1. March/2023/Paper_ 9700/42/No.2

Interferon-alpha (IFN-α) can be produced as a recombinant human protein to treat some types of cancer. The gene *IFNA2* codes for IFN-α.

One method of producing recombinant IFN-α uses genetically engineered *Escherichia coli* bacteria that contain recombinant plasmids. Each recombinant plasmid contains:

- the gene *IFNA2*
- three regulatory sequences of the *lac* operon (promoter, operator and *lacI*)
- a gene for antibiotic resistance, AMP^R.

Each of the sequences for the *lacI* gene and AMP^R gene contains its own promoter. As a result, these genes are always expressed in *E. coli* bacteria that contain this recombinant plasmid.

Fig. 2.1 is a diagram of the recombinant plasmid. The promoter regions of the *lacI* gene and AMP^R gene are **not** shown.

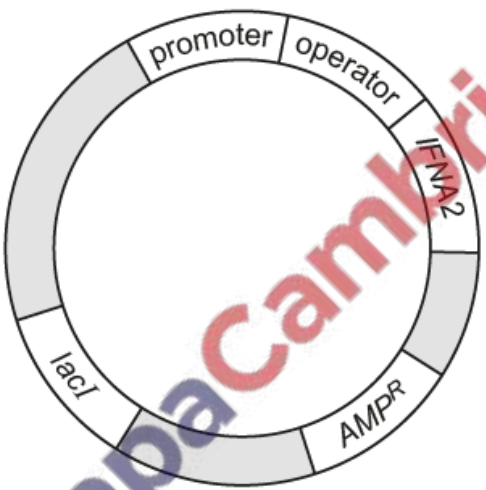

Fig. 2.1

(a) The start of transcription of the gene *IFNA2* by *E. coli* with the recombinant plasmid shown in Fig. 2.1 needs to be controlled to obtain an optimum yield of IFN-α.

Scientists investigated the effect of two inducers of transcription on the production of recombinant IFN-α:

- lactose, which is converted to allolactose in *E. coli*
- IPTG, which is a synthetic molecule with a very similar structure to allolactose. IPTG **cannot** be broken down by *E. coli*.

The scientists grew three cultures of *E. coli* containing the recombinant plasmid in the same growth medium. The growth medium contained glucose, amino acids, essential vitamins and minerals. The growth medium did **not** contain lactose.

After four hours, either lactose or IPTG at the same concentration was added to two of the cultures of *E. coli*. As a control, the third culture of *E. coli* was grown without adding lactose or IPTG.

The concentration of recombinant IFN-α in the cultures was measured at different times over a period of 28 hours. The results are shown in Fig. 2.2.

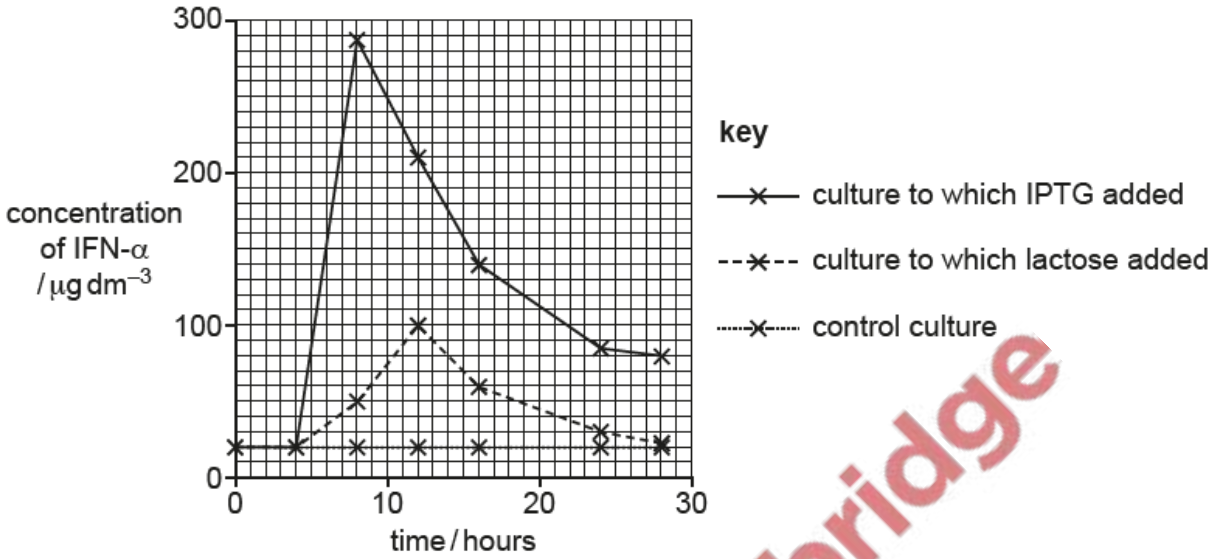

Fig. 2.2

(i) The regulatory sequences of the *lac* operon contained in the recombinant plasmid are involved in the control of transcription of the gene *IFNA2*.

Explain the role of the gene *lacI* in the control of transcription of the *IFNA2* gene between **0 hours** and **4 hours**.

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(ii) With reference to Fig. 2.2, describe the changes in the concentration of recombinant IFN-α in the culture containing IPTG from when IPTG was added at **4 hours** to the end of the experiment at **28 hours**.

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(iii) Suggest **one** reason for the difference between the concentration of recombinant IFN-α in the culture at **8 hours** in the presence of lactose and the concentration of recombinant IFN-α in the culture at **8 hours** in the presence of IPTG.

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(iv) Suggest **one** reason for the change in the concentration of recombinant IFN-α in the culture containing IPTG from **12 hours** to **16 hours**.

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(b) The gene AMP^R in the plasmid shown in Fig. 2.1 codes for a protein that provides resistance to the antibiotic ampicillin.

Suggest how AMP^R allows genetically engineered *E. coli* containing the recombinant plasmid to be identified.

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(c) Bacteria can evolve antibiotic resistance through natural processes.

Outline how bacteria can evolve to become resistant to antibiotics.

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[Total: 13]

2. March/2023/Paper_ 9700/42/No.3

Salmon can be genetically modified (GM) to produce increased quantities of growth hormone, which is a protein. GM salmon modified in this way have a faster growth rate and reach their maximum body mass at a younger age than non-GM salmon.

(a) Within any population of salmon there is variation in body mass. This is an example of continuous variation.

Explain what is meant by continuous variation **and** how it can be caused.

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(b) Scientists investigated whether injection of very young non-GM salmon with recom growth hormone could cause an increase in the growth rate of the salmon.

The scientists used two groups of non-GM salmon:

- a control group of salmon that were not injected with recombinant growth horr
- an experimental group of salmon that were injected with 1.0 μg of recom growth hormone at the start of the experiment and once a week for the ne weeks.

The mean body mass of the salmon in the two groups at the start of the experiment w same (5.3 g).

After six weeks, the body mass of every salmon was measured again. The resul summarised in Table 3.1.

Table 3.1

		no injection with recombinant growth hormone	**injected with recombinant growth hormone**
number of non-GM salmon (n)		28	27
body mass /g	range	6.5–8.6	7.2–12.7
	mean ($\bar{x}$)	7.7	9.4
	standard deviation (s)	0.4	1.1

A student decided that a *t*-test should be performed on the results shown in Table 3.1.

(i) Calculate the value of t for the results shown in Table 3.1 using the formula for the t-test:

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

Give your answer to **two** decimal places.

Show your working.

t = ...
[3]

(ii) The critical value at $p = 0.05$ for these data is 2.01.

The student used the results in Table 3.1 and the t-test to conclude that the injections of recombinant growth hormone cause an increase in the growth rate of the non-GM salmon.

Comment on the extent to which the conclusion made by the student can be supported.

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(iii) Suggest **one** advantage, **other** than cost, of farming GM salmon that produce increased quantities of growth hormone instead of farming non-GM salmon that are injected with recombinant growth hormone each week.

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[Total: 10]

3. March/2023/Paper_ 9700/42/No.4

Array comparative genome hybridisation (aCGH) is a technique involving the use of a microarray to analyse a genome or sections of a genome.

(a) Outline the steps required to prepare the genome of an individual so that the genome is ready for analysis using a microarray chip.

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(b) DiGeorge syndrome is a dominant inherited disease in humans. DiGeorge syndrome is caused by deletion of a large number of nucleotides from chromosome 22.

The number of nucleotides deleted varies between individuals in a range from 800 000 to 3 100 000. The largest deletions can cause the removal of up to 46 protein-coding genes from the chromosome.

Fig. 4.1 shows the results of aCGH using a microarray specific for the section of chromosome 22 within which the DiGeorge syndrome deletion occurs. The microarray analysed DNA from two individuals:

- one with DiGeorge syndrome
- one who did not have DiGeorge syndrome (control DNA for comparison).

In the aCGH results shown in Fig. 4.1:

- Each small circle represents the results from a single probe on the microarray.
- The *x*-axis shows the position of each probe on chromosome 22. The position is shown as distance along the chromosome in millions of nucleotides.
- A result close to 100% fluorescence on the *y*-axis means that the DNA from the individual with DiGeorge syndrome fluoresces at the same intensity as the control DNA for that probe.
- A result close to 50% fluorescence on the *y*-axis means that the DNA from the individual with DiGeorge syndrome fluoresces half as much as the control DNA for that probe.

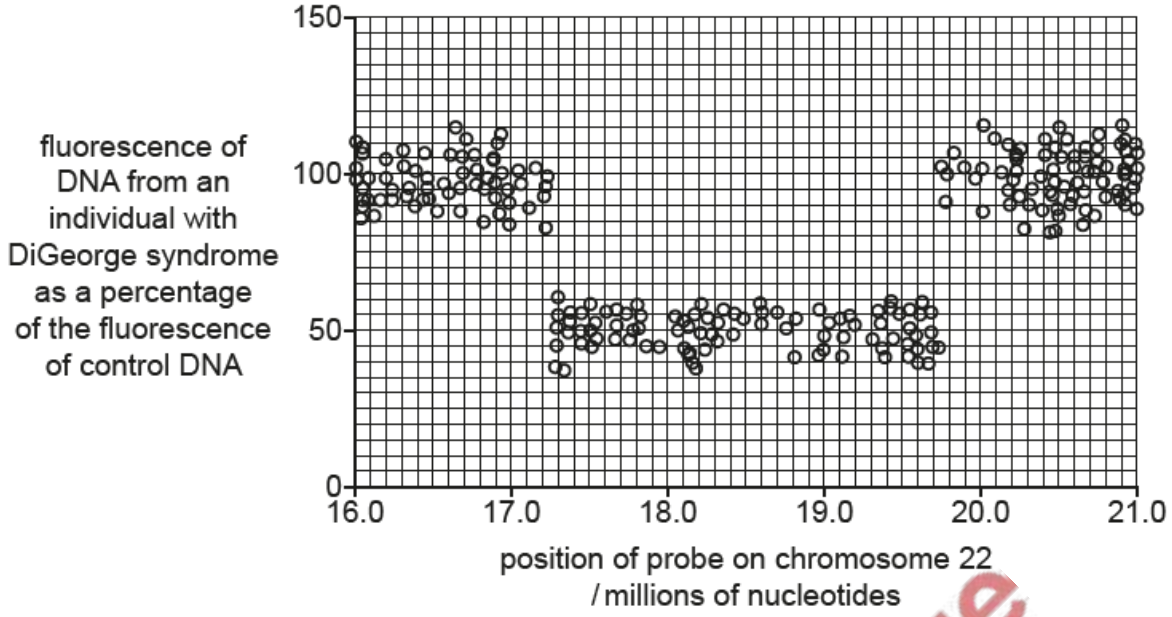

Fig. 4.1

(i) With reference to Fig. 4.1, estimate the number of nucleotides deleted from the affected chromosome 22 in the individual with DiGeorge syndrome.

Give your answer to the nearest 100 000 nucleotides.

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(ii) Explain how the microarray technique works to give the results shown in Fig. 4.1.

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(iii) Suggest why the phenotypes of two individuals with DiGeorge syndrome can be different.

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[Total: 10]

4. June/2023/Paper_ 9700/41/No.4

(a) Recombinant human proteins can be used to treat disease.

(i) Define the term recombinant DNA.

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(ii) From the 1920s until the 1970s, insulin obtained from the bodies of animals was used to treat diabetes. From the 1970s, recombinant human insulin was used instead.

Explain the advantages of using recombinant human insulin to treat people with diabetes.

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(b) Insulin is composed of two polypeptide chains, the A chain and the B chain, that are linked by disulfide bonds.

Variations in amino acid sequence occur:

- in the insulin molecules of different animals
- in new versions of human insulin that have been engineered to control blood glucose concentration more effectively than normal recombinant human insulin. These new versions of human insulin are called analogues.

Table 4.1 shows the amino acid positions where variation occurs in different animal and human analogue insulin molecules. The dashes indicate a missing amino acid.

Table 4.1

amino acid position	type of insulin					
	human	cow	pig	cat	short-acting analogue	long-acting analogue
A8	threonine	alanine	threonine	alanine	threonine	threonine
A10	isoleucine	valine	isoleucine	valine	isoleucine	isoleucine
A18	asparagine	–	asparagine	histidine	asparagine	asparagine
A21	asparagine	asparagine	asparagine	asparagine	asparagine	glycine
B3	asparagine	asparagine	asparagine	asparagine	asparagine	–
B28	proline	proline	proline	proline	lysine	proline
B29	lysine	lysine	lysine	lysine	proline	lysine
B30	threonine	alanine	alanine	alanine	threonine	threonine
B31	–	–	–	–	–	arginine
B32	–	–	–	–	–	arginine

(i) Cats with diabetes can be successfully treated with insulin injections. Cat insulin is **not** available, but vets can choose from the other types of insulin shown in Table 4.1.

Identify the type of insulin that is most suitable for treating cats.

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(ii) Suggest ways in which analogue insulin molecules can be produced by genetic engineering techniques.

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(c) Information about amino acid and nucleotide sequences is stored in computer databases.

Outline the advantages of using databases of nucleotide sequences to investigate evolutionary relationships between species.

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[Total: 11]

5. June/2023/Paper_ 9700/42/No.4

Genetic technology uses many different enzymes and techniques.

(a) Restriction endonucleases are used in genetic modification. These enzymes occur naturally in prokaryotic cells. More than 3500 different restriction endonucleases have been identified and it is thought there are many more to discover.

Name **two** domains that are a source of restriction endonucleases.

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(b) Originally, the method used to obtain a restriction endonuclease was to:

- grow large numbers of the specific prokaryotic cells that are the source of the enzyme
- break open the cells and extract and purify the restriction endonuclease.

This original method produced only a small quantity of restriction endonuclease and was **not** economical.

The newer method for large-scale production is to:

- obtain the gene coding for a specific restriction endonuclease
- introduce the gene into *Escherichia coli*, with a promoter that allows the gene to be expressed continuously.

The newer method increases the quantity of specific restriction endonuclease produced.

Suggest **and** explain the steps needed to carry out the newer method for large-scale production of a specific restriction endonuclease.

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(c) Describe the advantages of databases for the study and use of restriction endonucleases.

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(d) Electrophoresis is a technique used in genetic technology.

Paper chromatography is a technique used to investigate the photosynthetic pigments found in chloroplasts.

Compare the similarities and differences between electrophoresis and chromatography.

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[Total: 12]

6. June/2023/Paper_ 9700/43/No.3

Gene expression in a cell is controlled. When a gene is expressed (switched on), the gene is transcribed. When a gene is **not** expressed (switched off), the gene is **not** transcribed.

Environmental changes can cause some genes to be switched on or switched off.

(a) An example of control of gene expression in prokaryotes is regulation in the *lac* operon.

The *lac* operon is a length of DNA that is made up of different parts.

Fig. 3.1 shows a simple diagram representing the *lacI* (regulatory) gene and the *lac* operon.

Fig. 3.1

(i) Outline the main features of the *lac* operon.

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(ii) Explain the role of the *lacI* gene in the regulation of the *lac* operon.

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(b) The *lac* operon codes for inducible enzymes. Repressible operons code for repressible enzymes.

Suggest **and** explain why it is an advantage to a prokaryote to have a repressible operon.

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[Total: 10]

7. June/2023/Paper_ 9700/43/No.4

One cause of the genetic disease severe combined immunodeficiency (SCID) is a mutation in the *ADA* gene. This mutation results in a deficiency of the enzyme adenosine deaminase (ADA).

Although ADA is found throughout the body, it is especially active in lymphocytes. The absence of functional ADA causes the build-up of toxic metabolites that kill lymphocytes and damage organs.

Babies are often diagnosed with SCID by six months old. Treatment can greatly improve the life expectancy of children with SCID.

Some treatment options are available.

- Enzyme replacement therapy with recombinant human ADA made by genetically modified (GM) *Escherichia coli*. Weekly intra-muscular injections are given.
- Bone marrow transplant if a well-matched donor, such as a close relative, can be found.
- Gene therapy.

(a) Suggest **and** explain why it may be more appropriate to use enzyme replacement therapy to treat SCID instead of a bone marrow transplant.

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(b) Outline the procedure used for gene therapy treatment of a person with SCID.

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(c) Suggest the social and ethical implications of gene therapy for SCID that need to be considered before treatment is carried out.

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[Total: 10]