OCR
RECOGNISING ACHIEVEMENT

ADVANCED GCE **2805/04**

BIOLOGY

Microbiology and Biotechnology

WEDNESDAY 18 JUNE 2008 Afternoon

Time: 1 hour 30 minutes

Candidates answer on the question paper.
Additional materials: Electronic calculator
Ruler (cm/mm)

CUP/T44527

Candidate Forename		Candidate Surname	

Centre Number						Candidate Number				

INSTRUCTIONS TO CANDIDATES

- Write your name in capital letters, your Centre Number and Candidate Number in the boxes above.
- Use blue or black ink. Pencil may be used for graphs and diagrams only.
- Read each question carefully and make sure that you know what you have to do before starting your answer.
- Answer **all** the questions.
- Do **not** write in the bar codes.
- Do **not** write outside the box bordering each page.
- Write your answer to each question in the space provided.

INFORMATION FOR CANDIDATES

- The number of marks for each question is given in brackets [ ] at the end of each question or part question.
- The total number of marks for this paper is **90**.
- You will be awarded marks for the quality of written communication where this is indicated in the question.
- You may use an electronic calculator.
- You are advised to show all the steps in any calculations.

FOR EXAMINER'S USE		
Qu.	**Max.**	**Mark**
1	**9**	
2	**15**	
3	**19**	
4	**17**	
5	**19**	
6	**11**	
TOTAL	**90**	

This document consists of **20** printed pages and **4** blank pages.

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Answer **all** the questions.

1 This question concerns biotechnology in food production.

(a) Bromelain, found in pineapples, has a similar meat tenderising action to the papain enzyme present in papaya.

The following cooking hint appeared in a student recipe book:

> Rub pineapple onto cheap cuts of raw meat,
> then leave the meat for some time before cooking.

Explain the reasons behind this cooking hint.

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.....[4]

(b) For each of the following descriptions, **(i)** to **(v)** below, suggest an appropriate term.

(i) A substitute for meat produced by culturing *Fusarium graminearum*.

.....[1]

(ii) The continuous fermentation vessel used to culture *F. graminearum*.

.....[1]

(iii) The liquid produced after grist is mixed with hot water in a mash tun.

.....[1]

(iv) The enzyme added to coagulate milk in cheese production.

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(v) The chemical produced by *Lactobacillus* and *Streptococcus* that gives yoghurt its characteristic flavour.

.....[1]

[Total: 9]

2 **(a)** In this question, one mark is available for the quality of spelling, punctuation and grammar.

Fig. 2.1 represents changes that occur in the growth and reproduction of a bacterial cell that possesses plasmids. The plasmids carry the genes, *amp* and *strep*, which code for antibiotic resistance to ampicillin and streptomycin respectively.

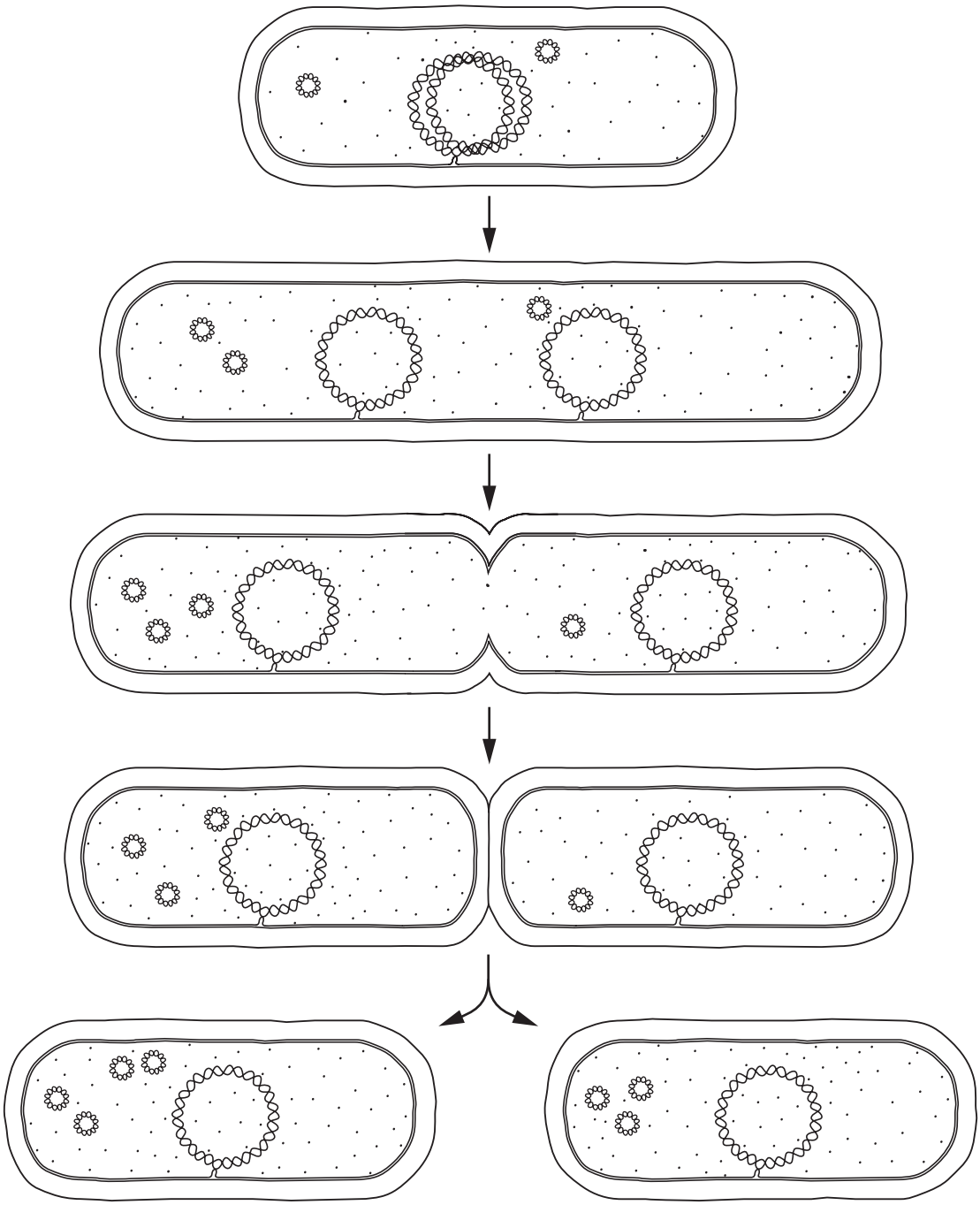

Fig. 2.1

Describe the sequence of events occurring in Fig. 2.1.

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Quality of Written Communication [1]

(b) Explain how plasmids have been of **both** benefit and harm to humans.

benefit.....

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harm .....

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.....[4]

(c) Mitosis and cell division in an animal cell differ from the sequence of events occurring in Fig. 2.1.

List **three** features of mitosis and cell division in an animal cell that are **not** observed during asexual reproduction in bacteria.

1 .....

2 .....

3 .....[3]

[Total: 15]

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3 One method of plant tissue culture uses protoplasts to produce whole plants. Protoplasts are cells that have had their cell walls removed, leaving the cell contents surrounded only by the plasma (cell surface) membrane.

A student used a haemocytometer to count protoplasts suspended in a 21% sucrose solution.

Fig. 3.1 shows part of the haemocytometer. The depth of the suspension in the haemocytometer chamber is 0.1 mm.

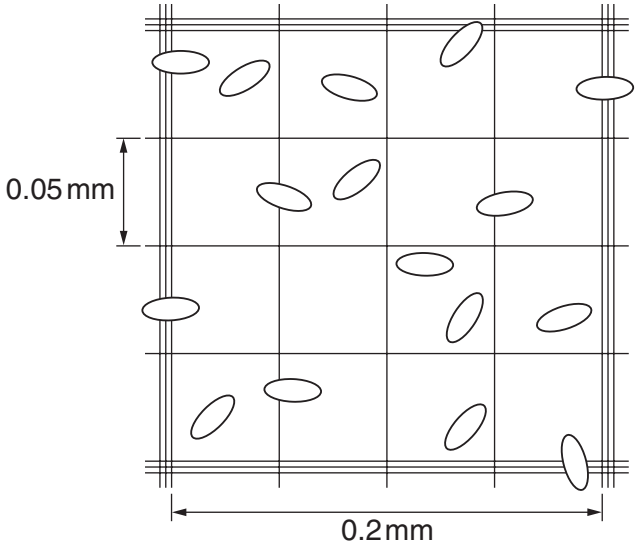

Fig. 3.1

(a) Explain why the student:

(i) suspended the protoplasts in 21% sucrose solution;

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(ii) agitated the suspension gently before removing a sample;

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(iii) transferred the sample to the haemocytometer chamber and then waited five minutes before counting.

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.....[4]

(b) With reference to Fig. 3.1, calculate the number of protoplasts in 1 cm^3 of suspension. Show your working.

Answer = ... protoplasts per cm^3 [2]

(c) Outline how the student could produce whole plants from the protoplast suspension.

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(d) State **one** advantage of using protoplasts, rather than explants, when carrying out a plant tissue culture procedure.

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Pathogenic fungal protoplasts are useful in research. Using similar methods to those which produce plant protoplasts, their DNA can be isolated for genome analysis.

The fungal protoplasts are obtained by incubating the cells with a commercial preparation that has a range of enzyme activity. As fungal cell wall components vary, each preparation is specific to particular fungal species.

The enzymes present in four different commercial preparations are shown in Table 3.1.

Table 3.1

preparation	enzymes present
A	cellulase, chitinase
B	cellulase, glucanase
C	chitinase, glucanase, lipase
D	chitinase, glucanase

The effectiveness of using each of the four different preparations to obtain fungal protoplasts was investigated.

Different groups of cells from the same fungal species were separately incubated with each preparation for six hours. The relative numbers of protoplasts formed in the four preparations at three hours and at six hours were determined using a haemocytometer.

The results of the investigation are shown in Fig. 3.2.

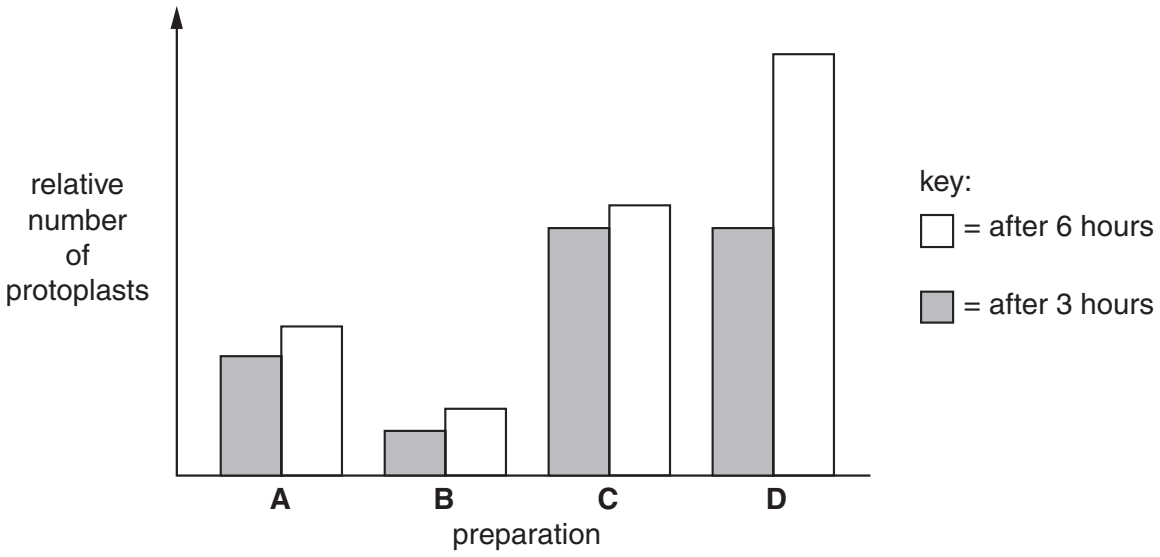

Fig. 3.2

(e) State **one** variable, apart from incubation time, that should be controlled during the investigation. Justify your choice.

variable .....

justification .....

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.....[2]

(f) Comment on the results of the investigation.

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[Total: 19]

4 **(a)** In recent years there has been increasing interest in the use of microorganisms to produce fuels for use in vehicles. The yeast, *Saccharomyces cerevisiae*, and the bacterium, *Zymomonas mobilis*, are able to ferment sugars to alcohol.

The National Alcohol Programme, which started in Brazil in 1975, has led to the majority of Brazil's vehicles having engines that can use:

- ethanol alone
- gasohol
- flex fuel (ethanol alone, petrol alone or any mixture of the two).

The alcohol is produced on a large scale by continuous fermentation using *S. cerevisiae*.

(i) State what is meant by *gasohol*.

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.....[1]

(ii) State **one** advantage of using gasohol instead of using petrol.

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.....[1]

(iii) Using batch fermentation gives a better control of contamination.

Suggest why **continuous** fermentation is chosen for alcohol production in Brazil.

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(b) State whether you would choose to use immobilised cells or cells free in the fermenter medium for the **continuous** fermentation of ethanol. Give a reason for your choice.

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(c) Table 4.1 shows the results of laboratory-scale investigations into two yeast strains and two bacterial strains that produce ethanol.

Table 4.1

feature	strain **J**	strain **K**	strain **L**	strain **M**
ethanol tolerance / % (by mass)	11	11	12	13
maximum temperature for optimum growth rate / °C	33	30	40	37
range of carbohydrates that can be used by microorganism	sucrose glucose fructose galactose maltose maltotriose xylulose	glucose fructose galactose maltose maltotriose xylulose	glucose fructose sucrose	glucose fructose sucrose
ability to flocculate	low	high	low	low

Using the data in Table 4.1, choose a strain, **J**, **K**, **L**, or **M**, which you consider to be suitable for ethanol production. Give reasons for your choice.

strain.....

reasons.....

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.....[3]

(d) A commercial strain of *S. cerevisiae* is currently used in large-scale ethanol production even though a strain of *Z. mobilis* has been identified that has a better laboratory-scale profile.

Suggest why this *Z. mobilis* strain is still **not** used in the commercial production of ethanol.

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.....[1]

(e) *S. cerevisiae* and *Z. mobilis* are considered to have a high tolerance to ethanol compared to other microorganisms. Ethanol passes freely through the phospholipid bilayer; as concentrations of ethanol increase, the membrane becomes increasingly leaky.

Read the statements below and then answer the questions **(i)** to **(iii)** that follow.

- When *S. cerevisiae* is grown in 7.5% ethanol, the oleic acid content of the phospholipid bilayer doubles compared with growth in 0% ethanol.
- This increase is due to a replacement of palmitic acid with oleic acid.
- The molecular structures of palmitic acid and oleic acid are shown in Fig. 4.1.

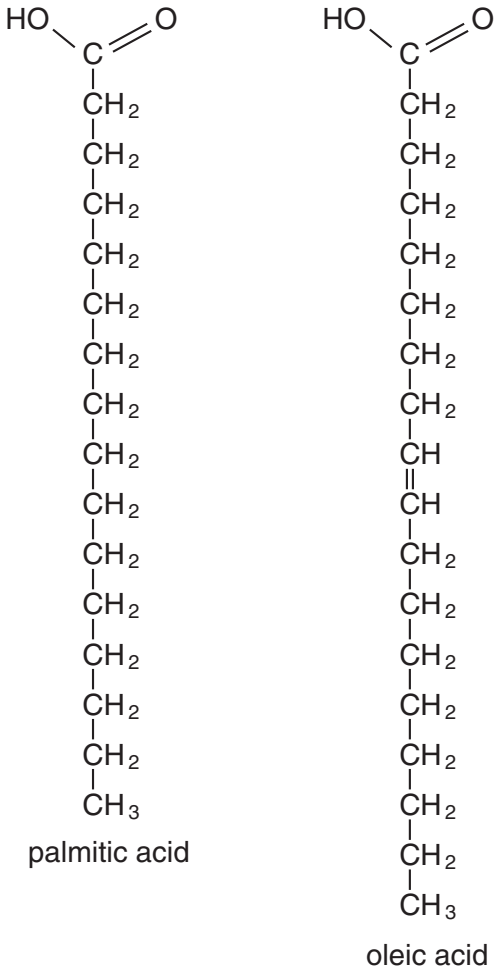

Fig. 4.1

- When *S. cerevisiae* is grown in ethanol, the membrane content of ergosterol increases.
- When *Z. mobilis* is grown in ethanol, the membrane content of hopanoids increases.
- Ergosterol and hopanoids are similar to cholesterol.

(i) Using Fig. 4.1, state how the structure of oleic acid differs from that of palmitic acid.

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.....[2]

(ii) A student suggested that the replacement of palmitic acid by oleic acid 'helps to increase ethanol tolerance of *S. cerevisiae* because there is an increase in hydrophobic interaction'.

Explain whether or not you would support the student's suggestion.

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.....[3]

(iii) Explain how the increase in ergosterol and hopanoid content in membranes could increase ethanol tolerance in *S. cerevisiae* and *Z. mobilis*.

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[Total: 17]

5 Fig. 5.1 summarises the large-scale production of three proteins of medical importance, insulin, human growth hormone (HGH) and human factor VIII. **P**, **Q** and **R** are enzymes.

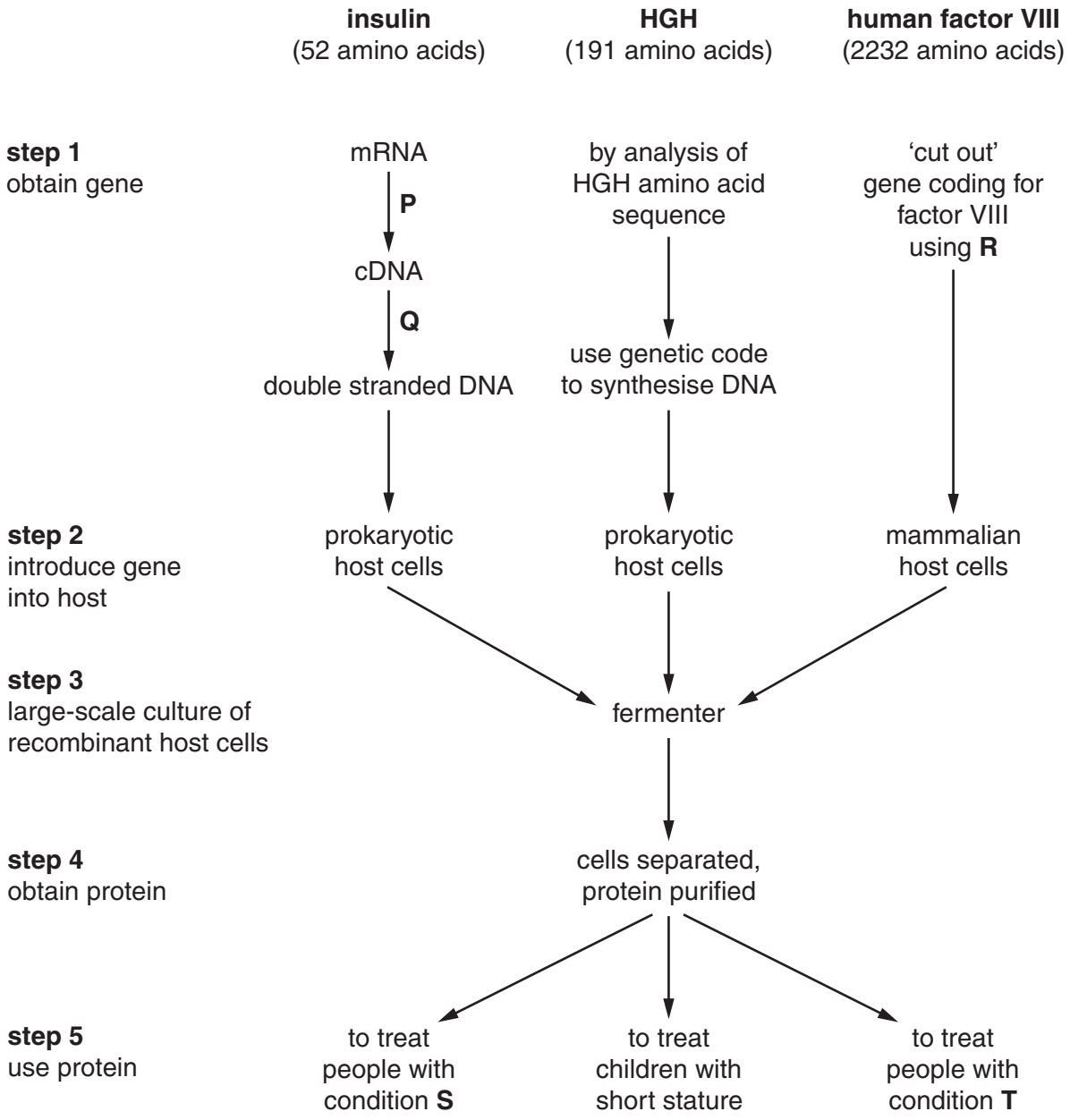

Fig. 5.1

(a) Name the enzymes **P**, **Q** and **R** in **step 1** of Fig. 5.1.

P .....

Q .....

R ..... [3]

(b) Suggest why the method used to synthesise the gene coding for HGH is **not** used to obtain the gene coding for human factor VIII.

.....[1]

(c) Table 5.1 shows the genetic code (mRNA codons) that can be used to synthesise the gene coding for HGH in **step 1**.

Table 5.1

first position	second position				third position
	U	C	A	G	
U	phe	ser	tyr	cys	U
	phe	ser	tyr	cys	C
	leu	ser	STOP	STOP	A
	leu	ser	STOP	trp	G
C	leu	pro	his	arg	U
	leu	pro	his	arg	C
	leu	pro	gln	arg	A
	leu	pro	gln	arg	G
A	ile	thr	asn	ser	U
	ile	thr	asn	ser	C
	ile	thr	lys	arg	A
	met	thr	lys	arg	G
G	val	ala	asp	gly	U
	val	ala	asp	gly	C
	val	ala	glu	gly	A
	val	ala	glu	gly	G

(i) The sequence of the first five (N-terminal) amino acids in human HGH is met-phe-pro-thr-ile.

Using the genetic code in Table 5.1, work out a sequence of bases in the synthetic DNA strand that would correspond to the first five amino acids.

amino acid sequence: met – phe – pro – thr – ile

RNA base sequence: ……–……–……–……–……

DNA base sequence: ……–……–……–……–…… [3]

(ii) The recombinant HGH produced in **step 4** is identical to human HGH. However, the synthetic gene coding for HGH may possess a different nucleotide sequence to the actual human gene.

Explain this statement.

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.....[2]

(d) Name the conditions, **S** and **T**, in **step 5** of Fig. 5.1.

S .....

T .....[2]

(e) In this question, one mark is available for the quality of use and organisation of scientific terms.

With reference to Fig. 5.1, describe **and** explain the sequence of steps that can be taken to obtain the genetically-modified host cells for the production of insulin (**steps 1** and **2**).

Details of the large-scale fermentation process and purification, **steps 3** and **4**, are **not** required.

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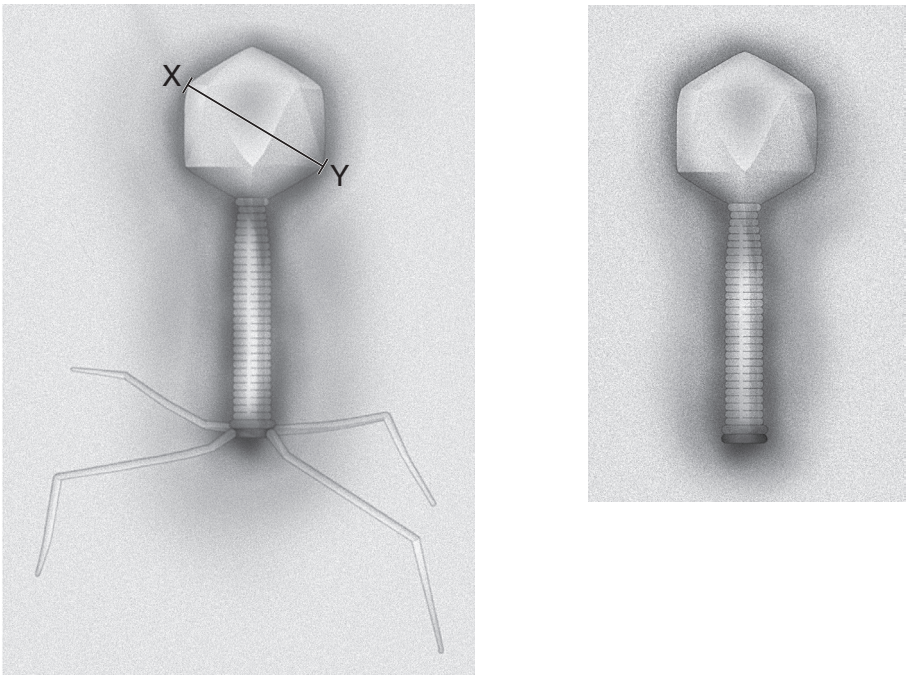
Quality of Written Communication [1]

[Total: 19]

6 Lambda is a bacteriophage that uses *Escherichia coli* as its host cell.

Fig. 6.1**A** is an electron micrograph (EM) of a wild-type bacteriophage lambda.
Fig. 6.1**B** is an EM of a laboratory-cultured lambda.



A **B**

Fig. 6.1

(a) The actual diameter, **X** – **Y**, is 50 nm.

Calculate the magnification of the bacteriophage, lambda, shown in Fig. 6.1**A**. Show your working.

Answer = x ..... [2]

(b) State the main difference, **visible in Fig. 6.1**, between the wild-type and laboratory-cultured bacteriophage lambda.

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.....[1]

(c) Suggest how laboratory-cultured bacteriophage lambda binds to cells of its host, *E. coli*.

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.....[1]

(d) Explain why it is sometimes difficult to distinguish between a plasmid and the DNA of bacteriophage lambda inside an *E. coli* cell when viewed using an electron microscope.

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.....[2]

(e) The passage below is a description of bacteriophage lambda that appeared in a magazine for non-scientific readers. The terms that have been highlighted have **not** been written using the correct scientific terminology.

> The **complicated germ** has a **head** which is made of 72 **building blocks** and contains **the molecule of inheritance**. It acts to infect a **larger and different type of germ** known as *E. coli* and, unlike the **microbe responsible for AIDS**, does not have an **outer covering** surrounding it.

Re-write the passage using the correct scientific terminology.

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[Total: 11]

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