VICTORIA JUNIOR COLLEGE
BIOLOGY DEPARTMENT
JC2 PRELIMINARY EXAMINATIONS 2016
Higher 2

CANDIDATE NAME

CLASS | 1 | 5 | S | | |

INDEX NUMBER | | |

BIOLOGY 9648/03

Paper 3 **19 September 2016**

Additional Materials: Answer Booklet/ Paper **2 Hours**

READ THESE INSTRUCTIONS FIRST

Write your CT GP/ INDEX NO. and name on all the work you hand in.
Write in dark blue or blue pen.
You may use a soft pencil for any diagrams, graphs or rough working.
Do not use any staples, paper clips, highlighters, glue or correction fluid.

Answer **all** questions.

At the end of the examination, fasten all your work securely together.

The intended number of marks is given in brackets [ ] at the end of each question.

For Examiner's Use	
Section A	
1	
2	
3	
Planning Question	
5	
Total	

Answer **all** questions

Question 1

(a) The bacterial plasmid, pBR322, was used as a vector for Gene X as shown in Fig. 1.1 below.

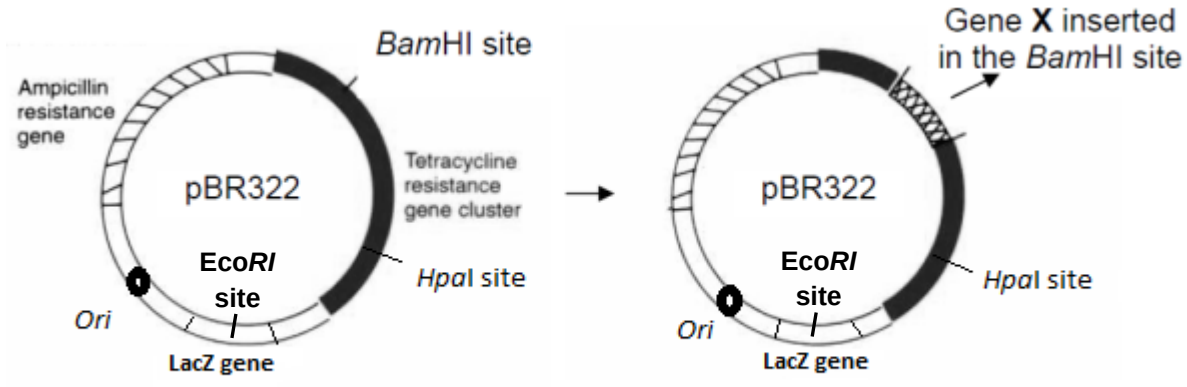

Fig. 1.1

Gene X was inserted in the *Bam*HI restriction site. pBR322 also contain the *Eco*RI and *Hpa*I restriction sites. The target sites for these restriction enzymes are shown in the table below. The lines drawn in each sequence show where the enzyme cuts the DNA molecule.

restriction enzyme	specific target base sequence of DNA
EcoRI	G | A A T T C C T T A A | G
BamHI	G | G A T C C C C T A G | G
HpaI	G T T | A A C C A A | T T G

(i) With reference to Fig. 1.1, explain how two properties of plasmid pBR322 allow it to be used as a vector.

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…… [2]

(ii) Outline the steps taken to produce the recombinant plasmid shown in Fig. 1.1.

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…… [2]

(iii) Explain the disadvantage that would arise if gene X was to be inserted into the *Hpa*I restriction site instead of the *Bam*HI site.

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…… [2]

(b) Calcium chloride heat shock treatment was then used to introduce the recombinant plasmid into *Escherichia coli*. However, the process of creating recombinant plasmids is typically not 100% efficient. Often, a mixture of re-annealed plasmid and re-annealed DNA is produced along with the recombinant plasmid. These may be taken up by the bacteria as well. This necessitates the process of selecting for the bacteria that have successfully taken up the recombinant plasmid. As such, the bacteria was first plated onto a nutrient agar plate containing ampicillin. Replica plating was subsequently carried out onto a nutrient agar plate containing tetracycline. Bacterial growth on both plates is shown in Fig. 1.2.

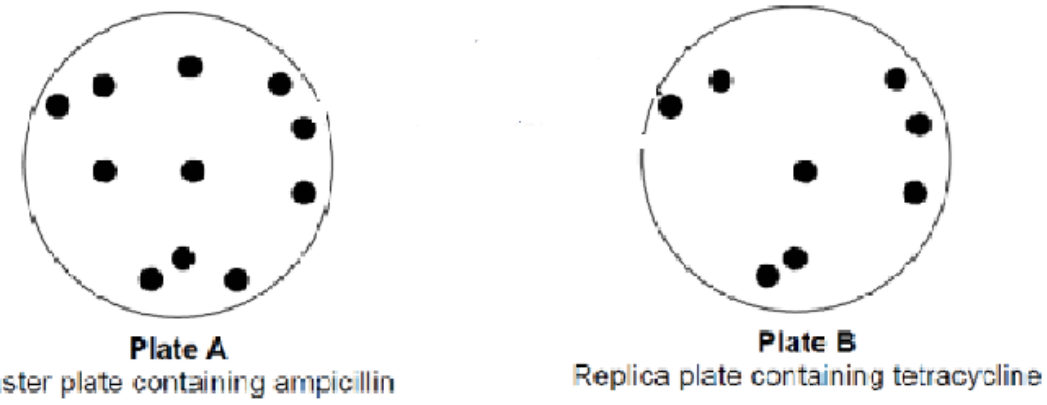

Plate A
Master plate containing ampicillin

Plate B
Replica plate containing tetracycline

Fig. 1.2

With reference to Fig 1.2,

(i) Circle the colonies that were successfully transformed with the recombinant plasmid.

[1]

(ii) Account for the difference in colony numbers in Plate A and B.

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…… [3]

(c) In a separate cloning experiment, the same plasmid pBR322 was used to introduce another gene, Gene Y, into a batch of E.coli cells. Fig. 1.3 shows the results of plating the transformed E.coli cells onto an agar plate with the appropriate substances.

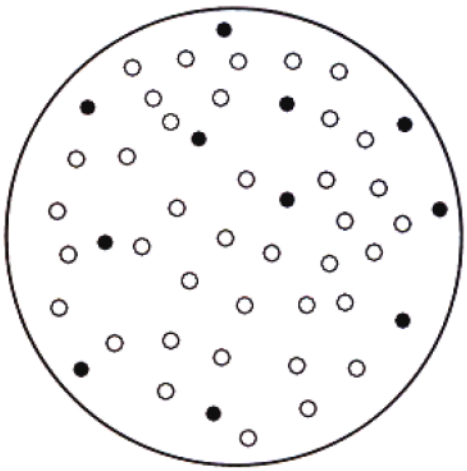

Fig. 1.3

(i) Explain why the colonies differ in colours in Fig. 1.3.

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…… [4]

(ii) Suggest why replica plating was not necessary in this experiment.

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…… [1]

(d) In another cloning experiment, another plasmid pBR33 was used to introduce Gene Z into a different strain of *E.coli* bacteria. Gene Z was inserted into one of the three selection markers found in pBR33 – neomycin resistance gene, kanamycin resistance gene and streptomycin resistance gene.

The bacteria were then plated onto nutrient agar plate containing neomycin. Replica plating was subsequently carried out onto nutrient agar plate containing streptomycin. Bacterial growth on the two plates is shown in Fig. 1.4 below.

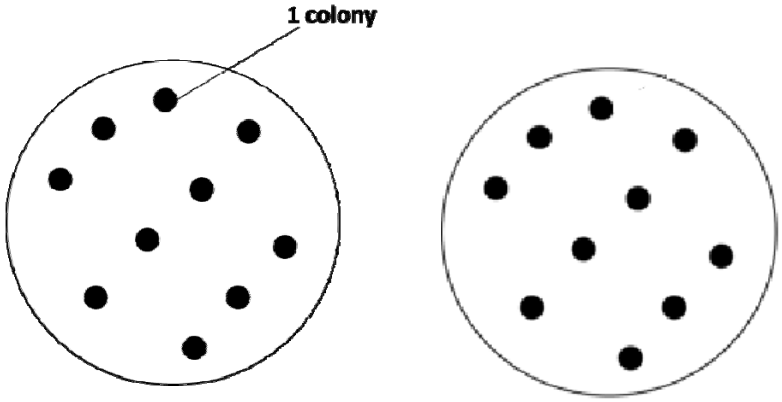

Fig. 1.4

Account for the results obtained in Fig. 1.4.

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…… [3]

[Total 18]

Question 2

Explain why two primers are used for polymerase chain reaction.

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…… [2]

Duchenne muscular dystrophy is a genetic disease in which there is a progressive loss of muscle mass, leading to physical weakness, difficulty in standing and walking, and eventually paralysis and death. Early symptoms of the disease can only be observed between the ages of 2 and 3 in most patients. A group of doctors and medical biologists discovered a RFLP marker, found on the same chromosome as the disease gene, which can be used in the screening of the disease during pregnancy.

To investigate the effective of the RFLP marker in disease screening, samples of DNA were obtained from a family known to have the disease. The RFLP locus was isolated and amplified using polymerase chain reaction, which was then mixed with *Bam*HI restriction enzymes. The pedigree tree of the family and results of gel electrophoresis are shown in Fig. 2.1.

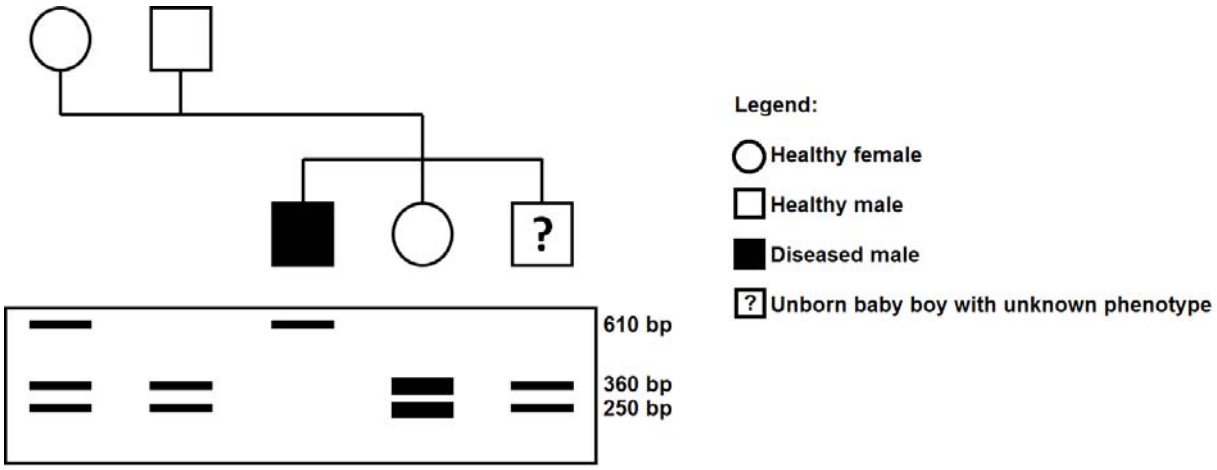

Fig. 2.1

(a) With reference to Fig. 2.1,

(i) state the mode of inheritance of the Duchenne muscular dystrophy.

…… [1]

(ii) explain why there are different fragment lengths after restriction digest.

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…… [3]

(iii) Explain the difference in the band patterns between the father and the daughter.

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…… [3]

(b) Some years later, the baby boy with unknown phenotype is born and has reached two years of age. Clinical diagnosis reveals that he too suffers from Duchenne muscular dystrophy.

(i) Explain why the band pattern of the baby boy is different from that of his older brother even though both are with the disease. Assume no new mutations occurred in the disease gene or RFLP locus.

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…… [2]

(ii) Suggest an ethical implication that may arise due to the use of this RFLP marker to screen for Duchenne muscular dystrophy.

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……. [1]

[Total: 12]

Question 3

(a) Patients with severe combined immunodeficiency disorder (SCID) are vulnerable to serious infections and death. There are two main types of SCID, X-SCID and ADA-SCID. Besides the location and difference in their modes of inheritance, give two differences between the two types of SCID.

Difference	X-SCID	ADA-SCID
1		
2		

[2]

(b) Gene therapy can be used to treat SCID by introducing retrovirus containing the normal gene into hematopoietic stem cells (HSCs). The HSCs are then infused into the patient.

Explain how this choice of vector and target cells may theoretically lead to long term treatment of the disease.

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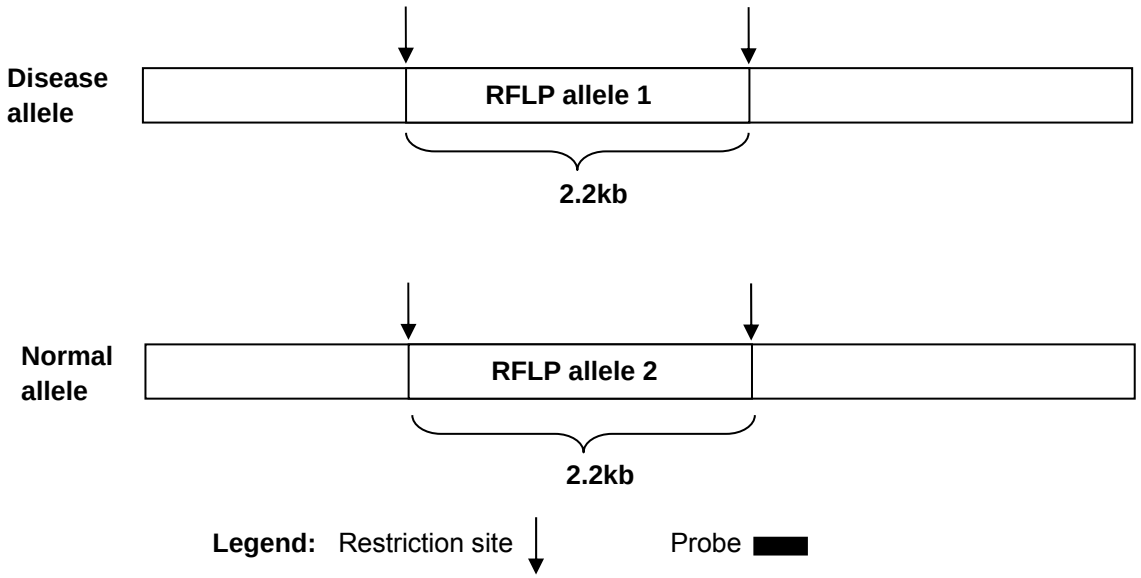
……. [2]

(c) In one attempt to treat ADA-SCID, hematopoietic stem cells (HSCs) from the bone marrow of baby X patient were collected and treated with retrovirus containing the ADA gene before infusing them back.

Genomic DNA from the T lymphocyte cells of baby X was later extracted. The extracted DNA was digested with a restriction enzyme and subjected to Southern blot analysis using a specific probe that binds to a known RFLP marker found within the gene associated with the disease.

The RFLP allele 1 associated with the disease allele gives rise to a 0.8kb band while the RFLP allele 2 associated with the normal allele gives rise to a 2.2kb band.



(i) With the information provided, draw to indicate the position of another restriction site and the position where the probe binds to. You should also indicate the length of the restriction fragments that would be produced. [1]

The RFLP band patterns from baby X's normal brothers, Tom and John, are shown in Fig. 3.1.

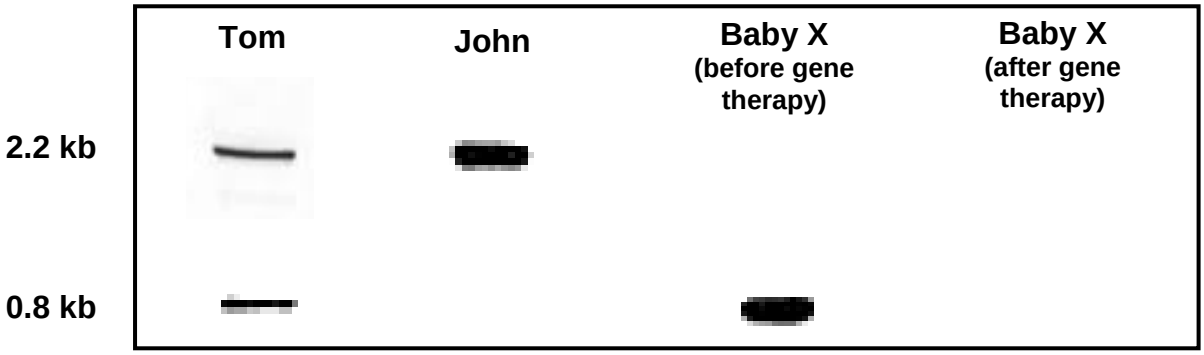


Fig 3.1: Results of Southern Blot analysis of DNA extracted from T lymphocytes

(ii) With reference to Fig 3.1, explain the difference in the band pattern of Tom and John.

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…… [2]

(iii) Draw the expected band pattern of baby X after gene therapy treatment. [1]

Over the next 360 days after infusion, the total number of various blood cells (e.g. red blood cells and white blood cells) of baby X were recorded at regular intervals.

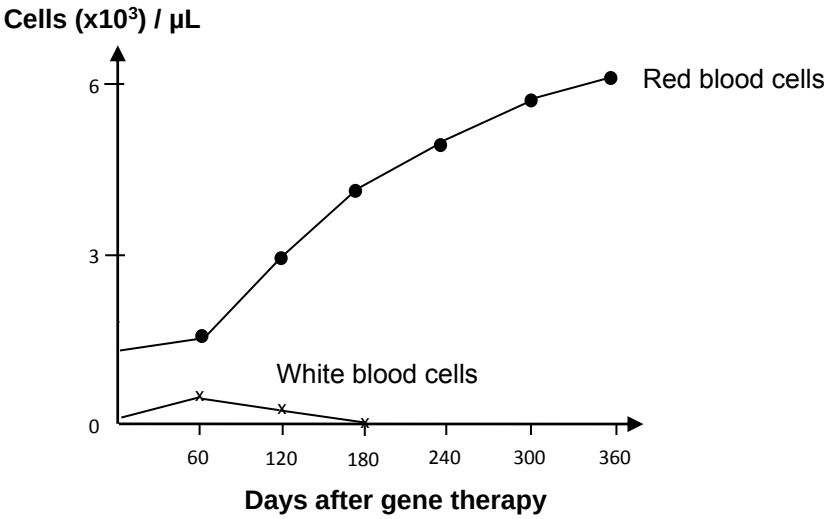

Fig 3.2

(iv) With reference to Fig 3.2, describe the effectiveness of the treatment in baby X.

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…… [1]

(v) Suggest a reason for your answer. [1]

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…… [1]

[Total 10]

Planning Question

4. An orange plantation owner wants to find out the amount of ascorbic acid (vitamin C) that his breed of oranges produces. He believes that his oranges produce the most vitamin c compared to the standard orange breeds which typically contain 0.8 to 1.6 $mmolL^{-1}$.

The amount of ascorbic acid present in a sample can be determined using a bioassay method. At pH 8, ascorbic acid reduces solutions of the dye dichlorophenol indophenol (DCPIP) from blue to colourless. For the bioassay to work, the pH of the samples must be adjusted to pH 8. Ascorbic acid does not chemically change when neutralised by sodium hydroxide, or when boiled.

Using this information and your own knowledge, design an experiment to determine the validity of the plantation owner's claim that orange juice from his plantation contains higher concentrations of ascorbic acid.

Your planning must be based on the assumption that you have been provided with the following equipment and materials which you must use:

- 100 cm^3 of 5.0 $mmolL^{-1}$ stock solution of ascorbic acid, adjusted to pH 7
- 100 cm^3 distilled water
- 100 cm^3 molten agar containing DCPIP
- Sterile petri dishes
- 1ml syringe
- plastic straw to create wells in the agar plate
- Ruler / 2mm graph paper
- Labels
- Timer, e.g. stopwatch
- Bunsen burner
- Normal laboratory glassware e.g. test tubes, beakers, graduated pipettes, droppers, glass rods etc
- 10 cm^3 orange juice, supplied by the plantation owner
- 10% sodium hydroxide solution
- pH indicator paper to indicate alkaline pH

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it,
- be illustrated by relevant diagrams, if necessary,
- identify the independent and dependent variable,
- describe the method with the scientific reasoning used to decide the method so that results are as accurate and reliable as possible,
- show how you will record your results and the proposed layout of tables and graphs,
- use correct technical and scientific terms,
- include references to safety measures to minimize any risk associated with the proposed experiment.

[Total: 12]

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Free-response question

Write your answers to this question on the separate paper provided.

Your answer:

- should be illustrated by large, clearly labelled diagrams, where appropriate.
- must be in continuous prose, where appropriate.
- must be set out in section (a), (b), etc., as indicated in the question.

5 **(a)** Outline the procedure for the production of human growth hormone by genetic engineering techniques. [8]

(b) Describe the benefits of the Human Genome Project. [8]

(c) Discuss the ethical concerns that have arisen from genetically modified organisms. [4]

[Total: 20]