Cambridge International A Level

Cambridge International Examinations
Cambridge International Advanced Level

CANDIDATE NAME

CENTRE NUMBER

CANDIDATE NUMBER

2847631226

BIOLOGY **9700/52**

Paper 5 Planning, Analysis and Evaluation **May/June 2014**

1 hour 15 minutes

Candidates answer on the Question Paper.

No Additional Materials are required.

READ THESE INSTRUCTIONS FIRST

Write your Centre number, candidate number and name on all the work you hand in.
Write in dark blue or black pen.
You may use a soft pencil for any diagrams, graphs or rough working.
Do not use staples, paper clips, highlighters, glue or correction fluid.
DO **NOT** WRITE IN ANY BARCODES.

Answer **all** questions.

Electronic calculators may be used.
At the end of the examination, fasten all your work securely together.
The number of marks is given in brackets [ ] at the end of each question or part question.

This document consists of **9** printed pages and **3** blank pages.

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CAMBRIDGE International Examinations

[Turn over

1 **(a)** A group of students was given the task of planning a method to compare the effect of two different restriction enzymes on some DNA extracted from peas.

The students used the following procedure to extract and digest DNA from the peas.

- 3 g of sodium chloride was dissolved in 90 cm^3 of distilled water in a beaker.
- 10 cm^3 of liquid detergent was added to the salt solution and stirred.
- 50 g of peas were ground up using a glass rod and then added to the salty detergent solution.
- The beaker containing the mixture of peas and salty detergent solution was left in a water bath at 60°C for **exactly** 15 minutes.
- The mixture was then cooled in an ice-water bath for 5 minutes, stirring frequently.
- The mixture was filtered into another beaker.
- 10 cm^3 of filtrate was transferred into a boiling tube and 2–3 drops of a protease solution were added. This was left for a few minutes.
- Ice-cold ethanol was then poured down the side of the boiling tube to form a layer on top of the filtrate. This was left undisturbed for a few minutes to form a layer of precipitated DNA.
- The precipitated DNA was separated from the cold ethanol layer by twisting the DNA onto a glass hook.
- The DNA was mixed with 20 cm^3 EDTA buffer solution.
- 5 cm^3 samples of the EDTA buffer solution containing the DNA extract were placed into three separate test-tubes, 1, 2 and 3.
- Two different restriction enzymes, *Eco* RI and *Hin* dIII, were added to the samples as shown in Table 1.1.

Table 1.1

sample	1	2	3
restriction enzymes added	*Eco* RI	*Hin* dIII	*Eco* R1 and *Hin* dIII

- These were left for three hours to complete digestion of the DNA into fragments of different sizes.

Suggest a reason for each of the following steps in the procedure for extracting DNA.

(i) using detergent .....

.....[1]

(ii) leaving at 60°C .....

.....[1]

(iii) filtering the mixture .....

.....[1]

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(iv) adding protease .....

.....[1]

(b) The students used gel electrophoresis to separate the DNA fragments produced by the digestion with the restriction enzymes.

Describe the main stages used in gel electrophoresis that the students could use to separate and locate the DNA fragments.

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.....[5]

(c) **(i)** Identify a dependent variable that the student could measure.

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.....[1]

(ii) State two variables that the students should standardise to ensure that the results from the different samples shown in Table 1.1 can be compared.

1. .....

.....

2. .....

.....[2]

(d) Fig. 1.1 shows the results of the electrophoresis.

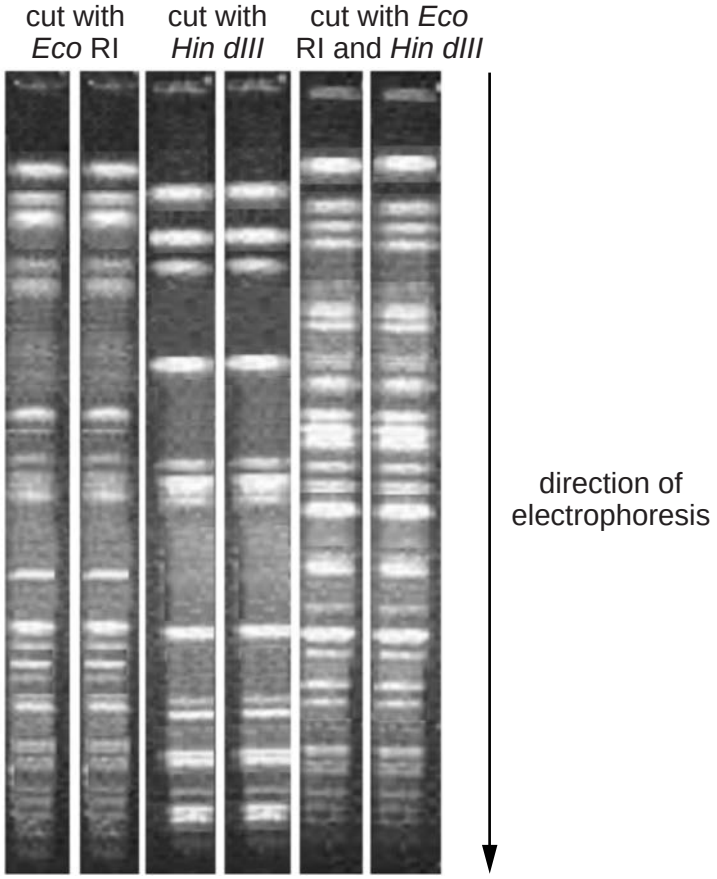


Fig. 1.1

The students concluded that the DNA of peas had more restriction sites for *Eco* RI than for *Hin* dIII and that some of the *Hin* dIII sites were within fragments produced by *Eco* RI.

(i) State **one** piece of evidence in Fig. 1.1 to support each of these conclusions:

there are more restriction sites for *Eco* RI than for *Hin* dIII

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there are some *Eco* RI sites within fragments produced by *Hin* dIII.

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.....[2]

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(ii) Suggest **one** reason why the results in Fig. 1.1 may **not** support the student's conclusions.

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.....

.....[1]

(e) Gel electrophoresis is also used in the sequencing of DNA.

A method used for sequencing DNA is described below.

- A DNA molecule is first heated so it separates into two strands.
- DNA polymerase is then used to synthesise DNA using normal nucleotides and modified nucleotides.
- The four different modified nucleotides are each labelled with a different coloured fluorescent dye.
- These are: **red** CTP **green** TTP **blue** GTP **yellow** ATP
- The addition of modified nucleotides to the DNA strand stops the addition of any more nucleotides.

Fig. 1.2 shows the formation of a three nucleotide fragment from normal nucleotides and a modified nucleotide.

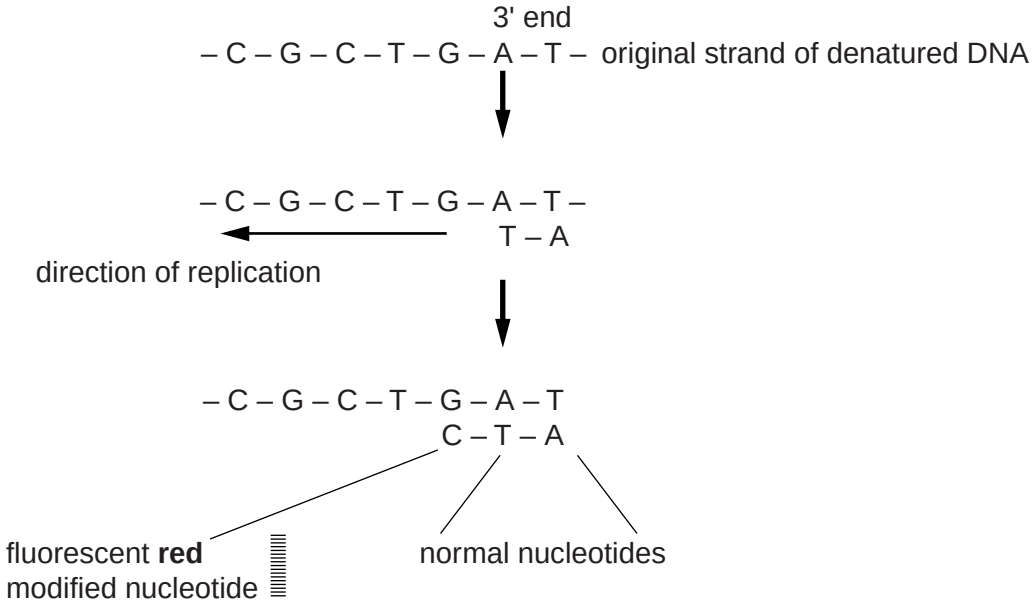


Fig. 1.2

- Fragments of different lengths are produced depending on the position of the modified nucleotides.
- The DNA fragments synthesised are separated by gel electrophoresis and a laser light used to identify the different colours.

Fig. 1.3 shows the results of sequencing part of a DNA molecule.

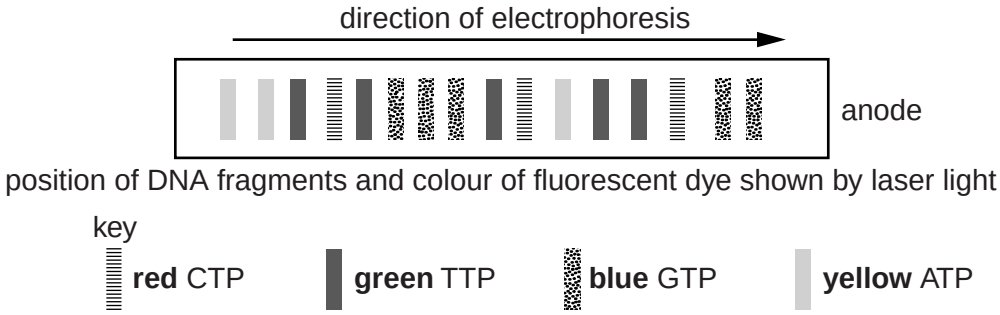


Fig. 1.3

(i) Use the information in Fig. 1.3 to work out the sequence of the DNA template, starting from the 3' end.

.....[1]

(ii) Explain how you worked out this sequence.

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.....[3]

[Total: 19]

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2 **(a)** An investigation into the efficiency of nitrogen use by *Sorghum bicolor* was carried out in a glasshouse.

- Four varieties of *Sorghum,* **W**, **X**, **Y** and **Z**, were grown in pots.
- Eight replicates of each variety were watered using a nutrient medium containing all the plant requirements **except** nitrogen. These were the **low nitrogen** plants.
- Another eight replicates were watered with a nutrient medium containing all the required nutrients including nitrogen. These were the **high nitrogen** plants.
- The efficiency of nitrogen use was determined from the activity of three enzymes used in photosynthesis.

Fig. 2.1 shows the reactions in mesophyll cells catalysed by two of these enzymes.

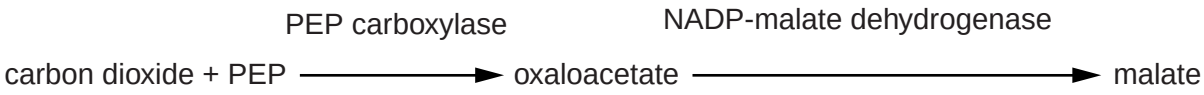


Fig. 2.1

In the bundle sheath cells malate is broken down to release carbon dioxide which is assimilated by the third enzyme, RuBP carboxylase/oxygenase (Rubisco).

(i) Identify the **two** independent variables in this investigation.

1. .....

2. .....[2]

(ii) Describe **one** way in which this experimental design helps to ensure the reliability of the results.

.....

.....[1]

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(b) Table 2.1 shows the results of this investigation.

Table 2.1

	mean enzyme activity of leaf samples ± S_M / nmol min^{-1} mg^{-1} protein					
	PEP carboxylase		NADP-malate dehydrogenase		Rubisco	
variety	high nitrogen	low nitrogen	high nitrogen	low nitrogen	high nitrogen	low nitrogen
W	29.1 ± 8.3	41.1 ± 5.6	2662 ± 331	338 ± 20	13.1 ± 0.4	1.5 ± 0.6
X	17.1 ± 1.6	8.1 ± 0.9	1979 ± 161	2468 ± 256	16.4 ± 2.9	2.0 ± 0.2
Y	44.4 ± 6.0	10.8 ± 2.4	927 ± 126	1936 ± 146	19.8 ± 1.2	3.3 ± 0.3
Z	59.4 ± 2.8	7.5 ± 1.2	1477 ± 89	2547 ± 350	23.0 ± 1.2	2.4 ± 0.1

(i) Calculate the mean percentage decrease in activity of Rubisco between high and low nitrogen conditions for variety **Y**. Give your answer to the **nearest whole number**. Show your working.

.....% [2]

(ii) With reference to Table 2.1 explain what standard error (S_M) shows about the results of this investigation.

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.....[2]

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(c) A number of *t*-tests were carried out to find out if the difference in activity of the enzymes was significantly different in the four varieties tested.

The tests carried out were between the following pairs of varieties:

W and **X**	**W** and **Y**	**W** and **Z**
X and **Y**	**X** and **Z**	**Y** and **Z**

(i) With reference to the activity of NADP-malate dehydrogenase in low nitrogen, shown in Table 2.1, identify the *t*-test(s) in which the difference in activity of NADP-malate dehydrogenase is **not** likely to be significant.

Show your answer by underlining the test(s). Give a reason for your answer.

.....

.....[2]

(ii) State why 14 degrees of freedom was used for each of the *t*-test calculations.

.....

.....[1]

(d) Using the information in Table 2.1, suggest which one of the varieties appears to be anomalous. Give a reason for your answer.

.....

.....[1]

[Total: 11]

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