

2011 'A' Level  
**H2 Biology**  
Mark Scheme

**PAPER 1 (MCQ)**

1	<b>D</b>
2	<b>B</b>
3	<b>B</b>
4	<b>C</b>
5	<b>C</b>

21	<b>B</b>
22	<b>D</b>
23	<b>B</b>
24	<b>B</b>
25	<b>A</b>

6	<b>B</b>
7	<b>A</b>
8	<b>D</b>
9	<b>A</b>
10	<b>A</b>

26	<b>C</b>
27	<b>D</b>
28	<b>B</b>
29	<b>D</b>
30	<b>C</b>

11	<b>A</b>
12	<b>B</b>
13	<b>A</b>
14	<b>B</b>
15	<b>A</b>

31	<b>A</b>
32	<b>D</b>
33	<b>C</b>
34	<b>C</b>
35	<b>C</b>

16	<b>D</b>
17	<b>D</b>
18	<b>C</b>
19	<b>B</b>
20	<b>C</b>

36	<b>D</b>
37	<b>A</b>
38	<b>A</b>
39	<b>D</b>
40	<b>B</b>

## PAPER 2 (CORE)

### QUESTION 1

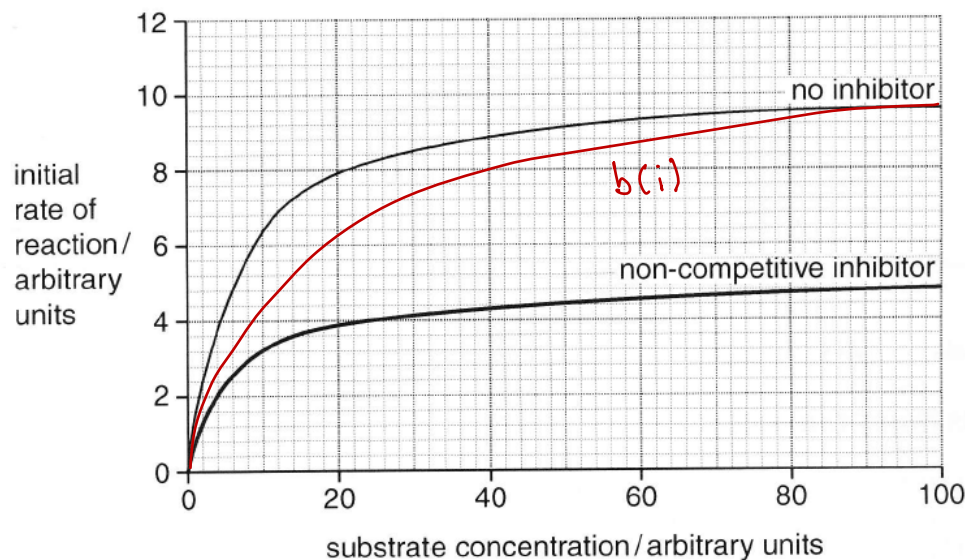
#### (a)

- 1 Rate of reaction **increases rapidly** from 0 to 4 a.u. at **low** substrate concentration of 0 to 4 a.u.
- 2 Rate of reaction slows down as substrate concentration increases and eventually level-off to a **plateau** of 9.6 a.u. at a **high** substrate concentration of 80 a.u.

**Examiner's comment:** this required students to mention both the rapid initial increase followed by a levelling off to a plateau as well as quoting figures from both low and high substrate concentrations.

#### (b)

##### (i)



##### (ii)

- 1 a competitive inhibitor has a close structural resemblance to the substrate, and therefore competes with the substrate for the same active site of enzyme
- 2 inhibitor binds to the active site, rate of reaction decreases
- 3 binding of the inhibitor to the active site is temporary and not permanent. At high substrate concentration, effect of inhibition is negligible / increasing the substrate concentration can decrease the effect of inhibition.

#### (c)

- 1 toxin permanently binds to the active site of the enzyme and prevents hydrolysis of acetylcholine
- 2 acetylcholine accumulates and remains bound to receptors on the post-synaptic membrane
- 3 ligand-gated sodium ion channels would stay open, leading to repeated action potentials

**QUESTION 2****(a)**

- A – (large ribosomal subunit ) ribosome
- B – polypeptide
- C – transfer RNA (tRNA)
- D – messenger RNA (mRNA)

**(b)**

- 1 Attachment of an amino acid to its corresponding tRNA with specific anticodon by aminoacyl tRNA synthetases
- 2 which have an active site with 3D conformation complementary to the 3D conformations of the amino acid **and** the corresponding tRNA
- 3 catalyzes covalent attachment of the specific amino acid to its tRNA,
- 4 resulting in the formation of an aminoacyl-tRNA with 3D conformation that is no longer complementary to the conformation of the active site (and is released from the enzyme).

**(c)**

- 1 The rRNA in ribosomes holds the tRNA and mRNA together in close proximity, via complementary base pairing using hydrogen bonds
- 2 positions the new amino acid for addition to the carboxyl end of the growing polypeptide
- 3 rRNA peptidyl transferase activity catalyzes formation of a peptide bond between the new amino acid and the polypeptide chain

**QUESTION 3****(a)**

Haemagglutinin

- 1 Binds to the sialic acid on host cell membrane
- 2 aids in the binding and entry into host cell

Neuraminidase

- 3 Removes sialic acids on the envelopes of newly assembled viruses
- 4 aids in the release of newly assembled viruses from host cells and from each other

**(b)**

- 1 phospholipids
- 2 glycoproteins
- 3 cholesterol

**Examiner's comments:** Any two components of the virus's enveloped derived from host cell membrane.

**(c)**

- 1 Haemagglutinin on the envelope of influenza virus binds to the sialic acid receptor on the cell surface membrane of the target cell
- 2 host cell membrane invaginates, engulfing the virus to form an endocytotic vesicle within the host cell
- 3 endosome membrane fuse with the viral envelope for the release of viral nucleocapsid
- 4 viral capsid is enzymatically removed for the release of the viral genome into the cytoplasm

**(d)****(i)**

- 1 influenza viruses from 3 different species infect a single cell
- 2 during assembly of new virus, there is reassortment / random assembly of RNA segments from the 3 different influenza
- 3 giving rise to novel combinations, antigenic shift

**(ii)**

- 1 Mutations in key viral genes in human H1N1 due to an error-prone RNA polymerase (which has no proof-reading capability)
- 2 leading to subsequent potential for change in number and sequence of amino acids in polypeptide chain thus affecting the tertiary structure / 3D conformation of the protein structure (eg. glycoprotein haemagglutinin)

**QUESTION 4****(a)**

- 1 Base mutation where there is a substitution of a single nucleotide (G in proto-oncogene ras to T in ras oncogene) causes change in a codon
- 2 resulting in change of amino acid from glycine to valine (in ras protein)
- 3 R-groups of glycine and valine are different and each form different type of bonds with other amino acids
- 4 results in different tertiary structure / 3D conformation which affects protein interactions with other molecules and alters the protein's activity (resulting in ras protein triggering the kinase cascade even in the absence of growth factor resulting in increased cell division).

**(b)**

- 1 gain of function mutations were usually dominant and require only a single mutation in a gain of function mutation
- 2 loss of function mutations were usually recessive and both genes required mutant alleles in loss of function mutation to have an effect
- 3 gain of function mutations result in gene product / protein e.g. ras protein gaining a new and abnormal function
- 4 while loss of function mutations result in gene product / protein e.g. p53 having less or no function

(c)

Any 2 points:

- 1 Radiation / UV light / X-ray / ionizing radiation
- 2 Carcinogens
- 3 Viruses

(d)

- 1 translocation of *c-myc* proto-oncogene from chromosome 8 to chromosome 14 does not result in a change in gene sequence (Protein encoded carry out normal functions)
- 2 mutation of *H-ras* proto-oncogene results in a change in gene sequence, resulting in a change in amino acid sequence (hence mutated protein gained a new / abnormal function)
- 3 different regulatory sequence involved in the transcription of translocated *c-myc* proto-oncogene sequence
- 4 same regulatory sequence involved in the transcription of *H-ras* proto-oncogene and the oncogene

(e)

- 1 DNA replication has taken place during interphase before cell division.
- 2 Chromosomes only become visible as they condense and shorten prior to cell division
- 3 results in each chromosome consisting of two sister chromatids joined at the centromere.

**Examiner's comments:** Candidates **mistakenly referred to the pair of homologous chromosomes** rather than addressing why each homologous chromosome appeared as a double structure in Fig. 4.3.

(f)

- 1 *c-myc* oncogene has less effect compared to *H-ras* oncogene in causing tumours in mice. (*state general trend for individual oncogene*)
- 2 100% of mice without tumours in the first 100 days and 65% of mice without tumours at the end of 200 days for *c-myc* oncogene **vs** 75% of mice without tumours in the first 50 days and 45% of mice without tumours at the end of 200 days for *H-ras* oncogene
- 3 The combined effects of both oncogenes in causing tumours in mice is much greater than the sum of the individual tumour-causing effect of each oncogene (*state general trend for combined oncogenes*)
- 4 Only 30% of mice without tumours in the first 50 days, and 0% mice without tumours at about 180 days

**Examiner's comments:** Candidates needed to study Fig. 4.4 carefully before answering the question. Candidates **making full use of the data** presented in Fig. 4.4 to quote figures invariably achieved higher marks.

**QUESTION 5****(a)****(i)**

- 1 The fixed position of an allele or gene
- 2 on a particular chromosome

**(ii)**

- 1 Alternative form of a gene
- 2 Present on the same gene locus (on a pair of homologous chromosomes)
- 3 May be dominant, recessive or co-dominant

**(b)**Symbols $X^H$  – X chromosome with allele for normal blood clotting (no haemophilia) $X^h$  – X chromosome with allele causing haemophiliaAllele  $X^H$  is dominant to allele  $X^h$ 

	Beatrice		x		Henry
Parental phenotypes:	Carrier		x		Normal
Parental genotype:	$X^H X^h$				$X^H Y$
Parental gametes:	$X^H$ $X^h$				$X^H$ Y
F <sub>1</sub> genotypes:	$X^H X^H$	$X^H X^h$		$X^H Y$	$X^h Y$
F <sub>1</sub> phenotypes:	Unaffected female	Carrier female		Unaffected male	Male with Haemophilia
F <sub>1</sub> phenotypic ratio:	1 :	1 :		1 :	1

Alexander:  $X^H Y$  ; unaffected maleEugenie:  $X^H X^H$  ; unaffected female or $X^H X^h$  ; carrier unaffected female (both genotypes required)Leopold Arthur:  $X^h Y$  ; Haemophilia maleMaurice:  $X^h Y$  ; Haemophilia male**Examiner's comments:** Credit lost for incorrect symbols and not giving both possible genotypes for Eugenie.**(c)**

- 1 Queen Victoria
- 2 mutation occurs in her ovaries

**QUESTION 6****(a)**

- 1 Ions are charged
- 2 Cannot pass through hydrophobic core, consisting of fatty acid tail, of the phospholipid bilayer.

**Examiner's comments:** A number of candidates described sodium and potassium ions as polar, rather than charged, and were not aware of the significant difference between these two terms.

**(b)**

- 1 Facilitated diffusion through protein channels
- 2 Active transport by protein pumps / carrier proteins

**(c)**

- 1 A – nucleus (of Schwann cell) ;
- 2 B – myelin sheath ;

**(d)**

- 1 ATP synthesis during aerobic respiration used to drive  $\text{Na}^+$ - $\text{K}^+$  pump
- 2 to restore unequal distribution of  $\text{Na}^+$  and  $\text{K}^+$  ions in the intracellular and extracellular fluid at resting potential so that stimulus can trigger action potential.
- 3 Vesicle transport via the cytoskeleton along axon to carry vital proteins and organelles for axon function requires ATP.

**(e)(i)**

- 1 -70mV ;

**(ii)**

- 1 Sodium-potassium pump takes two  $\text{K}^+$  in and pumps three  $\text{Na}^+$  out of neurone through active transport result in net loss of positive charges from the neuron
- 2 More ungated  $\text{K}^+$  channels compared to ungated  $\text{Na}^+$  channel results in more  $\text{K}^+$  leaving the neuron than  $\text{Na}^+$  entering into neurone via facilitated diffusion due to the concentration ion gradients, result in net loss of positive charges from neuron.
- 3 Presence of negatively charged anionic protein within neurone

**QUESTION 7****(a)**

- 1 Classification is the organisation of species according to particular characteristics
- 2 Classification may not take into consideration the 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

**(b)**

- 1 Molecular evidence is quantifiable
- 2 protein, nucleic acid sequence data are precise and accurate and easy to quantify/convertible to numerical form for mathematical and statistical analysis;
- 3 Molecular evidence is objective and described in an unambiguous manner as it is based strictly on heritable material
- 4 Morphological evidence could be due to convergence/ some morphological characteristics may be analogous
- 5 Molecular evidence can be used as a molecular clock to date evolutionary event
- 6 By determining the number of neutral mutations that do not confer any selective advantage or disadvantage
- 7 are fixed/accumulated at a relatively constant rate for any particular gene
- 8 in a common gene/amino acid sequence in each organism



**QUESTION 8****(a) Compare the Krebs cycle and the Calvin cycle.****[6]**

*This column for  
reference only; no  
need to write in exam*



Description	Krebs cycle	Calvin cycle
Occurrence:	<b>1</b> Occurs in the mitochondrial matrix.	<b>1</b> Occurs in the stroma of chloroplasts.
CO <sub>2</sub> :	<b>2</b> Results in release of CO <sub>2</sub> via oxidative decarboxylation.	<b>2</b> Results in the use of CO <sub>2</sub> , CO <sub>2</sub> is fixed by Rubisco (CO <sub>2</sub> uptake)
Electron carriers: (NADH or NADPH)	<b>3</b> Formation of reduced NAD.	<b>3</b> Uses reduced NADP.
Type of reaction:	<b>4</b> Involves dehydrogenation - removal of hydrogen atoms from substrates in Krebs cycle / oxidation.	<b>4</b> Involves reduction - transfer of hydrogen atoms from NADPH to substrates in Calvin cycle.
Starting material and regeneration:	<b>5</b> Oxaloacetate (the starting point) was regenerated.	<b>5</b> Ribulose bisphosphate (the starting point) was regenerated.
ATP:	<b>6</b> There was synthesis of ATP by substrate level phosphorylation.	<b>6</b> Uses ATP in the reduction of PGA to PGAL, and the regeneration of RuBP.
Overall reaction:	<b>7</b> Overall is catabolic – which results in the breakdown of carbohydrate molecules to simple inorganic ones.	<b>7</b> Overall is anabolic – results in the synthesis of carbohydrates molecules from simple inorganic one.

**(b) Discuss the roles of NAD and NADP.****[6]**

- 1 (NAD and NADP are) Coenzymes that carry protons and electrons ;

NAD:

- 2 Reduced NAD provides hydrogen atoms which split into hydrogen ions and electrons.
- 3 Reduced NAD transfers electrons through the ETC for chemiosmosis / oxidative phosphorylation ;
- 4 Protons and electrons donated from substrates in the glycolytic pathway, Link reaction and Krebs cycle ;

NADP:

- 5 Reduced NADPH is final electron acceptor in the light reactions / non-cyclic photophosphorylation ;
- 6 Reduced NADP / NADPH carries hydrogen to reduce CO<sub>2</sub> in Calvin cycle ;

**(c) Outline the main features of photo-phosphorylation.****[8]**

- 1 Light energy absorbed by chlorophyll and other accessory pigments in photosystems ;
- 2 Electrons in pigments boosted from ground to higher energy levels / excited states ;
- 3 Energy transferred from pigment to pigment till the special chlorophyll a in the reaction centres of photosystems ;
- 4 Excited electron donated by special chlorophyll a to ETC ;
- 5 Energy levels of electrons decreases as they are passed down ETC from one carrier to another ;
- 6 Energy lost used to pump protons across thylakoid membrane into thylakoid space from stroma to establish proton gradient ;
- 7 Protons diffuse across ATP synthase complex down concentration gradient ;
- 8 Release energy which is used to synthesise ATP ;
- 9 ref. ATP synthase ;

**QUESTION 9****(a) Compare the structure of prokaryotic and eukaryotic genomes.****[6]**For every comparison: $\frac{1}{2}$  mark for correct *comparison* $\frac{1}{2}$  mark for correct information

	<b>Prokaryotic chromosome</b>	<b>Eukaryotic chromosome</b>
1. Chromosome structure*	Circular DNA molecule.	Linear DNA, each contained in a different chromosome.
2. Packaging of DNA*	Naked DNA with no introns; does not form chromatin.	Eukaryotic DNA with introns and exons is complexed with histones and other proteins to form chromatin.
3. Introns	Coding sequence proceeds from start to finish without interruption by introns.	Presence of introns within genes.
4. Regulatory sequences	Small amount of non-coding DNA consists mainly of regulatory sequences, such as promoters)	More complex regulatory sequences (eg. enhancers and silencers)
5. Repetitive sequences	Few repetitive DNA sequences.	Many highly repetitive DNA sequences
6. Coding and non-coding DNA	Most of DNA are coding sequences (codes for protein, tRNA, or rRNA).	Most of DNA are non-coding.
7. Presence and absence of operons*	Two or more genes may be expressed and regulated as a unit (genomes arranged in operons)	Absence of operons
8. Origins of replication*	One	Many
9. Telomeres*	Absent	Present
10. Centromere*	Absent	Present
11. Genome size	Smaller genomes, 0.6 Mb to 10 Mb	Large genomes, being less than 10 Mb – 100,000 Mb
12. Gene coding sequence	Shorter gene sequences	Longer gene sequences

\* Mentioned in A Level Examiner's Report.

**(b) Discuss the role of telomeres and centromeres.****[6]****Telomeres**

- 1 Disposable, repetitive sections of DNA at the end of chromosomes
- 2 stabilized the ends of the chromosome (by binding to proteins forming a protective nucleoprotein cap)
- 3 stopped the ends of chromosomes sticking together
- 4 prevent unintentional cell death/apoptosis/programmed cell death

**Centromeres**

- 5 holds the two sister chromatids together
- 6 site of assembly of the kinetochore
- 7 which attaches to the kinetochore microtubules of the mitotic spindle

**(c) Outline the main features of DNA Replication.****[8]**

- 1 Helicase binds to origins of replication, unwinds double-stranded DNA
- 2 DNA unzips, breaking hydrogen bonds between the bases, exposing the two parental strands as template strands/separating DNA strands
- 3 single-stranded DNA binding proteins bind to and stabilize the single-stranded DNA / prevent it from renaturing
- 4 DNA primase then attaches to the DNA (template) strands and synthesize a short RNA primer complementary to the template
- 5 DNA polymerase III adds complementary deoxyribonucleotides to the free 3' OH end of an already existing chain (via complementary base-pairing to deoxyribonucleotides in the existing chain)
- 6 catalyzes formation of the phosphodiester bond between two nucleotides
- 7 DNA polymerase I hydrolyses RNA portion of the RNA-DNA hybrid and fills in gaps with the complementary DNA bases
- 8 One of the daughter strand, called the leading strand, is synthesized continuously while the other daughter strand, called the lagging strand, is synthesized discontinuously in the form of short fragments called Okazaki fragments due to the anti-parallel nature of the DNA molecule
- 9 DNA ligase catalyzes formation of a phosphodiester bond between free hydroxyl group at the 3' end of one DNA chain and a free phosphate group at the 5' end of an adjacent DNA chain / between two Okazaki fragments

**PAPER 3 – Applications Paper and Planning Question****QUESTION 1****(a)**

- 1 isolate mature mRNA of human insulin
- 2 from the cytoplasm of cells of the pancreas
- 3 addition of reverse transcriptase
- 4 to produce double-stranded cDNA

**(b)**

- 1 protein sequencing performed to find out the sequence of the 14 amino acids of somatostatin, hence the mRNA codons can be known
- 2 artificial synthesis of the DNA sequence coding for the mRNA is performed

**(c)(i)**

- 1 protect bacteria from attack by viruses/bacteriophages
- 2 cleave any foreign DNA that enters bacterial cell  
/ by cutting up intruding DNA from other organism

**(ii)**

- 1 sticky end 1 – AATTC
- 2 sticky end 2 – CCTAG

**(iii)**

- 1 ensure that the gene coding for somatostatin is inserted in the correct orientation into a cloning vector

**(d)**

- 1 cut plasmid using *EcoRI* and *BamHI* restriction enzymes to produce sticky ends
- 2 the prepared gene for somatostatin is added to the cut plasmid, annealing by hydrogen bonding between complementary base pairs
- 3 DNA ligase is added to seal nicks in sugar-phosphate backbone by forming phosphodiester bonds

**(e)**

- 1 small plasmid allows inserts of larger sizes
- 2 self-replicating plasmid results in multiple copies of the plasmid and inserted foreign gene within one bacterium

**QUESTION 2****(a)****(i)**

- 1 Use of attenuated virus such as retrovirus allows the integration of viral genome manipulated to carry the normal allele of the *Rpe65* into the host genome without causing the viral disease in the host cell.
- 2 The normal allele can be stably propagated by chromosomal replication following cell division for long-term stable expression, thus provides the possibility of a permanent cure for a disease.
- 3 Use of attenuated viral such as adenovirus which does not integrate its viral genome into the host's chromosome but persists extrachromosomally in the cells
- 4 eliminates the problem of insertional mutagenesis by the vector.
- 5 Viral entry into cells occurs by receptor-mediated endocytosis
- 6 high transduction efficiency, e.g. retroviruses infects specific cells and introduces its genome into cell.
- 7 Adenoviruses are large viruses
- 8 potential for accepting large inserts (up to 35 kb) allowing ease of manipulating and introducing allele into host cell.

**(ii)**

(any 2)

- 1 No viral genes that may cause disease / No infectious capability
- 2 No insertional mutagenesis / No mutagenic possibility
- 3 No immune response elicited
- 4 Ease of manipulation and production of liposome to carry normal allele (of *Rpe65* gene) compared to viral delivery system / large scale production possible

**Examiner's comments:** A significant number of candidates did not use all of the information in the question, since in (i) and (ii) both delivery systems were being used for the normal allele of the *Rpe65* gene. Therefore in this question, the size of gene that can be carried is not relevant.

**(b)**

- 1 Inserted DNA does not integrate into chromosomal DNA or the introduced gene may not segregate equally to daughter cells if the cells are actively dividing. Hence, expression of inserted genes is transient.
- 2 Repeated treatments involving gene transfer may be necessary.
- 3 Mutations due to random integration of the transgene and this could result in the inactivation of a tumor suppressor gene or activation of an oncogene leading to cancer.

**(c)****(i)**

- 1 collection of all the coding and non-coding DNA sequences of a human genome
- 2 useful when the cell type expressing the therapeutic allele is unknown, or unable to obtain the cell type

- 3 used a radioactive probe (with sequence complementary to that in the therapeutic allele) to hybridize to therapeutic allele for identification / screening of the library

**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.

(ii)

- 1 a collection of host cells, usually bacterial cells, each containing a cloning vector carrying a particular segment of cloned DNA molecules made by reverse transcription from mRNA.
- 2 represents expressed genes (in a particular type of cell) that are subset of the whole genome.
- 3 useful if the cell type expressing the therapeutic allele is known
- 4 used a radioactive probe (with sequence complementary to that in the therapeutic allele) to hybridize to therapeutic allele for identification / screening of the library

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

**QUESTION 3****(a)****(i)**

- 1 Aseptic / sterile environment
- 2 Recombinant corn cells placed on nutrient medium to provide nutrients (e.g.  $K^+$ ), sugars, e.g. sucrose as a source of energy and stimulates mitosis / cell division.
- 3 Callus formed and subculture callus into another medium containing appropriate growth regulators such as auxins and cytokinins at varying concentrations
- 4 to stimulate differentiation of cells in culture to form roots and shoots into plantlets which are transfer to soil to allow further growth.

**(ii)**

Any two:

- 1 Not all beads coated with recombinant plasmid
- 2 Penetration of the beads can cause damage to the cells
- 3 Shallow penetration of the beads

**Examiner's comments:** A significant number of candidates did not appear to understand the information given in the question. When beads of tungsten are fired at very high speed at corn cells, the cell wall, cell surface membrane or nuclear envelope will not be 'too strong to prevent entry'.

**(b)****(i)**

- 1 Development of wasp larvae into adult wasps is dependent on the survival of caterpillars. Wasp development is prevented when Bt crop cause Bt-susceptible caterpillars to die but wasp developed into adult wasp in Bt-susceptible caterpillars feeding on non-Bt crop.  
63% of parasitized caterpillars had adult wasps emerged.
- 2 54% and 56 % of parasitized caterpillars had adult wasps emerged from Bt-resistant caterpillars which ate Bt and non-Bt crop, respectively. (Growing Bt crop could eliminate Bt-susceptible insects in the food chains.)

**(ii)**

- 1 Percentage of parasitised caterpillars from which adult wasps emerged is higher (2 and 9% higher)  
in both Bt-resistant and Bt-susceptible caterpillars that ate non-Bt crop than Bt-resistant caterpillars which ate Bt crop.
- 2 In Bt-resistant caterpillars that ate Bt crop, percentage of parasitized caterpillars from which adult wasps emerged is **2% lower than** those which ate non-Bt crop even though Bt-resistant caterpillars are able to survive eating Bt crop and allow wasp development to adults.



**QUESTION 5****(a)**

- 1 loading dye, containing glycerol and a tracking dye such as bromophenol blue or xylene cyanol, is added to the DNA samples to help DNA to sink into the wells and allow the process of electrophoresis to be tracked, respectively.
- 2 DNA samples loaded into wells at one end of an agarose gel.
- 3 Gel is submerged in a electrophoresis buffer solution, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE), that provide ions to conduct electricity from cathode to anode.
- 4 Electric current is applied through electrodes at opposite ends of the gel
- 5 Negatively-charged DNA fragments migrate to the positively charged electrode.
- 6 Gel electrophoresis separates DNA fragments by size, the smaller fragments will be able to move through the agarose matrix faster than the larger ones when subjected to an electric field.
- 7 Invisible DNA bands can be revealed under UV light by staining the gel with a dye such as ethidium bromide.

**Examiners' comment:**

The roles of the buffer and loading dye were often described in insufficient detail.

**(b)****What is meant by RFLP**

- 1 restriction enzymes only cut DNA at specific restriction sites
- 2 polymorphisms / genetic variation / different alleles between individuals exists
- 3 resulted in difference in nucleotide sequence due to mutation
- 4 hence differences in the restriction sites on homologous chromosomes / may be due to addition or deletion of restriction sites
- 5 resulting in different lengths of restriction fragments obtained / different restriction fragment patterns.

**How RFLP can be detected**

- 6 restriction enzymes are used to digest DNA strands into fragments
- 7 digestion of genomic DNA produces numerous fragments / a smear after gel electrophoresis
- 8 agarose gel electrophoresis is performed to separate the DNA fragments based on size
- 9 The gel is treated with an alkaline solution (i.e. sodium hydroxide) to cause the double-stranded DNA to denature / separate into single strands.
- 10 DNA fragments are transferred onto nitrocellulose membrane
- 11 then baked to permanently crosslink the DNA to the membrane.
- 12 membrane is treated with a single-stranded radioactive probe which is complementary to the target sequence / marker / restriction fragment of interest
- 13 after hybridisation, excess probe is washed off from the membrane
- 14 autoradiography – pattern of hybridization is visualized on X-ray film
- 15 distinguish the restriction fragments of interest from among many other restriction fragments

**(c)**

- 1 haemoglobin has a quaternary structure, containing 2 identical  $\alpha$ -chains + 2 identical  $\beta$ -chains
- 2 Sickle-cell disease is caused by a mutation in a single nucleotide
- 3 T at the 17th nucleotide of the normal  $\beta$ -globin allele is replaced by A.
- 4 results in the deletion of a *DdeI* restriction site in the sickle-cell mutant  $\beta$ -globin allele
- 5 nucleic acid hybridization is used to compare restriction fragments produced from different samples of genomic DNA
- 6 radioactive single-stranded DNA probe is complementary to the  $\beta$ -globin gene
- 7 restriction digestion of normal  $\beta$ -globin allele using *DdeI* will yield a large fragment and two smaller fragments of 201bp and 175bp - Unaffected individuals (2 normal alleles) will show 3 bands
- 8 restriction digestion of sickle-cell mutant  $\beta$ -globin allele using *DdeI* will yield a large fragment and one other fragment of 376bp – Sickle-cell individuals (2 mutant alleles) will show 2 bands
- 9 Heterozygous carriers of the sickle-cell allele (1 normal allele + 1 mutant allele) will show 4 bands (large fragment + 376bp fragment + 201bp fragment + 175bp fragment)

**Examiners' comment:**

The majority of answers were in terms of the disease mentioned in the syllabus - sickle cell anaemia and these were often extremely detailed. Candidates who wish to describe other diseases should ensure that the complete details of restriction enzymes, fragment lengths etc. are known to the same degree as they are known for the well documented example of sickle cell anaemia.