each other by forming hydrogen bonds between complementary sticky ends
6 DNA ligase added to seal the nicks by formation of phosphodiester bonds between adjacent nucleotides (on the sugar phosphate backbone)

Examiner's comments
Most candidates fully described one method of solving the problem of inserting a blunt-ended gene into a plasmid. Most also correctly identified the enzyme needed for the method chosen. A few candidates mixed up different methods within a single strategy or constructed unnecessarily complex protocols.

Many candidates exceeded the requirements of the question and went on to describe transformation and selection of recombinant colonies.

(c) Eukaryotic genes cannot be expressed directly in the bacterial plasmid because of differences between prokaryotes and eukaryotes, including the presence of introns.

Outline these problems and explain how they are overcome in order to allow expression of eukaryotic genes in plasmids within E.coli cells. [7]

Problems

1. **Presence of introns in most eukaryotic genes**

- Bacterial cells may not express the gene correctly as they do not have spliceosomes.
- Eukaryotic introns will be used in translation, causing the wrong amino acids to be incorporated into the protein or presence of stop codon in intron leads to premature termination of translation

2. **Different promoters and other DNA control sequences.**

- Bacterial cells may not be able to recognize eukaryotic promoter sequences and other control elements.

3. **Lack of post-translational modification**

- Protein may not necessarily be functional in bacterial cells as bacteria do not have rough endoplasmic reticulum or Golgi apparatus; and may not be able to attach the sugars or lipids correctly to the protein.

4. **Difficulty in separating the desired protein from the others made by the bacteria.**

- Purification process is time-consuming and expensive, but comparatively easier than isolating proteins from tissues of animals (e.g. insulin from pig pancreases)

Solutions

5. **Cloning the cDNA of the gene.**

- cDNA (synthesized from a mature mRNA template via reverse transcriptase) is used to produce the recombinant plasmid.
- Introns are absent, hence there is no need for splicing.

6. **Use of an expression vector to overcome differences in promoters and other control sequences**

- Bacteria can express any eukaryotic cDNA gene if the vector contains a prokaryotic promoter and any other control elements necessary for the gene's transcription and translation.
- The bacterial host recognizes this promoter and can express any foreign gene linked to that promoter.

7. **Targeting proteins for secretion outside cell by bacteria**

- Use of bacterial signal sequences to target proteins for secretion outside cell by bacteria will help to simplify purification process

Examiner's comments

The majority of candidates developed detailed responses that showed sound understanding of the problems of expressing eukaryotic genes in prokaryotes and approaches to overcome these. The question already alerted candidates to the occurrence of introns in eukaryotic genes, which provided a strong starting point for many responses.

Some candidates illustrated their responses with particular examples, including insulin and human growth hormone. While such examples can be useful, care must be taken to ensure that only those aspects relevant to the overall answer are selected. Full accounts of particular examples inevitably include much detail not needed to address the broader question.