1 Gene sequencing is an important technique in molecular biology.

Fig. 3.1, **on page 3 of the Insert**, shows part of a computerised graph obtained from an automated gene sequencing machine.

- The section of the DNA molecule represented in Fig. 3.1 is from base position 117 (on the left of the graph) to base position 137 (on the right of the graph).
- The bases in the DNA sequence are labelled with four different coloured fluorescent dyes.
- The identities of some of the bases (117 to 119 inclusive and 129 to 137 inclusive) are indicated below the graph.

(a) Use Fig. 3.1 to identify the order of bases from positions 120 to 128.

.....	.....	.....	.....	.....	.....	.....	.....	.....
120	121	122	123	124	125	126	127	128

[1]

(b) To produce the type of graph shown in Fig. 3.1, the automated gene sequencing machine needs to be loaded with the following:

- the DNA to be sequenced
- short primer sequences specific to the DNA to be sequenced
- many normal DNA nucleotides
- some chain-terminating DNA nucleotides labelled with coloured dyes
- the enzyme *Taq* polymerase.

A regular cycle of temperature changes allows many DNA fragments of different lengths to be built up by the polymerase chain reaction (PCR).

Fig. 3.2 (**on the next page**) shows the end parts of the sequences of seven of these different length fragments, labelled 1 to 7. The end parts of the sequences for fragments 1 to 4 are complete but those for fragments 5 to 7 are not.
These seven fragments correspond to the **last seven peaks** on the right hand side of the graph in Fig. 3.1.

The letters in boxes represent labelled chain-terminating DNA nucleotides.
The letters not in boxes represent normal DNA nucleotides.

(i) Use the information in Fig. 3.1 to fill in the missing nucleotide bases on fragments 5 to 7 on Fig. 3.2.

You should distinguish between the normal and labelled nucleotides in the sequence for each fragment.

1 – **[T]**

2 – **T** **[A]**

3 – **T** **A** **[T]**

4 – **T** **A** **T** **[T]**

5 – **T** **A**

6 – **T** **A**

7 – **T** **A**

[2]

Fig. 3.2

(ii) Explain how the automated sequencing machine orders the DNA fragments from the PCR reaction into the size order shown in Fig. 3.2.

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..... **[3]**

(c) Gene sequencing can help us to understand how an individual's genome affects their body's response to drugs.

One research study has looked at the effectiveness of drugs used to treat asthma in children. Asthma is a condition in which the bronchioles become reduced in diameter. This results in the child finding it difficult to breathe.

(i) Using your knowledge of the structure of bronchioles, suggest how their diameter might become reduced.

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.....**[2]**

(ii) Explain why it is difficult to expel air from the lungs if the bronchioles become reduced in diameter.

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..... **[1]**

(d) Asthma in children may be treated with drugs. One of the most commonly used drugs is salmeterol.

Salmeterol acts by binding to protein receptors in the lining of the bronchioles. However, in approximately 14% of children with asthma, salmeterol is not very effective. This is thought to be the result of a genetic mutation in these children.

Suggest why this mutation reduces the effectiveness of salmeterol.

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..... **[3]**

(e) In a recent medical trial, 62 children with this genetic mutation were studied.

- Their asthma was not controlled well by salmeterol.
- 31 children continued using salmeterol and the remaining 31 were given an alternative drug, montelukast.
- Montelukast is not routinely prescribed because salmeterol is far more effective for most children with asthma.

(i) After one year, the children taking montelukast had better control of their asthma and were able to reduce their use of montelukast.

Suggest why these children responded better to montelukast than to salmeterol.

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..... **[2]**

(ii) Comment on the reliability of the results of this medical trial.

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..... **[1]**

(iii) It is proposed that a simple saliva test could identify those children who have the mutation.

What would be the source of the genetic material used in this test?

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..... **[1]**

[Total: 16]

2 **(a)** The Oxford Botanic Garden was founded in 1621 to grow plants for the teaching of medicine. Since that time it has seen many changes. When the ideas of Linnaeus were adopted in the 18th century, the plants were dug up and re-planted in family groups according to his new system of taxonomy.

Recently, the plants have once again had to be re-organised:

- DNA sequencing techniques, together with cladistic analysis, have provided a radical new view of plant evolutionary relationships.
- The same techniques have also improved the ability of researchers to pinpoint new cures for diseases, by examining the closest relatives of plants already known to have medicinal properties.

(i) Comment on what the different arrangements of plants in the Oxford Botanic Garden over time tell us about the nature of scientific knowledge.

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..... **[1]**

(ii) Suggest **two** purposes of a plant collection in a modern botanic garden.

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..... **[2]**

(b) DNA sequencing techniques have provided new information about plant relationships.

Outline the **roles** of each of the following procedures **in sequencing a genome**:

(i) the polymerase chain reaction (PCR)

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..... **[2]**

(ii) electrophoresis

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..... **[2]**

(iii) digestion of DNA by restriction enzymes.

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..... **[2]**

(c) Suggest why a genome has to be fragmented before sequencing.

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..... **[2]**

(d) Table 5.1 lists some plants considered for genome sequencing by the 'Floral Genome Project'. The chromosome numbers and genome sizes in mega base pairs (Mbp) are shown.

One Mbp is equal to 1 000 000 base pairs of DNA.

Name	Chromosome Number(s)	Genome Size (Mbp)
Amborella	2n = 26	870
sweet rush	2n = 18	392
monkey flower	2n = 28	430
blueberry	2n = 12, 4n = 24, 6n = 36	1078

Table 5.1

(i) The sequencing method that will be used is only able to sequence fragments of DNA with a maximum length of 750 base pairs.

Calculate the minimum number of DNA fragments that would need to be sequenced to read the genome of *Amborella*.

Show your working.

Answer = ... **[2]**

(ii) Monkey flower and blueberry belong to the same taxonomic group within the plant kingdom. Only one of the pair was chosen for further sequencing work.

Using the data in Table 5.1, suggest reasons why monkey flower was chosen instead of blueberry.

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..... **[2]**

(iii) Use your knowledge of the effects of polyploidy in bread wheat to suggest one way in which the fruit of a hexaploid (6n) blueberry might differ in appearance from that of a diploid (2n) blueberry.

..... **[1]**

(e) DNA sequence information is most useful when used with the phylogenetic (cladistic) approach to classification.

How does the phylogenetic approach to classifying species differ from the biological species concept?

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..... **[2]**

[Total: 18]

3 **(a)** Genetic modification of organisms uses a "toolkit" that includes:

- enzymes that cut DNA
- enzymes that join sections of DNA together
- vectors that introduce DNA into new host cells.

Some of the enzymes and vectors that are important in genetic modification are given an identifying letter in Table 4.1.

Table 4.1

	enzymes
A	reverse transcriptase
B	DNA polymerase
C	DNA ligase
D	restriction endonuclease
E	RNA polymerase

	vectors
J	plasmid
K	virus
L	*Agrobacterium tumefaciens*
M	BAC
N	bacteriophage

Select **one** correct letter from Table 4.1 to fit each of the following statements.

An enzyme that cuts DNA .....

An enzyme that joins sections of DNA together .....

A vector to introduce foreign DNA into bacteria .....

A vector to introduce foreign DNA into plant cells .....

A vector to introduce foreign DNA into animal cells ..... **[5]**

(b) Discuss the **potential benefits** to mankind and the **ethical concerns** raised by the following examples of genetically modified organisms:

- rice modified for increased vitamin A content ('Golden Rice™')
- humans having somatic gene therapy treatment for a genetic disease.

In your answer you should give a balanced account of the benefits and concerns for each example of genetic modification.

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[Total: 14]