1. 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.

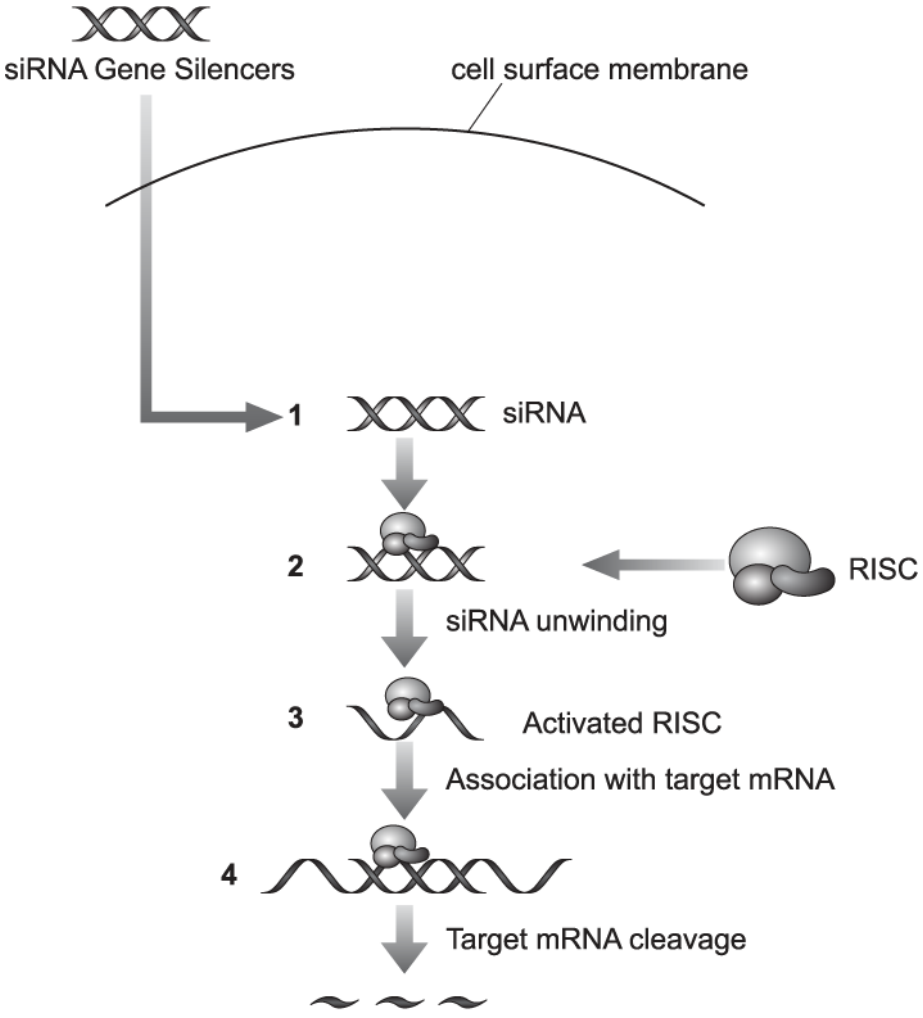


Fig. 36.1

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

ACGGGAAGGGCCCGAGCACGGA

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]

2. * A student wrote the following statement:

Bacteria can be harmful and cause disease, but some bacteria can play important roles in the environment, for example, recycling nutrients. In recent years, scientists have developed techniques to genetically alter bacteria. These genetically modified bacteria have allowed us to produce useful substances.

Using the ideas in the student's statement, outline the relationship between humans and bacteria.

---

[6]

3(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.

---

[2]

(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.

[5]

(c). The introduction of normal, functional copies of the CFTR gene into cells allows the cells to produce temporarily a functioning protein.

- 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.

Suggest why the failure of the CFTR gene to incorporate into the cell genome means that the beneficial effect of the gene therapy is only temporary.

---

[2]

4(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.

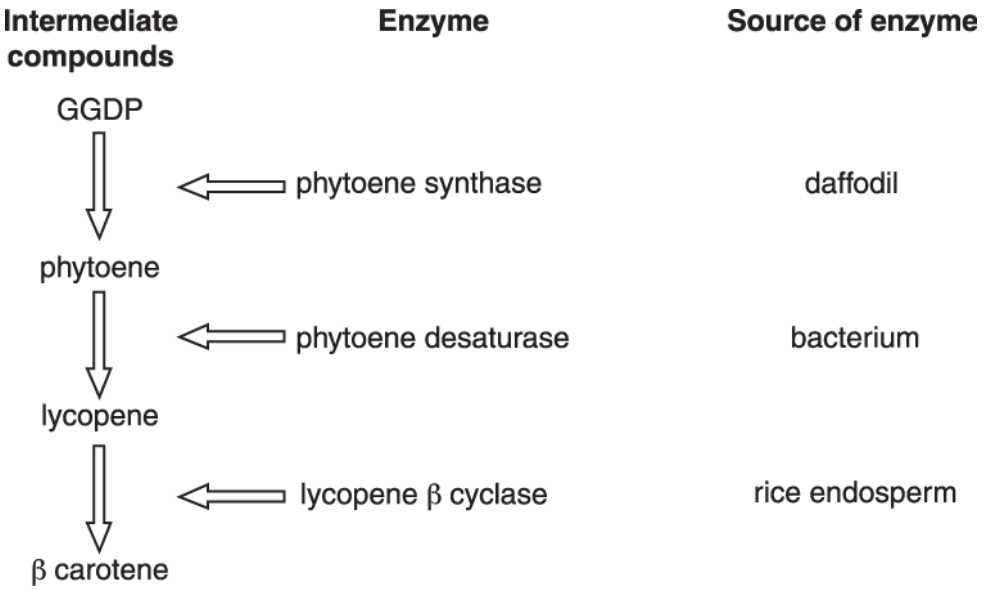
Name the enzyme used to seal a DNA fragment into a plasmid.

---

[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.



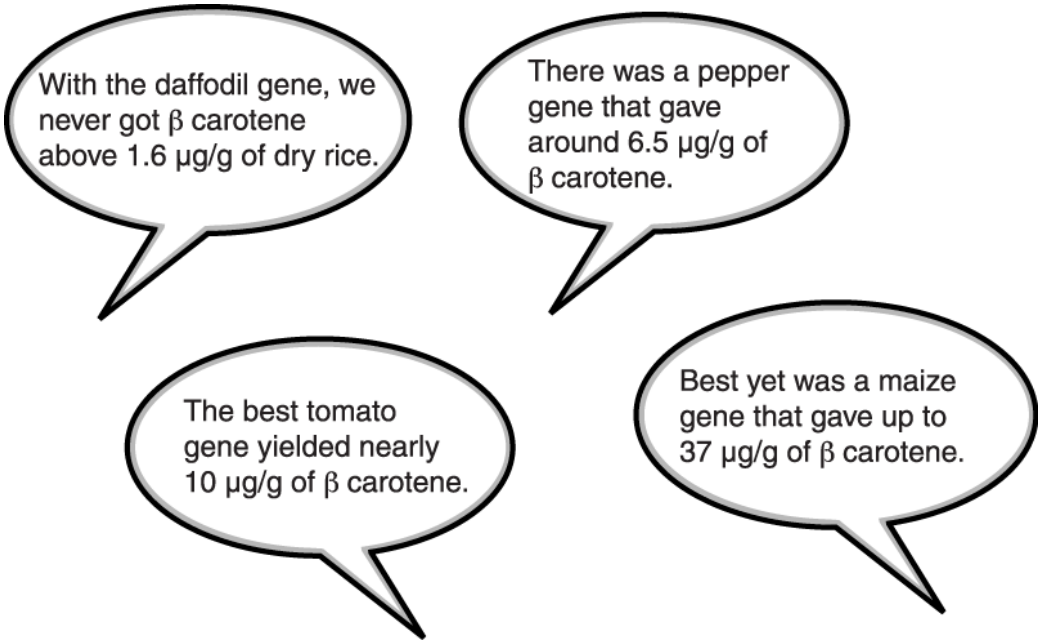
At first, conversion to β carotene was very inefficient. Analysis of quantities of intermediate compounds in the rice showed a build-up of GGDP and little phytoene.

(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]

(e). Genetic engineering is successful in isolating healthy alleles of a gene and putting them into suitable vectors. This opens exciting possibilities for treating human genetic diseases.

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

---

[2]

5.

(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]**

END OF QUESTION PAPER

Question			Answer/Indicative content	Marks	Guidance
1		i	*ACGGGAAGGGCCCGAGCACGGA* 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 *idea that* no new cells are infected	3	IGNORE 'virus cannot spread' as this is given in the question
			Total	5	

Question			Answer/Indicative content	Marks	Guidance
2			*** 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. *There is a well-developed line of reasoning which is clear and logically structured. The information presented is relevant and substantiated*. **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. *There is a line of reasoning presented with some structure. The information presented is in the most-part relevant and supported by some evidence*. **Level 1 (1–2 marks)** Simple comments about humans and bacteria made with connections not always made. Little exemplification. *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*. **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**	

Question			Answer/Indicative content	Marks	Guidance
3	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; *idea that* (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.

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; 3 enzyme binds to / AW, palindromic sites / specific recognition sites; 4 (restriction enzyme) gives plasmid complementary sticky ends (to those on CFTR gene); 5 plasmid and CFTR gene (sticky ends) anneal / hydrogen bonds form between (complementary) base pairs; 6 DNA ligase used to seal up (sugar phosphate) backbone; 7 (ligase) condensation reaction / forms phosphodiester bond / joins the sugar phosphate backbone;	4	

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 Examiner's Comments 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).

Question			Answer/Indicative content	Marks	Guidance
	c		(introduced) DNA / CFTR gene, broken down in cytoplasm; (DNA broken down) by lysosomes; *idea that* 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 Examiner's Comments 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.
			Total	13	

Question			Answer/Indicative content	Marks	Guidance
4	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. *'bacteria take up plasmids with antibiotic resistance and transfer resistance'*. 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.

Question			Answer/Indicative content	Marks	Guidance
		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	*phytoene synthase* is, limiting / in low quantities / low activity; little, phytoene / substrate, for *phytoene desaturase*; little, lycopene/ substrate, for *lycopene ? cyclase;*	2 max	**Examiner's Comments** This proved to be a difficult question for two marks. Most students scored one by recognising that *phytoene synthase* 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.

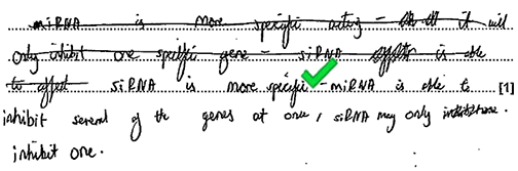
Question			Answer/Indicative content	Marks	Guidance
		ii	different base sequences (in the different genes/ DNA**);** different amino acid sequences (in the different enzymes); different, tertiary/3D, structures/ shape (in the different enzymes);	2 max	ACCEPT different, triplet /codon/ nucleotide, sequences. 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.

Question			Answer/Indicative content	Marks	Guidance
	d		*For:* relief of, vitamin A deficiency / symptoms of vitamin A deficiency; *Against:* 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: *For* ? 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. *Against* ? 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.

Question			Answer/Indicative content	Marks	Guidance
	e			2 max	*one mark for somatic (S) and one mark for germ line (G)* IGNORE ref to legality / ethical issues
			S1 cannot be inherited OR G1 can be inherited;		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 introduces (functional), gene/allele, into, patient/body cell /non reproductive cell OR G2 introduces, (functional), gene/allele, into sperm / egg / zygote/ embryo;		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.
			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;		
			Total	12	

Question			Answer/Indicative content	Marks	Guidance
5		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) x 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 Examiner's Comments 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.

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 <u>Examiner's Comments</u> 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 

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  miRNA is more specific as it will only inhibit one specific gene – siRNA affects it as to effect siRNA is more specific – miRNA is able to [1] inhibit several of the genes at once / siRNA may only inhibit one.
			Total	7	