

OCR A-LEVEL BIOLOGY B • H422

BIOLOGY



EXAM QUESTION PACK

5.1.3 GENE TECHNOLOGIES

SCORE

/163

PERCENTAGE

%

STEMUP

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1 *Taq* polymerase is an enzyme used during the polymerase chain reaction (PCR).

- At optimum temperature, *Taq* can replicate DNA at a rate of 150 nucleotides per second.
- The error rate of *Taq* has been measured at 1 in 9000 nucleotides.

Which of the options, **A** to **D**, is the estimated number of errors that could be produced by *Taq* during 20 minutes of PCR at optimum temperature?

- A 180
- B 60
- C 20
- D 1200

Your answer

[1]

2 Somatic cell gene therapy has been used for treating people with mitochondrial myopathy, a disease of the muscles caused by damage to the mitochondria.

Which of the following statements is / are correct?

- 1: DNA sequences coding for the functional gene are placed into muscle cells.
 - 2: Meiosis will ensure that all muscle cells receive a copy of the functional gene.
 - 3: The functional gene will be passed on to the offspring of the treated individual.
- A 1, 2 and 3 are correct
 - B only 1 and 2 are correct
 - C only 2 and 3 are correct
 - D only 1 is correct

Your answer

[1]

- 3 Sequencing the mitochondrial genome has helped our understanding of the function of mitochondria and the basis of mitochondrial disease.

PCR is used to amplify the mtDNA in a sample before sequencing.

- (i) In PCR, the DNA is placed in a buffered solution in a thermocycler.

State the **three** other components that are placed in the buffered solution in PCR.

1.
2.
3.

[3]

- (ii) A sample contained 1500 molecules of mtDNA.

Calculate the number of mtDNA molecules you would expect after 15 cycles of PCR.

Express your answer as a $\log_{10}$ value.

number of molecules = ... [2]

- (iii) Place the events, **A** to **G**, in the correct order in the boxes below to describe the process of gel electrophoresis. The first one is done for you.

A DNA fragments are placed in wells by the cathode

B when the current is stopped, shorter fragments will finish nearer the anode

C UV light can be used to visualise banding patterns

D this sentence should be straight to E

E this sentence should be straight to D

F DNA fragments can be extracted for further analysis

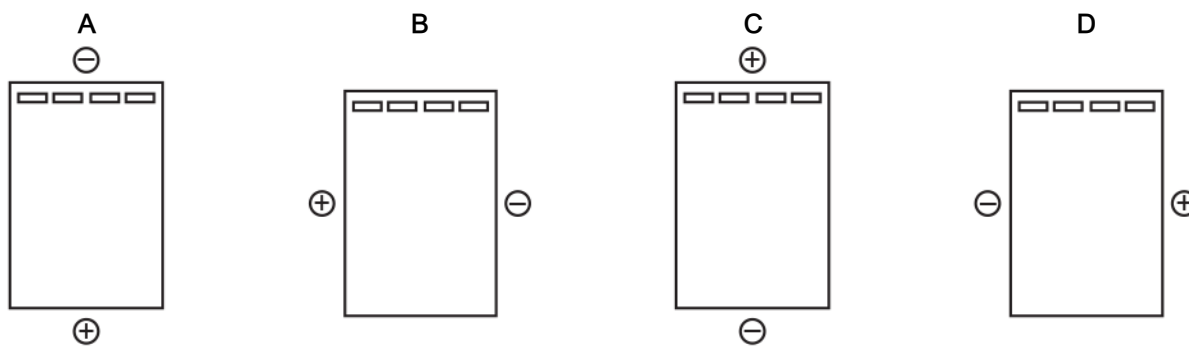
G the DNA fragments begin moving towards the anode

E						
---	--	--	--	--	--	--

[4]

- 4 The diagrams A to D represent different setups of an agarose gel electrophoresis experiment.

Each diagram shows an agarose gel and the positive and negative electrodes. DNA samples are loaded into the four wells at the top of each gel.



Which of the diagrams, A to D, shows the correct positioning of electrodes?

Your answer

[1]

5 Which of the following statements is/are true of variable number tandem repeats (VNTRs) and single nucleotide polymorphisms (SNPs)?

- 1: Both VNTRs and SNPs are only found in intronic regions of DNA.
- 2: VNTRs are used in forensics to identify potential crime suspects.
- 3: VNTRs are generally more polymorphic than SNPs.

- A 1, 2 and 3 are correct
- B Only 1 and 2 are correct
- C Only 2 and 3 are correct
- D Only 1 is correct

Your answer

[1]

6(a) CRISPR is an adaptive system in bacteria that protects against invading viruses by destroying viral DNA upon entry into cells.

CRISPR is an acronym for clustered regularly interspaced short palindromic repeats.

CRISPR has been adapted into a laboratory tool for genetic modification in eukaryotic cells. Using purified Cas9 protein and artificially-synthesised guide RNA molecules, scientists can target genes of interest.

In eukaryotic cells, DNA breaks induced by Cas9 are repaired by the cell's own (imperfect) DNA repair mechanisms. This leads to the generation of mutations at the site of the DNA break, and the gene function is lost.

RNA interference (RNAi) is another laboratory tool that scientists can use to target genes of interest.

Using the information provided, and your knowledge of RNAi, discuss the advantages **and** disadvantages of CRISPR and RNAi for the study of gene function.

CRISPR -----

RNAi -----

[4]

(b) Using the most appropriate word(s), complete the sentences below about the production of mature mRNA.

A molecule of pre-mRNA contains sequences that code for amino acids, known as , and sequences that are non-coding, known as The process of removes non-coding regions and joins coding regions to produce a molecule of mature mRNA. In this way, a gene may code for several different proteins.

[3]

7 The polymerase chain reaction (PCR) involves a repeating sequence of temperature changes.

Which of the options, A to D, occurs at a temperature of 72 °C?

- A annealing of primers
- B detachment of primers
- C polymerisation of free nucleotides
- D separation of DNA strands

Your answer

[1]

8 Which of the statements, A to D, is true of single nucleotide polymorphisms (SNPs)?

- A All SNPs have four possible variations.
- B Genetic recombination can generate a SNP.
- C SNPs can influence banding patterns on a DNA fingerprint.
- D SNPs occur in exons only.

Your answer

[1]

- (i) The roots of bean plants form nodules due to infection by the nitrogen-fixing bacteria, *Rhizobium*.

In response to infection by *Rhizobium*, bean plant nodule cells produce protein called leghaemoglobin.

Researchers wanted to find out more about three genes that code for leghaemoglobin.

They used RNA interference (RNAi) to inhibit the production of leghaemoglobin using miRNA. They measured the relative transcript level of the leghaemoglobin genes of bean plants treated with miRNA (RNAi plants) and those of untreated bean plants.

The results are shown in Table 1.

Name of leghaemoglobin gene	Relative transcript level of gene	
	Untreated plants	RNAi plants
LjLb1	3.5	0.085
LjLb2	4.0	
LjLb3	2.0	0.045

Table 1

Transcript levels for gene LjLb2 in the RNAi plants were reduced by 97.4% compared with the untreated plants.

Calculate the relative transcript level for LjLb2 in the RNAi plant.

Show your working.

Answer = ... [3]

10 Freeman was working on developing drought-resistant varieties of alfalfa using selective breeding, but this has proved difficult.

Drought resistance depends on the ability to withstand several abiotic factors, such as high temperatures and high light intensity.

- (i) Use your knowledge of inheritance to suggest why it is difficult to study the genetic basis of drought resistance.

----- [2]

- (ii) Alleles of the *miRNA* 156 gene regulate a group of transcription factors in alfalfa. These transcription factors activate or inhibit promoters that control genes related to drought resistance.

Explain how the *miRNA* 156 gene could be used to investigate the genetic basis of drought resistance.

----- [2]

- (iii) Scientists have made a plasmid that produces more of the *miRNA* 156 gene product than normal and want to use this to develop a drought-resistant alfalfa plant.

Explain how they could incorporate the plasmid into alfalfa cells.

----- [2]

Neuropathic pain is a type of pain caused by neurones malfunctioning following nerve damage. When this happens, some voltage-gated sodium ion channels (VGSCs) in pain receptors open spontaneously.

- (i) Single nucleotide polymorphisms (SNPs) have been detected in the genes coding for several types of VGSC. It is thought that these SNPs might alter the response of different patients to drugs that block VGSCs.

Describe the steps involved in identifying the SNPs present in a DNA sample from a patient.

----- [2]

- (ii) Screening for other SNPs is used in the study, diagnosis, and treatment of cancer.

A mutation in either the *BRCA1* or *BRCA2* gene increases the risk of breast cancer.

Explain why screening for these mutations is only offered to individuals with a strong family history of breast cancer and a living relative with breast cancer.

----- [2]



Some patients with diabetes are treated with regular insulin injections.

Most insulin is now produced using recombinant DNA technology. Outline this process.

You should refer to the role of specific enzymes in your answer.

[6]

13 Scientists can identify an unknown species as follows:

- extract DNA from tissue, e.g. skin or hair
- amplify a length of DNA, known as a barcode, using the polymerase chain reaction (PCR)
- read the base sequence of the DNA.

The strip below represents a length of extracted DNA before PCR. Lines 1 to 4 represent primers.

Key:

white = same DNA sequence in all species

black = variable DNA sequence between species



Which of the options, **A** to **D**, gives the pair of primers that could be used to amplify DNA from all species?

- A** 1 and 3
- B** 1 and 4
- C** 2 and 3
- D** 2 and 4

Your answer

[1]

14 A gene mutation causes part of a DNA sequence to change from TAC to TAA.

TAA is a stop codon.

Which of the statements, **A** to **D**, is a correct explanation of why the stop codon may not affect translation of the mRNA sequence?

- A** TAA also codes for an amino acid
- B** TAA stops transcription, not translation
- C** the mutation in the mRNA sequence may be repaired
- D** the stop codon may not be present in the mature mRNA sequence

Your answer

[1]

15(a) Fig. 4.1 shows one complete cycle of the polymerase chain reaction (PCR).

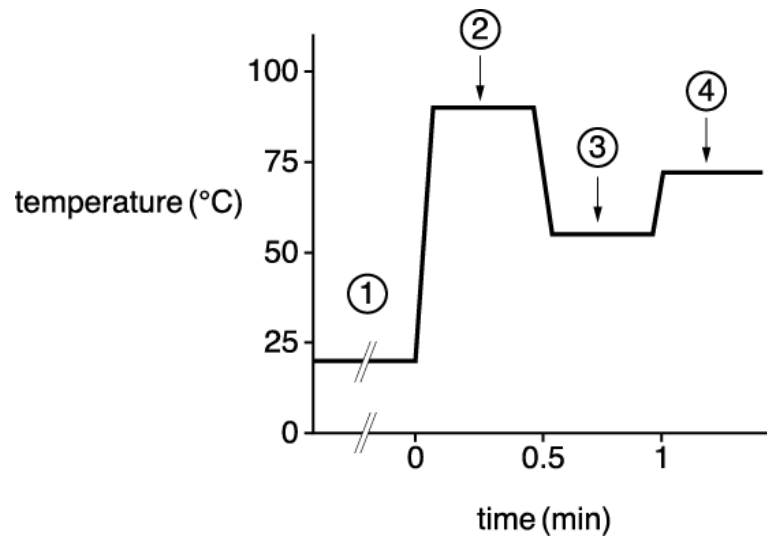


Fig. 4.1

For stages 2 and 3 in the cycle shown in Fig. 4.1, describe what is happening to the DNA.

Stage 2 _____

Stage 3 _____

[2]

(b) Stage 4 in Fig. 4.1 involves the addition of Taq, an enzyme obtained from the thermophilic bacterium, *Thermus aquaticus*.

(i) Explain the role of this Taq enzyme in PCR.

-----[1]

(ii) The bacterium, *Thermus aquaticus*, was originally isolated from hot springs.

Suggest why an enzyme from this bacterium is used in PCR.

-----[2]

(c) Fig. 4.2 is a graph showing the increasing mass of DNA over an increasing number of cycles of PCR.

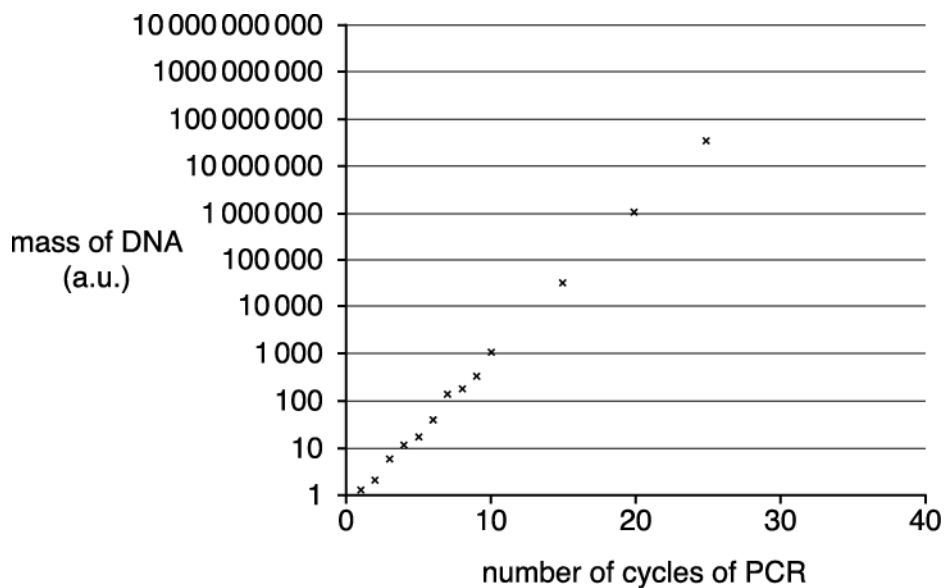


Fig. 4.2

(i) Using Fig. 4.2, estimate the mass of DNA after 30 cycles.

Give your answer in standard form.

mass of DNA a.u. [1]

(ii) Name the type of scale used for the numbers on the y axis of Fig. 4.2.

..... [1]

16 Gene therapy is under investigation as a treatment for patients with haemophilia A.

(i) Explain the difference in outcome between germ line and somatic gene therapy.

[2]

(ii) In gene therapy, structures known as vectors carry genetic material into a cell.

Viruses and liposomes (phospholipid vesicles) are two examples of such vectors.

Suggest **one** advantage and **one** disadvantage of using viruses rather than liposomes as vectors.

advantage -----

disadvantage -----

[2]

17 RNA interference can be carried out by a type of non-coding RNA molecule known as microRNA.

Some microRNA molecules are tumour suppressors.

Which of the options, **A** to **D**, is an action of a tumour suppressor microRNA molecule?

- A** degradation of a messenger RNA transcript encoding Myc
- B** degradation of a messenger RNA transcript encoding p53
- C** inhibition of translation from a messenger RNA transcript encoding Myc
- D** inhibition of translation from a messenger RNA transcript encoding p53

Your answer

[1]

Electrophoresis can be used to separate proteins. This technique has been used to investigate the different catalase isoenzymes present in some plant tissues.

Fig. 1.3 shows the appearance of a gel following the electrophoresis of a catalase isoenzyme purified from plant tissue.

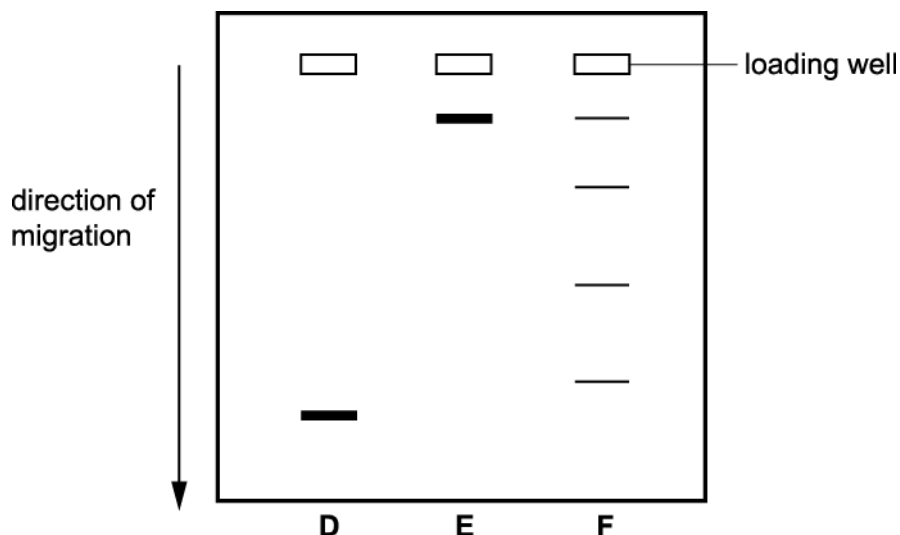


Fig. 1.3

- The distance moved by protein molecules depends on both the charge on the molecule and its size.
- The proteins are first treated with a detergent.
- The detergent covers the protein molecule, which denatures the protein and gives it a negative charge.
- The denatured proteins are loaded onto a gel and placed in an electric field and the molecules migrate.
- The gel is stained and molecules appear as separate bands.

Using this information, suggest which of the three tracks, D, E or F, represents the result obtained following the detergent treatment and electrophoresis of this catalase isoenzyme.

Explain your choice.

track _____

explanation _____

[3]

- 19 Women may become infertile several years before the menopause. Tests which predict the timing of the menopause would allow women to plan their pregnancies.

Recent genetic studies have identified *loci* which are associated with variation in the age of onset of natural menopause.

- Each *locus* is a short DNA sequence which differs by one nucleotide
- One variant increases the risk of an early menopause

Fig. 6.1 is a diagram of a DNA sequence

- The variation occurs at the point indicated by the arrow where the nucleotide can be either 'C' or 'T'
- The presence of 'C' at this point increases the risk of an early menopause.

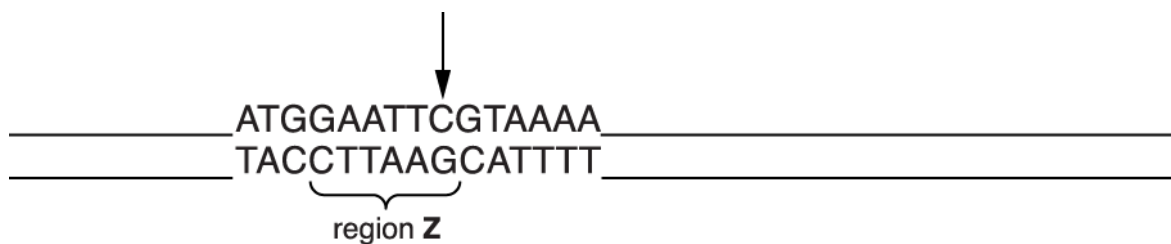


Fig. 6.1

- (i) What do the letters C and T represent in Fig. 6.1?

C

T

[1]

- (ii) Region Z is a nucleotide sequence that is complementary to the active site of an enzyme known as EcoRI. EcoRI is a type of enzyme that will cut the DNA strand.

State the following:

the type of enzyme that cuts DNA at a particular recognition site

.....
.....

the nature of the nucleotide sequences at the recognition site

the name of the bond in the DNA strand that the enzyme breaks

[3]

- (iii) Separate DNA samples of the 'C' variant and the 'T' variant were treated using EcoRI. The DNA was analysed using electrophoresis.

Describe **and** explain how the appearance of the pattern on an electrophoresis gel will differ between the DNA samples.

You should assume that:

- region Z occurs only once
- no other recognition sites for EcoRI are present.

[3]

20 The Polymerase Chain Reaction (PCR) can be used to test a blood sample for the presence of Human Immunodeficiency Virus (HIV).

Stages in the PCR test are listed in Table 1.1.

A	The beta haemoglobin gene is used as a positive control.
B	Viral RNA is converted into DNA.
C	The presence of both viral DNA and beta haemoglobin DNA indicates a positive test result.
D	HIV RNA is extracted from the person's blood.
E	DNA is amplified in a PCR machine.

Table 1.1

(i) Place the stages B to E in the correct order to describe the PCR test for HIV.

----- A -----

[2]

(ii) State one other test for HIV that could be performed on a sample of blood.

-----[1]

21(a) Many researchers are involved in investigating the potential use of stem cells to treat a variety of human conditions.

Stem cells can be produced from a variety of sources. One possible source is known as somatic cell nuclear transfer (SCNT).

In SCNT:

- the nucleus is removed from a donor’s oocyte
- a nucleus from the patient’s somatic cell is introduced into the oocyte
- the resultant cell is allowed to divide to produce several cells
- some of these cells will be used to produce cultures of stem cells.

Insert a tick (✓) against the term that best describes the production of stem cells using SCNT.

Term	Insert a tick (✓)
Genetic engineering	
Therapeutic cloning	
Reproductive cloning	
ICSI	
IVF	

[1]

(b) Stem cells have the potential to be used to treat patients with conditions such as Parkinson’s disease or Type 1 diabetes.

(i) Suggest the **type** of stem cell which is produced using SCNT.

[1]

(ii) State **two** properties of the stem cells produced by SCNT that would make them potentially suitable for treating conditions such as Parkinson’s disease or Type 1 diabetes.

1 -----

2 -----

[2]

22(a) The mutation responsible for cystic fibrosis occurs in a gene coding for a membrane transport protein called CFTR.

In March 2012, a gene therapy trial for the treatment of cystic fibrosis was launched in the United Kingdom. Patients received the treatment by inhaling small liposomes (phospholipid vesicles) containing molecules of DNA with a copy of the normal CFTR gene. The liposomes act as vectors and will deliver this DNA into the cells lining the lungs.

(i) Explain why a treatment such as that described above is an example of somatic gene therapy.

----- [2]

(ii) Suggest why it was necessary to enclose the DNA in liposomes for delivery into cells.

----- [2]

(b) The CFTR gene is found on chromosome 7 and is approximately 190 000 base pairs in length.

The copy of the normal CFTR gene was made using an enzyme called **reverse transcriptase**.

- Messenger RNA coding for the CFTR protein is isolated from cells.
- Reverse transcriptase is used to synthesise a DNA strand that is complementary to the mRNA molecule.
- The DNA molecule is made double stranded forming a complementary or **cDNA** molecule containing a copy of the gene.
- 'Sticky ends' are added to this cDNA molecule.
- This DNA is inserted into a plasmid to form a recombinant plasmid.

(i) Suggest why a cDNA copy of the CFTR gene will have fewer base pairs than the CFTR gene located on chromosome 7.

- (ii) Describe how the plasmid is treated in order to form a recombinant plasmid containing the cDNA CFTR gene.



In your answer you should refer to the action of the enzymes used to make the recombinant plasmid.

- The CFTR gene is **not** inserted into the genome of the cells.
- To maintain the production of the protein, the gene needs to be introduced into the cells lining the lungs over and over again.
- This means that repeated doses of gene therapy using fat globule vectors are required.

[2]

- Research is being carried out into the use of modified viruses to deliver the CFTR gene into cells.
- Viruses have been shown to be very successful vectors for gene therapy.
- Treatments using most viruses become less and less successful at delivering the gene into cells with each repeated treatment.

[2]

23 Following an MRI scan, a patient was found to have an area of her brain that was deprived of oxygen due to a blood clot. She was immediately 'thrombolysed' and then transferred to a specialist stroke ward.

- 'Thrombolysis' involves the injection of a substance known as a plasminogen activator.
- The activator converts the protein **plasminogen** in blood into an active form called **plasmin**.
- Plasmin dissolves blood clots.

(i) Suggest the type of molecule that plasmin might be **and** how it dissolves blood clots.

----- [2]

(ii) Plasminogen activator is a glycoprotein. The plasminogen activator used in hospitals has been manufactured using genetically modified cells.

What **type** of modified cell might be used in the manufacture of plasminogen activator?

Explain your answer.

----- [2]

(iii) The recommended dose of plasminogen activator is 0.9 mg for each kilogram of body mass.

- The patient was prescribed a dose of 67.5 mg.
- The patient is 1.5 m tall.
- BMI is calculated using the formula below:

$$\frac{\text{body mass (kg)}}{(\text{height (m)})^2}$$

Using the above information, calculate a BMI for Mrs G.

Give your answer to the nearest whole number.

Answer = _____

[2]

(iv) Using your calculation of BMI in part (iii), discuss the risk factors that may have contributed to Mrs G's stroke.

[2]

24(a) Choroideremia is an inherited sex-linked recessive condition that results in degeneration of the choroid layer in the eye. This condition leads to a gradual breakdown of the retina and eventual blindness.

Using the normal conventions for constructing genetic diagrams and the letters **E** and / or **e**, choose appropriate symbols to represent:

(i) the allele for choroideremia

-----[1]

(ii) the possible genotype(s) of a person who develops choroideremia

-----[1]

(iii) the genotype of a carrier of choroideremia.

-----[1]

(b) The gene involved in choroideremia codes for a protein called REP-1.

Many of the gene mutations that cause choroideremia result in the formation of a protein that is much smaller than normal REP-1. This smaller protein is known as a **truncated protein**.

(i) State the **organelle** in the cell where the following occurs:

a complementary RNA copy of the gene is synthesised -----

the REP-1 protein is synthesised -----

[2]

(ii) Suggest **how** a mutation in the REP-1 gene could lead to the formation of a truncated protein.

-----[2]

(c) A phase 1 clinical trial was carried out using gene therapy to treat choroideremia. An outline of the method used is given below.

- A complementary DNA (cDNA) copy of the REP–1 gene was inserted into a viral vector.
- The retina of the patient was detached to expose the choroid layer.
- A fine needle was used to inject the virus into the choroid layer.

(i) Name the enzyme used to create a cDNA copy of the REP–1 gene.

-----[1]

(ii) What type of gene therapy has been used in the trial?

-----[1]

(iii) Discuss the reasons why genetic diseases such as choroideremia are good choices for treatment using gene therapy.

-----[3]

- 25 This question is based on the Advanced Notice article **CHANGES IN THE NUCLEUS THAT LEAD TO CANCER**, which is an insert.

There are several different types of gene mutation. In substitution mutations one nucleotide is exchanged for another which produces a different base sequence.

Fig.1.1 represents part of a gene. The letters X and Y indicate two nucleotides that have been changed by a mutation.

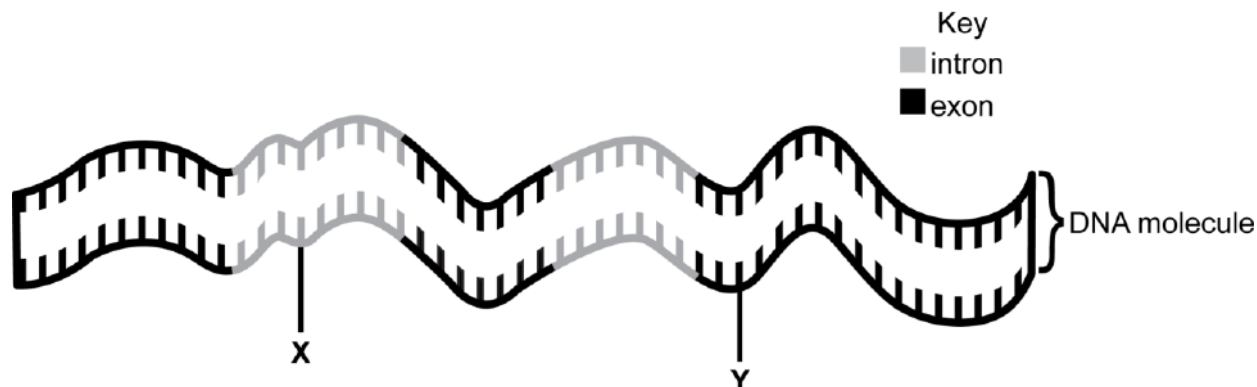


Fig. 1.1

- (i) Name one genetic disease caused by a **substitution** mutation. [1]

- (ii) Explain why a substitution mutation at point X will **not** result in a genetic disease. [2]

- (iii) The substitution mutation at point Y on the DNA molecule resulted in a change from the triplet AGA to AGG. This did **not** result in a genetic disease.

Suggest why **this** substitution mutation did **not** result in a genetic disease.

27(a) This question is based on the Advanced Notice article **MYOKINES**, which is an insert.

IL-6 is produced by muscle cells in response to physical exercise.

(i) Using the data in **Table 1.1**, determine the mode and mean IL-6 increase after a 1.5 hour cycle ride.

Determine the mode to **one** significant figure and the mean to **three** significant figures.

mode _____ mean _____

[3]

(ii) Based on the data in **Table 1.1**, how valid are the following conclusions?

The duration of exercise affects IL-6 concentration in blood.

Running has a greater influence than cycling on IL-6 concentrations.

[4]

(b)

- (i) Suggest how IL-6 increases the uptake of glucose from the blood plasma to muscle cells **and** how it increases glucose production in the liver.

----- [3]

- (ii) * *"IL-6 increases the risk of type 2 diabetes."*

Evaluate the evidence that supports or contradicts the above statement.

Use information from the article **MYOKINES**.

----- [6]

(c) Studies F and H in **Table 1.2** both used knockout mice.

(i) Outline **one** way in which a gene can be inactivated in the knockout procedure.

----- [1]

(ii) Suggest **one** reason why mice are used as the model organism in the knockout procedure.

----- [1]

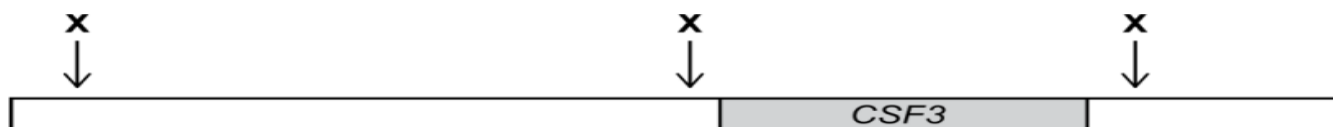
(d) Cytokines produced in muscles are called myokines. IL-6 is an example of a myokine.

Cytokines can act as chemical signals between immune cells.

Describe **one** specific role of cytokines in the immune system.

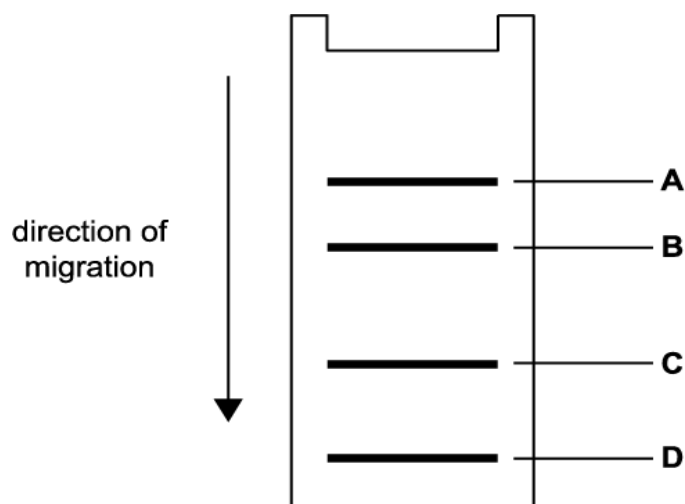
----- [2]

28 The diagram below represents an **isolated** length of DNA containing the gene, *CSF3*.



- *EcoRI* is a restriction enzyme that recognises specific sequences, labelled X, within the DNA.
- The DNA was cut at every point X with *EcoRI*, and the fragments separated by gel electrophoresis.

The result is shown below.



Which of the DNA bands, A to D, contains the gene, *CSF3*?

Your answer

[1]

29 The sympathetic and parasympathetic nervous systems affect heart rate.

Which of the options, A to D, would lead to a **decrease** in heart rate?

- A activation of the vagus nerve, releasing noradrenaline
- B activation of the vagus nerve, releasing acetylcholine
- C activation of the accelerator nerve, releasing noradrenaline
- D activation of the accelerator nerve, releasing acetylcholine

Your answer

[1]

30 The polymerase chain reaction (PCR) is used to produce multiple copies of a desired DNA sequence.

A DNA sequence encoding a gene was amplified from a single copy.

Which of the options, **A** to **D**, would be the theoretical number of copies of the gene after **12 cycles** of heating and cooling?

- A 13
- B 24
- C 144
- D 4096

Your answer

[1]

- 31 One mechanism for controlling gene expression in cells uses small, double stranded pieces of RNA known as siRNA.

- 1 siRNA molecules are introduced into the cell.
- 2 The siRNA molecules are combined with a protein complex called the RNA induced silencing complex (RISC) and one of the siRNA strands is destroyed.
- 3 The other strand remains bound to RISC and acts as a guide. RISC is now said to be activated.
- 4 This strand binds to complementary sequences on messenger RNA molecules in the cytoplasm causing them to be destroyed.

Fig. 36.1 shows the sequence of events for this mechanism.

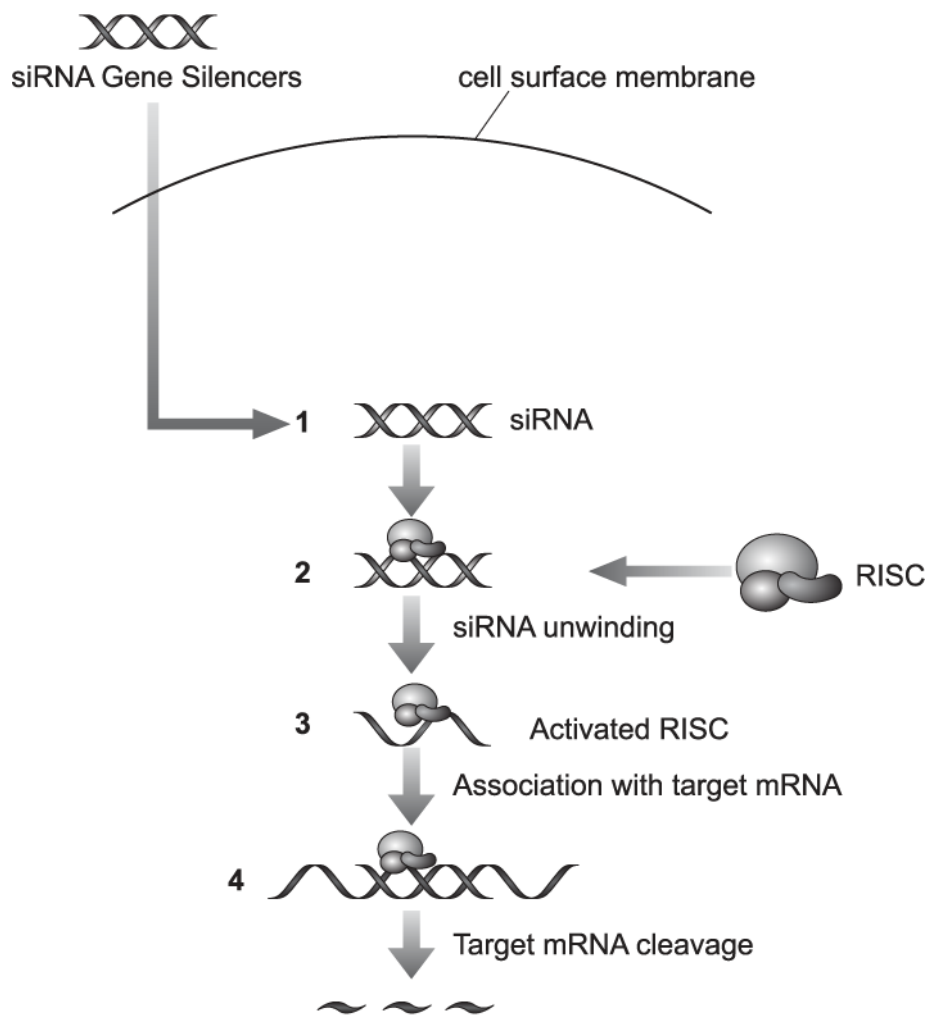


Fig. 36.1

- (i) An RNA strand on an activated RISC has the following sequence.

On the line above, write out the sequence that activated **RISC** would bind to on the mRNA molecule.

[1]

- (ii) What type of reaction is carried out by activated **RISC** on the bonds in the mRNA molecule?

[1]

- (iii) Clinical trialling is being carried out on the use of siRNA as an anti-viral therapy for the treatment of Hepatitis C infections.

Suggest how siRNA could prevent the spread of a virus within a person infected by the Hepatitis C virus.

----- [3]

32 Some of the stages in the genetic engineering of the bacteria *E. coli* to produce human growth hormones, HGH, are listed below.

- 1 A plasmid is cut with **enzyme 1** so the gene for HGH can be inserted.
- 2 A DNA copy of the messenger RNA for HGH is made using **enzyme 2**.
- 3 Many copies of the gene for HGH are made using PCR and **enzyme 3**.
- 4 The gene and plasmid are attached to each other using **enzyme 4**.

Select the row from **Table 20.1** to correctly identify enzymes 1, 2, 3 and 4.

Answer	Enzyme 1	Enzyme 2	Enzyme 3	Enzyme 4
A	DNA Ligase	DNA Polymerase	Reverse Transcriptase	Restriction Enzyme
B	Reverse Transcriptase	Restriction Enzyme	DNA Polymerase	DNA Ligase
C	Restriction Enzyme	Reverse Transcriptase	DNA Polymerase	DNA Ligase
D	DNA Ligase	Reverse Transcriptase	Restriction Enzyme	DNA Polymerase

Table 20.1

Your answer ☐

[1]

33(a) Genetic engineering often takes the form of extracting a gene from one organism to put into another organism. Genes can also be supplied by cDNA libraries.

Suggest one **other** way to obtain a gene.

----- [1]

(b) A useful vector for moving and storing genes is the bacterial plasmid. Plasmids are closed loops of DNA. Plasmids in bacterial cells are separate from the main chromosome.

(i) Bacteria can transmit plasmids from one cell to another, or take up plasmids from the surrounding medium.

What is the benefit to bacteria of having these abilities?

----- [2]

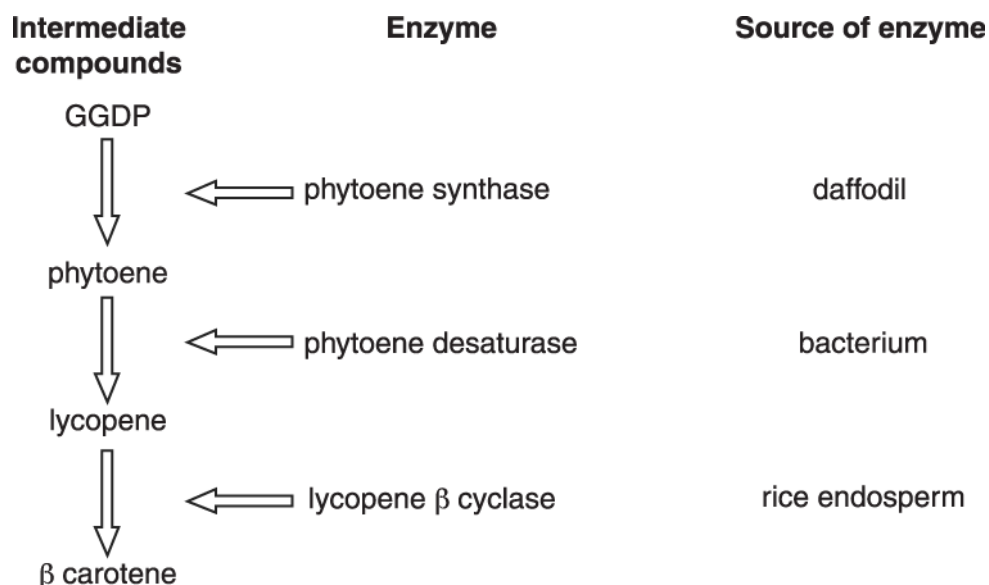
(ii) In genetic engineering, DNA fragments can be inserted into plasmids, which are then taken up by bacteria. The plasmid is cut open and the DNA fragment is sealed in using an enzyme.

Name the enzyme used to seal a DNA fragment into a plasmid.

----- [1]

- (c) Scientists used a transformed plasmid to insert genes into Golden Rice™, via the plantinfecting *Agrobacterium*.

The figure outlines the metabolic pathway by which early types of Golden Rice™ made β carotene, the precursor of vitamin A.

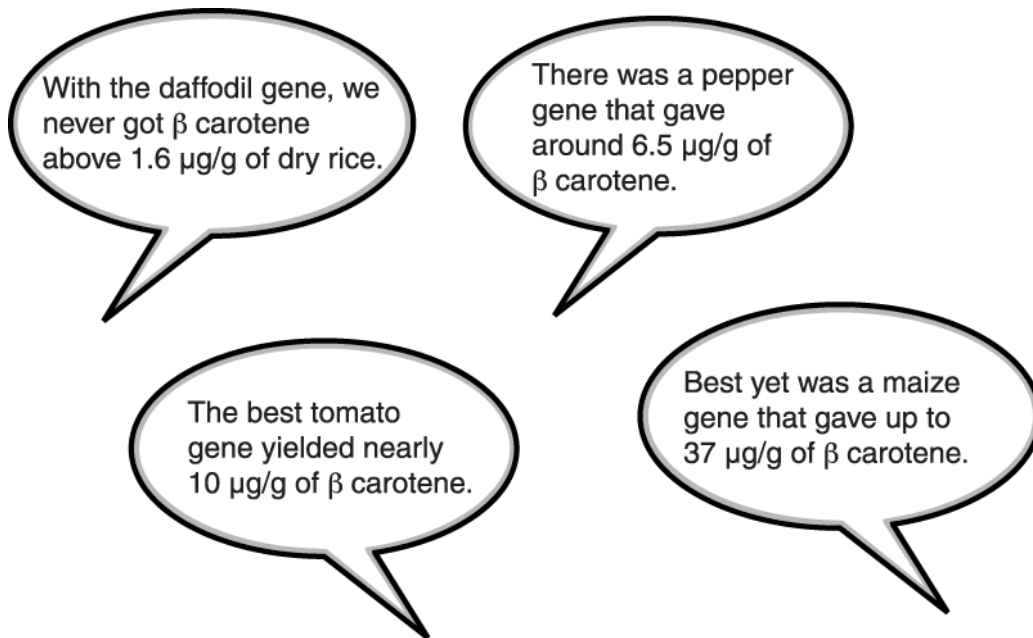


At first, conversion to β carotene was very inefficient. Analysis of quantities of intermediate compounds in the rice showed a build-up of GGDP and little phytoene.

- (i) Explain how the information above shows that the enzymes phytoene desaturase and lycopene β cyclase were **not** limiting the manufacture of β carotene.

[2]

- (ii) Phytoene synthase genes from other sources were then tried with these results:



The gene that makes phytoene synthase enzymes has slight differences between the species.

Suggest explanations for the different performances of these enzymes.

[2]

(d) State two ethical arguments, one **for** and one **against** this example of genetically manipulating a plant.

Argument for

Argument against

[2]

- Explain the difference between **somatic cell gene therapy** and **germ line cell gene therapy**.

[2]

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Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
1			C	1(AO2.6)	$((150 \times 60) \times 20) / 9000$
			Total	1	
2			D	1(AO2.5)	
			Total	1	
3		i	(phosphorylated) nucleotides ✓ <u>Taq</u> / thermostable , (DNA) polymerase ✓ (DNA) primers ✓	3 AO1.1	ALLOW nucleotide triphosphates
		ii	FIRST CHECK ON ANSWER LINE If answer = 7.69 OR $10^{7.69}$ award 2 marks amplification factor = 2^{15} (= 32 768) ✓ $\log_{10} (1\,500 \times 32\,768) = \log_{10} 49\,152\,000 = 7.69$ ✓	2 AO2.8	mark is for 2^{15} ALLOW $10^{7.69}$ ALLOW ECF from incorrect amplification factor
		iii	(E) A D G B C F ✓ ✓ ✓ ✓	4 AO1.2	If the order is incorrect, award one mark for each of the following: A first and F last A before G G before C
			Total	9	
4			A	1 AO1.2	
			Total	1	
5			C	1 AO1.1	
			Total	1	
6	a		<i>CRISPR</i> loss of function / knockout, so no residual gene activity ✓ effect is permanent ✓ CRISPR is specific to a particular DNA sequence / gene ✓ no protein synthesis ✓ <i>RNAi</i> some residual gene function / knockdown / ✓ effect not permanent ✓ miRNA not specific to a single gene ✓	max 4 AO2.7 3.2	ALLOW RNAi not as specific as CRISPR DO NOT ALLOW siRNA not as specific as CRISPR <u>Examiner's Comments</u> A lot of candidates scored no marks for this

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
			ref to low level of protein synthesis ✓		question, with a very small number gaining more than two marks. It was clear that many candidates did not understand the basic premise that both techniques targeted gene expression. Candidates need to appreciate that RNAi destroys mRNA but that multiple strands of mRNA are produced so that the gene is not permanently switched off whereas with CRISPR (they are told) the gene function is lost, and this would be permanent.  OCR support RNA interference case study is available on the delivery Guide for OCR AS/A Level Biology B (Advancing Biology) Gene technologies (5.1.3) https://www.ocr.org.uk/qualifications/as-a-level-gce-biology-b-advancing-biology-h022-h422-from-2015/delivery-guide/module-bb05-module-5-genetics-control-and-homeostasis/delivery-guide-bbdg029-gene-technologies-513 Exemplar 5 CRISPR • can make edits in DNA directly, so is a one-time solution to stop gene expression • mutations at site of DNA break could be unpredictable + harmful • could cause cancer RNAi • process would have to be repeated as only cuts mRNA, and transcription causes more mRNA to be synthesised • no risk of cancer In Exemplar 5. It is clear from the first two lines that the candidate understands that genes are permanently 'switched off' if the DNA is cut whereas RNAi cuts mRNA and more can be transcribed from the undamaged DNA.
	b		exons ✓ introns ✓ splicing ✓	3 AO1.1 1.2	Examiner's Comments Extrons was a common incorrect answer.
			Total	7	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
7			C	1	
			Total	1	
8			B	1	<u>Examiner's Comments</u> Many candidates confused SNPs with VNTRs and hence selected option C.
			Total	1	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
9		i	0.104 ✓✓✓	3	If answer is incorrect or missing, a maximum of 2 marks can be given for intermediate stages as follows: one mark for working such as • $4.0 - ((97.4 / 100) \times 4.0)$ • 4 & 0.026 • $100 - 97.4 = 2.6\%$ with 2.6% of 4.0 = 0.104 one mark for incomplete calculation • 3.896 one mark for correct answer but not quoted to 3dp e.g. 0.10 <u>Examiner's Comments</u> Candidates should provide answers that are to the same number of decimal places for other data in the same column. In this case the answer should be given to 3 decimal places.

Mark Scheme

Question	Answer/Indicative content	Marks	Guidance
ii	inhibits <u>translation</u> of mRNA ✓ (miRNA) binds at a <u>complementary</u> site (on mRNA) ✓ argonaute protein, breaks/cleaves, the mRNA strand ✓ AVP ✓	3 max	IGNORE references to 'miRNA inhibits mRNA' as this is given in the stem of the question DO NOT ALLOW references to inhibiting transcription Further detail e.g. • double stranded precursor binds to, dicer / endonuclease protein • dicer cuts precursor (into short segments) • dicer cuts precursor • (short double stranded) miRNA binds to argonaute protein • RNA induced silencing complex (RISC) formed • small sections of mRNA can be translated but will not result in formation of a, functional / complete, protein Examiner's Comments Some candidates did not score marks in this questions due to poor expression; for exampling muddling mRNA and miRNA in their answers. Others gave answers that related to enzyme inhibition which did not relate to the question. This area of the specification is new and examiners observed many answers that were lacking depth and detail. Exemplar 2 Double stranded RNA binds to a dicer which cleaves the RNA to around 200 nucleotide lengths. An argonaute protein binds to one of the RNA strands - RNA strand has a complementary sequence of nucleotides which will have complementary base pairing to the target mRNA. It binds from between mRNA and mRNA, miRNA cleaves the mRNA and prevents it from being translated.

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
		iii	miRNA less, precise / specific (than siRNA) ✓	1	DO NOT ALLOW 'miRNA is more accurate' ALLOW miRNA will bind to more than one mRNA ALLOW miRNA will inhibit, all / more, of the leghaemoglobin genes Examiner's Comments Again some less able candidates discussed enzyme inhibition rather than the difference between precision of miRNA and siRNA. Exemplar 3 <i>miRNA is more specific acting - that it will only inhibit one specific gene. siRNA affects is able to affect siRNA is more specific. miRNA is able to inhibit several of the genes at once / siRNA may only inhibit one.</i>
			Total	7	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
10		i	(drought resistance) involves many, genes / loci ✓ Involves (possible), multiple / variety, alleles (at each locus) ✓	2	ALLOW (drought resistance) is polygenic IGNORE different alleles <u>Examiner's Comments</u> Some candidates were able to appreciate the polygenic aspect of drought resistance and successfully linked the information in the stem of the question with genes and inheritance. Many candidates did not recognise the relevance of 'inheritance' and 'genetic basis' in the stem of the question and discussed their answers in terms of phenotype only e.g. not many plants survive, so there are very few that can be studied.
		ii	(gene/ transcription factor) can be used to identify, genes / alleles, involved / activated, in drought resistance ✓ promoters allow, gene expression / transcription ✓ remove / knockout, (miRNA 156) gene and observe, phenotype / drought resistance ✓	2	ALLOW RNA Polymerase binds to promoters <u>Examiner's Comments</u> Candidates struggled to understand the relevance of the miRNA gene and confused this with RNA. However, many candidates did discuss knocking out miRNA to observe the phenotype and so exhibited a good understanding of knockout technology.

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
		iii	place plasmid in <i>Agrobacterium</i> / use gene gun ✓ screen for, transgenic/ recombinant, cells ✓ details of selectable marker ✓	2 max	ALLOW use <i>Agrobacterium</i> as vector for 'place plasmid in <i>Agrobacterium</i>'. ALLOW a description of a gene gun IGNORE ref to viruses / liposomes IGNORE ref to antibiotics <u>Examiner's Comments</u> Few candidates scored full marks. Those that scored one mark usually referred to a gene gun for insertion of the plasmid. Most candidates did not appreciate that the alfalfa would be insertion into plant cells and referred to vectors relevant to animal cells e.g. viruses. There were a lot of descriptions using restriction enzymes and ligases to obtain the recombinant plasmid, not realising the focus of the question was on the incorporation of the plasmid.
			Total	6	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
11		i	DNA, cut / fragmented, using, restriction enzymes / endonucleases ✓ separate fragments using gel electrophoresis ✓ sequence fragments / add labelled probe ✓ compare to database / DNA samples from other individuals ✓	2 max	ACCEPT any valid method of labelling Examiner's Comments Candidates struggled with (c)(i) and could not discuss it in sufficient detail. Many candidates discussed electrophoresis separating the SNPs, not appreciating that it separates on the basis of size and a probe would need to be used to pick up individual SNPs. Indeed some candidates stated that the DNA ladder would identify the SNPs and did not seem to realise that a DNA ladder is also based on size only. Many candidates spoke about PCR being used to amplify the sample thus not realising that this would not be necessary as enough DNA would be available to proceed without performing PCR.
		ii	greater risk of, developing breast cancer / having mutated gene, if have family history ✓ high cost means screening must be restricted ✓ living relative needed to identify faulty gene ✓ (this gene) tested for in the individual / predictive testing ✓	2 max	Examiner's Comments For (c)(ii) most candidates achieved mp2 with a good understanding of the financial cost of testing everyone. Some candidates felt that the emotional impact of testing everyone should be considered and a few discussed the testing in terms of causing harm to the patient. Thus some candidates had not connected this question with the previous question of taking a blood sample and were visualising this method as a radioactive procedure. Many candidates wrote about the increased chance of inheriting the gene without realising that it is the mutated gene that is relevant, as everyone inherits these genes. The importance of the BRCA genes should not be restricted to their mutated forms and breast cancer.
			Total	4	

Mark Scheme

Question	Answer/Indicative content	Marks	Guidance
12	Summary of instructions to markers: <i>Read through the whole answer. (Be prepared to recognise and credit unexpected approaches where they show relevance.) Using a 'best-fit' approach based on the science content of the answer, first decide which of the level descriptors, Level 1, Level 2 or Level 3, best describes the overall quality of the answer.</i> <i>Then, award the higher or lower mark within the level, according to the Communication Statement (shown in italics):</i> <i>o award the higher mark where the Communication Statement has been met.</i> <i>o award the lower mark where aspects of the Communication Statement have been missed.</i> <i>The science content determines the level.</i> <i>The Communication Statement determines the mark within a level.</i> Level 3 (5 – 6 marks) A detailed outline, including the correct naming of enzymes and a correct description of their roles. Stages of the process are in the correct order, well detailed and with no major stages missing. <i>There is a well-developed line of reasoning which is clear and logically structured. The information presented is relevant and substantiated.</i> Level 2 (3 – 4 marks) Outline includes the correct naming of an enzyme and a description of its role. Stages of the process are in the correct order, although some stages may be missing. <i>There is a line of reasoning presented with some structure. The information presented is in the most-part relevant and supported by some evidence.</i>	6	Indicative scientific points may include: Gene - isolate human gene with restriction enzyme OR - reverse transcribe mRNA with reverse transcriptase Vector - cut open vector / plasmid with (same) restriction enzyme Gene and vector - joining of gene and vector with DNA ligase - annealing of complementary bases - molecular detail, e.g. ligation of sugar-phosphate backbones / formation of phosphodiester bonds - ref to recombinant vector / plasmid

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
			Level 1 (1 – 2 marks) No enzymes are named but the roles of the enzymes may be described. Stages of the process may not be in the correct order and some may be missing. <i>There is an attempt at a logical structure with a line of reasoning. The information is in the most part relevant.</i> 0 marks No response or no response worthy of credit.		IGNORE reference to DNA polymerase Bacteria • mix plasmid and bacteria • transform bacteria / electroporation Additional detail • e.g. addition of sticky ends with terminal transferase • reference to identification of transformed bacteria e.g. antibiotic resistance IGNORE ref to protein purification Examiner's Comments (c) was the second Level of Response question. Outlining the role of enzymes in recombinant DNA technology appeared to be more accessible than the first with significantly more candidates achieving the higher marks. Organised and well-structured responses were seen from many candidates with the attention to scientific detail proving to be the discriminating feature. The most common stage to be left out of the sequence was the transformation stage – even simple mixing of the bacteria and the plasmid together was often omitted. DNA ligase was sometimes omitted or inaccurately replaced with DNA polymerase. Descriptions of how the enzyme catalysed the formation of hydrogen bonds rather than phosphodiester bonds were not uncommon and less regularly seen errors included the injection of the plasmid into cells (sometimes even into human cells). It was pleasing to see a good range of additional material relating to identification of the transformed bacteria.
			Total	6	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
13			A ✓	1	Examiner's Comments Candidates scored highly on this question, most correctly identifying the pair of primers that could be used to amplify DNA from all species.
			Total	1	
14			D ✓	1	Examiner's Comments This question was assessing whether candidates understood that not all the DNA sequence is transcribed into mature mRNA. The most common incorrect response was that repair of the mRNA took place.
			Total	1	
15	a		<i>Stage 2</i> DNA is being denatured / 2 strands separate (by H-bonds breaking) ✓ <i>Stage 3</i> primers, anneal / bind, to complementary strands (of DNA) ✓	2	ALLOW DNA is being annealed
	b	i	(Taq DNA polymerase / it) catalyses the addition of nucleotides (to the primers) to form DNA strands / polynucleotides ✓	1	ALLOW extends the DNA from the primers (allowing for the formation of cDNA strands) ALLOW makes double stranded DNA from single stranded DNA template
		ii	(Taq DNA polymerase / it is) not (thermally) denatured ✓ by the high temperatures needed to separate the DNA strands / at 90° C ✓	2	IGNORE optimum at ~70°C
	c	i	1×10^9 (a.u) ✓	1	DO NOT ALLOW 1000 000 000 ALLOW answers in range 5×10^8 to 2.5×10^9
		ii	log(arithmetic) ✓	1	
			Total	7	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
16		i	germ line: all cells of organism will contain healthy gene ✓ somatic: only cells targeted will contain healthy gene / healthy gene not, inherited / passed to offspring ✓	max 2	
		ii	any 1 advantage: easy to deliver gene to cell / infection is efficient ✓ delivery to specific, cells / tissues ✓ any 1 disadvantage: infected cells could raise immune response ✓ only dividing cells would be infected ✓ ref to regain of viral virulence ✓	max 2	ALLOW patient concern over use of virus
			Total	4	
17			C ✓	1	
			Total	1	
18			(track) D ✓ <i>explanation - up to 2 marks</i> detergent destroys (secondary, tertiary and) quaternary structure ✓ (4) subunits smaller so migrate lower down gel ✓ all subunits are, same / a similar size / 492 amino acids ✓	max 3	If incorrect track identified then award up to two marks
			Total	3	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
19		i	C- cytosine AND T- thymine;	1	ACCEPT phonetic spelling for cytosine DO NOT CREDIT cysteine DO NOT CREDIT 'thiamine' Examiner's Comments Three AO1 marks were available on this question in (a)(i), (c)(i) and (c)(ii). Part (c)(i) was done well by most candidates although a few 'cytokines' and 'Thiamines' were seen.
		ii	restriction (endonuclease enzyme); palindrome / palindromic; phosphodiester / covalent (bond);	3	ACCEPT phonetic spelling throughout Examiner's Comments In (ii) the terms restriction enzyme and palindromic sequence were well known but even very good candidates seemed to think that it was hydrogen bonds that were being cut. This may have been a 'gut response' to a question on DNA but it was a widespread misconception which needs to be addressed in Centres.

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
		iii	<i>describe:</i> C variant gives 2 bands AND T variant gives 1 band; C variant band closer to bottom(of gel) / T variant band(s) closer to top (of gel); <i>explain:</i> <i>idea that</i> enzyme does not 'cut' T variant / enzyme cuts C variant; <i>idea that</i> GAATTT / T variant, no longer complementary to active site of, restriction / EcoR1, enzyme;	3	ACCEPT idea that more bands / lines seen in C variant CREDIT C variant (band(s)) moved further (down the gel towards anode / away from the wells) / T variant (band) moved less distance (down the gel) ACCEPT idea that restriction enzyme does not recognise / fit / bind to T variant region Examiner's Comments Part (iii) contained elements of stretch and challenge. Many candidates were clearly familiar with gel electrophoresis as a technique but relatively few could explain why the banding patterns would differ between the "C" and 'T' variant. There were some obvious misconceptions such as the restriction enzyme cutting out the whole of the palindromic sequence. Responses seem to suggest that a lot of 'rote' learning is taking place on these topics without necessarily developing the knowledge and understanding.
			Total	7	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
20		i	D first and B second; C last;	2	Examiner's Comments Most candidates were able to correctly sequence the stages.
		ii	(HIV) antibody test; (HIV) antigen test;	1 max	ACCEPT ELISA ACCEPT correct description (e.g. use antibodies which will attach to antigens from the virus). Examiner's Comments Most candidates could correctly name another test for HIV. Some imprecise answers such as 'blood test' failed to gain credit.
			Total	3	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance												
21	a		<table><thead><tr><th>Term</th><th>Insert a tick</th></tr></thead><tbody><tr><td>Genetic engineering</td><td></td></tr><tr><td>Therapeutic cloning</td><td>✓</td></tr><tr><td>Reproductive cloning</td><td></td></tr><tr><td>ICSI</td><td></td></tr><tr><td>IVF</td><td></td></tr></tbody></table> ;	Term	Insert a tick	Genetic engineering		Therapeutic cloning	✓	Reproductive cloning		ICSI		IVF		1	Examiner's Comments This question addressed AO1 and AO2 objectives. DO NOT CREDIT if more than one box has been ticked DO NOT CREDIT hybrid ticks Examiner's Comments While most candidates could identify therapeutic cloning, responses were seen that covered each of the options given with some candidates ticking more than one box. Part (b) was accessible although some candidates did make the same point twice regarding the ability to differentiate into a variety of different cells.
Term	Insert a tick																
Genetic engineering																	
Therapeutic cloning	✓																
Reproductive cloning																	
ICSI																	
IVF																	
	b	i	embryonic / pluripotent ;	1	Mark the first answer. If the answer is correct and an additional answer is given that is incorrect or contradicts the correct answer = 0 marks ALLOW totipotent / multipotent IGNORE omnipotent												
		ii	mark in any order can (continue to) divide ; pluripotent OR able to differentiate into / AW, (several) different types of, cells / tissues ; <i>idea that</i> have same antigens as patient / will not be rejected (by patient's immune system);	2	Mark the first answer on each prompt line. If the answer is correct and an additional answer is given that is incorrect or contradicts the correct answer = 0 marks IGNORE 'totipotent'												
			Total	4													

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
22	a	i	gene, only enters cells lining lungs / does not enter all cells / not in sex cells; (gene) is not passed on to, next generation / offspring; this is not germ line therapy;	2	Examiner's Comments In (i) a surprising number of candidates wrote about the gene not being incorporated into the sex chromosome or seemed to confuse autosomal chromosomes with somatic cells, for example 'Only targets ordinary cells because these are controlled by autosomal chromosomes not sex chromosomes'.
		ii	DNA will not cross (cell surface / plasma) membrane; as molecule is too large OR molecule is hydrophilic / water soluble; liposome fat globule can, cross / fuse with, cell surface membrane;	2	ACCEPT 'phospholipid bilayer' CREDIT the reverse argument e.g. molecule is not small enough / is not fat soluble CREDIT idea that endocytosis can happen Examiner's Comments In (ii) some candidates did not focus on the idea of getting the DNA into the cell and answered in terms of getting the DNA into the body and protecting it from an immune response. The idea of endocytosis was picked up by good candidates but without explaining why the nature of DNA made this necessary.
	b	i	gene (on chromosome 7) has exons and introns; <i>idea that</i> (final) mRNA, has introns removed; (final) mRNA / cDNA (gene), only has exons; (because) no enzyme in bacteria to, remove introns / edit mRNA;	2	Examiner's Comments This question addressed both AO1 and AO2. In (i) the examiners were looking for clear reference to exons and introns and good candidates picked up on this. Some responses were in terms of 'coding' and 'non coding' DNA which missed the point that the question was referring to the DNA within a single gene. A few candidates confused introns and exons with many more answering incorrectly in terms of 'missing' bases because of sticky ends.

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
		ii	1(plasmid is) cut using, restriction enzyme / endonuclease; 2(restriction enzyme) hydrolyses / AW, phosphodiester bond / sugar phosphate backbone; 3enzyme binds to / AW, palindromic sites / specific recognition sites; 4(restriction enzyme) gives plasmid complementary sticky ends (to those on CFTR gene); 5plasmid and CFTR gene (sticky ends) anneal / hydrogen bonds form between (complementary) base pairs; 6DNA ligase used to seal up (sugar phosphate) backbone; 7(ligase) condensation reaction / forms phosphodiester bond / joins the sugar phosphate backbone;	4	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
		ii	QWC;	1	Award if the following mps are awarded mps 1 and 2 OR mps 6 and 7 <u>Examiner's Comments</u> In (ii) there was much confusion in the use of terminology and the action of enzymes. Many candidates are under the false impression that restriction enzymes cut out a section of the plasmid. Others described the use of a vector to get the gene into the plasmid. A common misunderstanding was that DNA ligase was required for annealing, rather than annealing being a stage which did not require an enzyme with the ligase being used after annealing to form covalent bonds by a condensation reaction to reform the sugar-phosphate 'backbone'. It was clear that many candidates had not thought synoptically about the enzymes involved in genetic engineering and it was very rare to see any reference to condensation reactions (ligase) or hydrolysis reactions (restriction enzymes).

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
	c		(introduced) DNA / CFTR gene, broken down in cytoplasm; (DNA broken down) by lysosomes; <i>idea that</i> mitosis occurs in epithelial cells / epithelial cells will be dividing; (introduced) DNA / CFTR gene, not, replicated / copied (during interphase); CFTR gene not present in daughter cells;	2	ACCEPT description of cells in lining of airways or lungs CREDIT reverse argument 'only genome is copied' DO NOT CREDIT ref to DNA replication in mitosis <u>Examiner's Comments</u> This was a stretch and challenge question and some candidates certainly answered along the right lines – that cells would be dividing and that, as it is not in the genome, the gene would not be replicated and so not passed on to daughter cells. However, careless structuring of responses meant some otherwise excellent candidates referring to the gene not being replicated in mitosis and this could not be credited. Weaker candidates did not read the stem of the question which indicated that the gene was in the cells and answered in terms of the gene being broken down in the body by the immune system.

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
	d		virus stimulates, an immune response / antibody production; <i>idea that</i> second / subsequent, doses produce greater amounts of antibodies; <i>idea that</i> antibodies, bind to virus / stops it entering the cell;	2	CREDIT correct reference to secondary immune response <u>Examiner's Comments</u> This was synoptic with F222. The majority of candidates were aware that an immune response would be triggered but many did not address the question stem which was why repeated doses were ineffective and good references to the secondary immune response were only rarely seen.
			Total	15	

Mark Scheme

[illegible]

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
		iii	33;;	2	Correct answer = 2 marks If answer is incorrect: Award one mark for correct calculation of weight ($67.5 \div 0.9 = 75$) OR for a BMI given as 33.3(3333333) OR for correct BMI calculated from an incorrect body weight (e.g. a body mass divided by 2.25 (height in metres squared) (given to the nearest whole number) <u>Examiner's Comments</u> In (iii) the calculation was done well with the majority of candidates stating the correct answer to the required degree of precision.

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
		iv	<i>for body mass calculated above 30</i> <i>idea that</i> (BMI means that, Mrs G is) obese / obesity is a risk factor; (risk of) high blood pressure / high (blood) cholesterol / atherosclerosis / described; <i>for body mass calculated less than 25,</i> <i>applying ecf</i> another risk factor (other than BMI) may have contributed to stroke; <i>(for any body mass calculation)</i> example of other risk factors (independent of BMI);	2	CREDIT reference to 'overweight' rather than obesity if BMI was calculated to be between 25 and 30 IGNORE reference to overweight (other than in above statement) ACCEPT (risk of) diabetes ACCEPT idea that this BMI does not pose an increased risk e.g. smoking / used HRT / age / family history <u>Examiner's Comments</u> Part (iv) asked them to interpret this answer and was synoptic to F222. A common mistake was to state that a BMI of 33 meant that the person was overweight rather than obese but otherwise this question was done well.
			Total	8	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
24	a	i	e;	1	CREDIT X ^e ACCEPT a single, lower case alternative letter IGNORE 'c' unless it is clearly 'lower case' IGNORE I ^e
		ii	X ^e Y and X ^e X ^e	1	ecf for the allele symbol in (i)
		iii	X ^E X ^e	1	ecf for the allele symbol in (i) Examiner's Comments While many were aware that 'e' would be the allele symbol for the condition, some put a genotype (ee) in (i) and only the better candidates went on to give the correct genotypes for (ii) and (iii).
	b	i	1. nucleus / nucleolus; 2. ribosomes;	1	ANSWERS MUST BE IN THIS ORDER ACCEPT RER / rough endoplasmic reticulum
		ii	<i>idea that</i> mutation (in DNA) leads to a, stop / termination, codon OR <i>idea that</i> DNA sequence is deleted OR <i>idea that</i> mutation leads to more RNA being spliced out; <i>idea that</i> translation is terminated by stop codon; no (more) amino acids are added (after the stop codon) / fewer amino acids (in truncated protein);	2 max	IGNORE ref to a base deletion in context of shorter DNA Examiner's Comments Part (i) was synoptic and answered well but in (ii) too many answers were given in terms of changing the sequence of amino acids rather than producing a protein with fewer amino acids.
	c	i	reverse transcriptase;	1	
		ii	somatic (gene therapy);	1	CREDIT augmentation (gene therapy)

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
		iii	<i>Idea that</i> disease involves a defect in a single gene; no other cause; <i>idea that</i> disease is easily identified in sufferers; gene / gene product, is known; gene can be delivered reliably to affected cells;	3	Mp2 ACCEPT idea that disease will be cured / vision will be restored. Mp3 CREDIT reverse argument 'easy to see if it has worked' ACCEPT 'easy to insert the gene' Examiner's Comments This was intended to be accessible so it was disappointing how many candidates did not 'spot' reverse transcriptase or the fact that this would be somatic gene therapy. In part (iii) many candidates did not pick up on the question rubric and answered in terms of germ line therapy or ethics. It would be a worthwhile discussion for Centres to have with their candidates as to what makes some genetic diseases targets for treatment using gene therapy while others are not.
			Total	12	
25		i	Sickle cell anaemia / PKU / Cystic Fibrosis	1	DO NOT ALLOW Huntington's ALLOW alternative correct suggestions
		ii	(X in intron) <i>idea that</i> code in this region does not appear in mature mRNA (1) <i>idea that</i> introns are transcribed but edited out (1)	2	ALLOW an explanation that this region does not code for amino acids
		iii	<i>idea that</i> genetic code is degenerate (1) <i>idea that</i> new triplet codes for an amino acid with a similar R group (1) no change in the tertiary structure of the protein (1)	2	ALLOW a description - some amino acids have more than one triplet ALLOW idea of 'little or no change' if second mark point is awarded ALLOW three dimensional protein structure DO NOT ALLOW 'no change in protein structure' without further qualification since 'structure' is given in the stem of the question
			Total	5	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
26			* Level 3 (5–6 marks) Candidate addresses all the ideas in the student's statement making clear connections between humans and bacteria and using examples to illustrate their answers. <i>There is a well-developed line of reasoning which is clear and logically structured. The information presented is relevant and substantiated.</i> Level 2 (3–4 marks) Candidate addresses some of the ideas in the student's statement making some connections between humans and bacteria and using at least one example to illustrate their answers. <i>There is a line of reasoning presented with some structure. The information presented is in the most-part relevant and supported by some evidence.</i> Level 1 (1–2 marks) Simple comments about humans and bacteria made with connections not always made. Little exemplification. <i>The information is basic and communicated in an unstructured way. The information is supported by limited evidence and the relationship to the evidence may not be clear.</i> 0 marks No response or no response worthy of credit.	6	Examples of relevant scientific points: Benefits from the use of bacteria • nitrogen cycling – including the role of putrefying, denitrifying, nitrogen-fixing and nitrifying bacteria. All benefitting food production • biotechnology – including genetic modification techniques that lead to benefits due to the production of drugs, insulin, the broadening of scientific research. Ideas might include the palindromic nature of recognition sequences for restriction enzymes and the need for reporter genes on plasmids. Challenges from interactions with bacteria • communicable diseases – including general mechanisms of pathogenicity of bacteria, causes, transmission, mode of infection, symptoms, treatment e.g. TB • the use of antibiotics and antibiotic resistance, including reference to TB and MRSA. Examples of technical terms that could be used in answers: Mycobacterium, communicable disease, prevalence, incidence, denitrification, saprotrophs, nitrification, named bacterial taxa, restriction enzymes, palindromic sequence, plasmid.
			Total	6	

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27	a	i	mode = 2 mean = 3.11	3	$(2 \times 9) + (2 \times 8) + (3 \times 11) + (6 \times 7) / 35 = 3.11$ Award one mark if a candidate has clearly taken the number of participants into account but has made an error in the calculation
		ii	<i>General points</i> <i>Idea of valid conclusions</i> would require information about the intensity of exercise <i>Idea that</i> (named) statistical test would confirm conclusion <i>Duration (up to a maximum of 2 marks)</i> positive correlation (suggests the conclusion is valid) many studies / repeats / AW, improves validity <i>idea of relationship / correlation</i> , clearer for cycling (than running) <i>Running vs cycling (up to a maximum of 2 marks)</i> <i>idea that</i> (conclusion is valid because) for a given duration, IL-6 increases more with running (than cycling) the number of participants varies, which makes comparison difficult	4	The general point marks can be scored for either section, but can only be scored once ALLOW 'meta-analysis' for 'many studies' ALLOW calculated example
	b	i	Any 3 from: <i>Glucose uptake</i> (IL-6) increases / AW, insulin production (IL-6) increases / AW, insulin receptor sensitivity (on muscle cells) <i>Glucose production</i> (IL-6) increases / AW, glucagon production glycogen converted to glucose (in liver cells)	3	ALLOW acts like a hormone ALLOW glycogenolysis
		ii	* Level 3 (5–6 marks) Candidate's evaluation demonstrates excellent judgement of the data, providing conclusions that address all the significant issues. <i>There is a well-developed line of reasoning which is clear and logically structured. The information presented is relevant and substantiated.</i>	6	Examples of relevant scientific points at L3 (in addition to points at L1 and L2) <i>experimental & epidemiological evidence</i> • discussion of <i>in vitro</i> v <i>in vivo</i> experiments (e.g. the conflicting results in A and B; <i>in vitro</i> experiments might lack important factors, for example, found in <i>in vivo</i>) • discussion of the link between TNF and IL-6 (e.g. G and H. IL-6 could, for

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			Level 2 (3–4 marks) Judgement is made on a range of aspects of the data, but conclusions are not comprehensive. <i>There is a line of reasoning presented with some structure. The information presented is in the most-part relevant and supported by some evidence.</i> Level 1 (1–2 marks) Simple conclusions are made, drawing on limited aspects of the data. <i>The information is basic and communicated in an unstructured way. The information is supported by limited evidence and the relationship to the evidence may not be clear.</i> 0 marks No response or no response worthy of credit.		example, be high in certain cases because it has a defensive role against TNF) i.e. detailed links made between studies in Table 1.2 and impact on diabetes risk - in depth discussion of correlation v causation (e.g. C and D show correlation but not causation). <i>biological knowledge</i> - detailed understanding of the causes and symptoms of type 2 diabetes. Examples of relevant scientific points at L2 (in addition to points at L1) <i>experimental & epidemiological evidence</i> - discussion of correlation v causation - linking exercise, low baseline IL-6 and possible effects on diabetes risk - quite detailed links made, some in detail, between studies in Table 1.2 and the impact on diabetes risk. <i>biological knowledge</i> - good understanding of the causes and symptoms of type 2 diabetes. Examples of relevant scientific points at L1 <i>experimental & epidemiological evidence</i> - basic links made between studies in Table 1.2 and the impact on diabetes risk. <i>biological knowledge</i> - some understanding of the causes and symptoms of type 2 diabetes.
	c	i	<i>idea that</i> (gene is) replaced / disrupted / AW, with, artificial / synthetic / AW, DNA	1	

Mark Scheme

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		ii	Any 1 from: (mice have) similar, genome / DNA sequences, to humans (mice have) similar, metabolism / physiology, to humans (mice are) easy / cheap, to, house / manipulate / breed	1	
	d		chemotaxis attraction of neutrophils (to site of infection) OR cause / stimulate, B cells / T cells, to, differentiate / proliferate cause / stimulate, B cells to release antibodies OR inhibit virus replication activation / AW, of T killer cells	2	ALLOW (undergo) clonal expansion
			Total	20	
28			B	1	
			Total	1	
29			B	1	
			Total	1	
30			D	1	
			Total	1	
31		i	ACGGGAAGGGCCCCGAGCACGGA UGCCCUUCCCGGGCUCGUGCCU	1	
		ii	hydrolysis	1	IGNORE 'cleavage'
		iii	Any 3 from: (activated) RISC, cleaves / AW, viral mRNA no viral proteins made no, viral particles / AW, assembled <i>idea that</i> no new cells are infected	3	IGNORE 'virus cannot spread' as this is given in the question
			Total	5	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
32			C	1	
			Total	1	

Mark Scheme

Question			Answer/Indicative content	Marks	Guidance
33	a		chemical synthesis / polynucleotide sequencing;	1	ACCEPT make an artificial (gene) / manufactured (gene) / synthetic (gene) IGNORE refs to gene bank, cDNA library, BAC's, using reverse transcriptase/ making cDNA from RNA Examiner's Comments Common wrong answers in part (b) included using a gene/DNA probe, transcription, reverse transcriptase and PCR. Very few suggested making an artificial/synthetic gene, or using polynucleotide sequencing.
	b	i	(bacteria) acquire / take up / gain, (useful) genes; example of useful gene; faster / without waiting for mutation;	2 max	ACCEPT sharing genetic information/ increase genetic variation / sharing DNA IGNORE 'transfer / passing on genes' ACCEPT (gene for) antibiotic resistance, enzyme to metabolize new nutrients DO NOT CREDIT 'become immune to antibiotics' Look for the idea of accelerated acquisition. e.g. quicker /in one generation Examiner's Comments Full marks were usually achieved through gaining mp1 and mp2. Mp3 was rarely awarded. A number of answers spent time rewording the question and failed to gain marks e.g. '<i>bacteria take up plasmids with antibiotic resistance and transfer resistance</i>'. A lot of candidates wrote about antibiotic resistance but failed to link it to a gene and so did not gain credit. Few candidates used the term 'immunity' which was pleasing to see since this misconception has often been seen in the past.

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		ii	(DNA) ligase;	1	Mark the first answer. If that answer is correct and an additional answer is given that is incorrect or contradicts the correct answer then = 0 marks. Most candidates gave the correct DNA ligase. Those that gained no marks typically gave no response or DNA polymerase as their answer. Examiner's Comments Most candidates gave the correct DNA ligase. Those that gained no marks typically gave no response or DNA polymerase as their answer.
	c	i	<i>phytoene synthase</i> is, limiting / in low quantities / low activity; little, phytoene / substrate, for <i>phytoene desaturase</i>; little, lycopene/ substrate, for <i>lycopene ? cyclase</i>;	2 max	Examiner's Comments This proved to be a difficult question for two marks. Most students scored one by recognising that <i>phytoene synthase</i> was the limiting factor because it was the enzyme that catalyses the first stage in a metabolic pathway. However the question asked why the other two enzymes were not limiting and only a few candidates were able to answer this.

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Question			Answer/Indicative content	Marks	Guidance
		ii	different base sequences (in the different genes/ DNA); different amino acid sequences (in the different enzymes); different, tertiary/3D, structures/ shape (in the different enzymes);	2 max	ACCEPT different, triplet /codon/ nucleotide, sequences. ACCEPT different primary structures ACCEPT refs to active site different shape Examiner's Comments The biochemistry required for this answer was surprisingly elusive. Some candidates tried to link their answer with active sites, but most of these failed to mention that it was the shape that was important. Of those candidates who recognised they needed some biochemistry here, most did not use the term 'sequence' when suggesting differences in DNA or protein primary structure. Many proposed different environmental conditions affecting the different plants, which did not gain credit.

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Question			Answer/Indicative content	Marks	Guidance
	d		<i>For:</i> relief of, vitamin A deficiency / symptoms of vitamin A deficiency; <i>Against:</i> expense of, seed to (poor) growers / grain to consumers; (uncontrolled) hybridization with other rice, species / types / varieties; unknown long-term effects on consumers' health;	2 max	IGNORE refs to other instances of genetic engineering. ACCEPT prevents blindness, improves immune system, increase vitamin A uptake IGNORE helps eyesight / prevents death ACCEPT refs to putting (non GM) farmers out of business IGNORE refs to gene crossing to different plant species. IGNORE refs to “against nature”, “playing God”, loss of biodiversity Examiner's Comments Part (e) was a straightforward question about advantages/disadvantages for GM crops but note two areas for improvement: <i>For</i> ? candidates must read and answer the question being asked. Answers in terms of making food more nutritious or supplying more vitamins were not credited as this question asked about golden rice and needed the advantage to be linked to reducing vitamin A deficiency, or symptoms of vitamin A deficiency. <i>Against</i> ? it is disappointing that a significant minority of candidates still thought 'playing God' was a legitimate scientific objection to genetic modification. If they did write about unknown consequences it was often in generalised terms such as a loss of biodiversity, or unknown long term effects, which did not gain credit. Some candidates correctly talked about hybridisation with wild rice species or unknown long term effects on human health. A few mentioned the cost of seed to farmers.

Mark Scheme

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	e		S1 cannot be inherited OR G1 can be inherited; S2 introduces (functional), gene/allele, into, patient/body cell /non reproductive cell OR G2 introduces, (functional), gene/allele, into sperm / egg / zygote/ embryo; S3 only some cells have (functional), gene/ allele OR G4 all cells have (functional), gene/ allele; S5 short lived / temporary / needs repeating OR G5 long lived / permanent / does not need repeating;	2 max	<i>one mark for somatic (S) and one mark for germ line (G)</i> IGNORE ref to legality / ethical issues S1 / G1 ACCEPT (gene / allele) passes e.g. S (gene / allele) does not pass to offspring S1 / G1 IGNORE (gene / allele) affects e.g. G (gene / allele) does not affect offspring S2 / G2 DO NOT CREDIT altering / removing / replacing, genes Examiner's Comments Many candidates reworded the stem of the question, saying that germ line gene therapy affects embryos and somatic gene therapy affects somatic cells. Others missed the term 'insertion of gene', instead writing about manipulating cells, or manipulated/altered DNA, which did not gain credit. The most common correct mark seen was the idea of no need to repeat the treatment for germ line gene therapy, or insertion of genes into embryos. Quite a few candidates then went on to discuss the ethics and legality between the two types of treatment, which gained no credit. Another common error was genes being altered or replaced. Some candidates found it difficult to specify the cells involved, giving the impression that they think a sperm cell is undifferentiated or not present in an adult's body.
			Total	12	