H

RAFFLES INSTITUTION

2015 Year 6 Preliminary Examination
Higher 2

CANDIDATE NAME

CIVICS GROUP | 1 | 5 | S | 0 | 3 |

INDEX NUMBER

BIOLOGY
Paper 3

9648/03
22nd SEPTEMBER 2015
2 hours

Additional materials: Answer Sheet

READ THESE INSTRUCTIONS FIRST

Write your index number, CT group & name on all the work you hand in.
Write in dark blue or black pen on both sides of the paper.
You may use a soft pencil for any diagrams, graphs or rough working.
Do not use staples, paper clips, highlighters, glue or correction fluid.

Sections A and B

Answer **all** questions.

At the end of the examination, **hand in your essay SEPARATELY.**
The number of marks is given in brackets [ ] at the end of each question or part question.

For Examiner's Use	
Section A	
1	**/12**
2	**/13**
3	**/15**
4	**/12**
Section B	
5	**/20**
Total	**/72**

This document consists of **13** printed pages.

For Examiner's Use

Section A

Answer **all** the questions in this section.

1 Plasmid **X** is used to clone a gene of interest. **Fig. 1.1** shows a diagram of plasmid **X**. The restriction sites of four restriction enzymes are shown in **Table 1.1**.

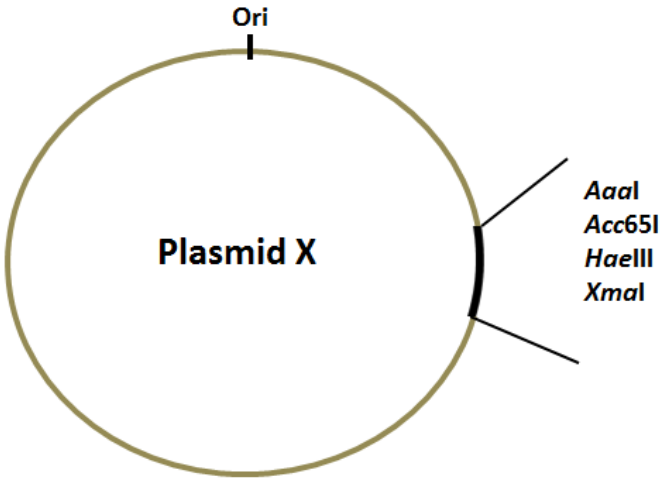

Fig. 1.1

Restriction Enzyme	Restriction Sites
*Aaa*I	5' ---T GTACA--- 3' 3' ---ACATG T--- 5'
*Acc*65I	5' ---G GTACC--- 3' 3' ---CCATG G--- 5'
*Hae*III	5' ---GG CC--- 3' 3' ---CC GG--- 5'
*Xma*I	5' ---C CCGGG--- 3' 3' ---GGGCC C--- 5'

Table 1.1

(a) **(i)** With reference to the information in **Table 1.1**, describe the characteristics of a restriction site. [2]

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(ii) The restriction enzyme *Puv*II recognises a 6 base pair sequence on double stranded

DNA. The first three bases of one strand are given, complete the restriction site for *Puv*II. [1]

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5′ C A G _ _ _ 3′
3′ _ _ _ _ _ _ 5′
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(iii) Discuss the significance of having the multiple cloning site on plasmid **X**. [1]

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(b) To clone a gene of interest into a plasmid, a researcher used restriction enzyme *Aaa*I to digest the plasmid. However, he used restriction enzyme *Acc*65I to isolate the DNA fragment.

(i) Explain whether or not the DNA fragment can be cloned into the plasmid to form a recombinant plasmid. [3]

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(ii) In another experiment, *Hae*III was chosen over the other 3 restriction enzymes to produce restriction fragments.

Explain an advantage of using *Hae*III in cloning. [2]

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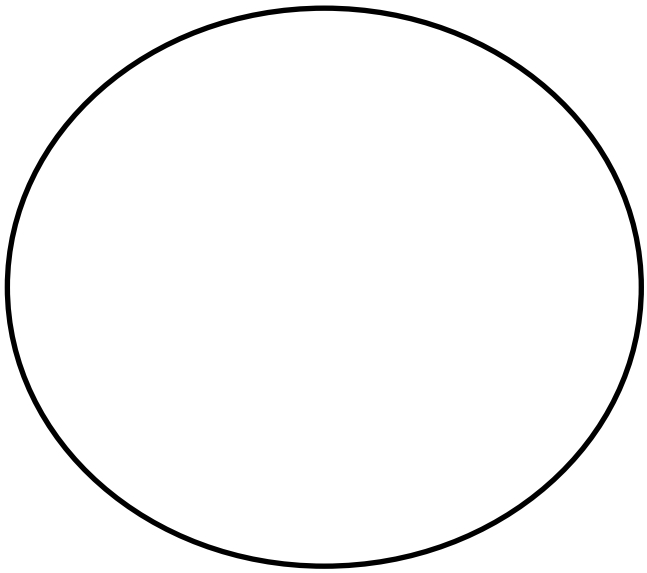
(c) Plasmid **Y** was digested with *Eco*RI, *Bam*HI, and both restriction enzymes together. All reactions are allowed to run to completion and **Table 1.2** shows the sizes of the resulting DNA

fragments:

*Eco*RI	*Bam*HI	*Eco*RI and *Bam*HI
7.0 kb	4.0 kb	4.0 kb
1.0 kb	2.8 kb	2.0 kb
	1.2 kb	1.0 kb
		0.8 kb
		0.2 kb

Table 1.2

(i) Using the circle below, draw a restriction map for plasmid **Y**. [2]



(ii) During the digestion of the DNA by *Hae*III, regulation of temperature is important. An experiment was carried out to investigate the rate of digestion by restriction enzyme, *Hae*III, over a range of temperatures.

Explain the significance of carrying out this experiment. [1]

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[Total : 12]

2 Restriction fragment length polymorphism (RFLP) can be used to detect variations in genomes. The technique is widely used in molecular biology.

(a) Explain what RFLPs is. [3]

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(b) Women with mutations in the *BRCA–1* gene found on chromosome 17, have been found to have a significantly increased risk for breast and ovarian cancers. These mutations are often heritable. Women with family members who show incidences of breast and ovarian cancers sometimes choose to get tested to determine if they have a mutated *BRCA-1* gene. Genetic testing typically involves RFLP analysis.

A radioactive probe, **P**, revealed two RFLP alleles that are tightly linked to the *BRCA-1* gene. Both RFLP alleles are shown in **Fig. 2.1**. The arrows represent the restriction sites of the restriction enzyme used. The size of each resulting fragment (in kb) is indicated.

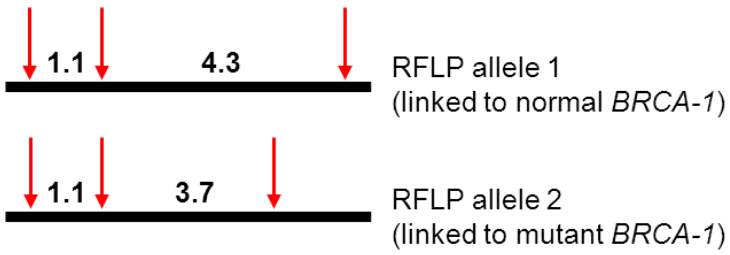

Fig. 2.1

Fig. 2.2 shows the pedigree of a family with some female members with incidences of breast and/or ovarian cancer, as well as the results of their RFLP analysis. The pedigree and RFLP

analysis was prepared at the request of individual **III-1** who requested medical advice about her risk for developing cancer. Each person's DNA sample is shown directly below the individual.

The underlined numbers indicate the age at which cancer developed in individuals **I-2**, **I-5**, **II-3**, **II-4**, **III-2** and **III-5**, where **Br** indicates incidence of breast cancer and **Ov** for incidence of ovarian cancer. The current ages of **II-2** and **III-1** have also been indicated.

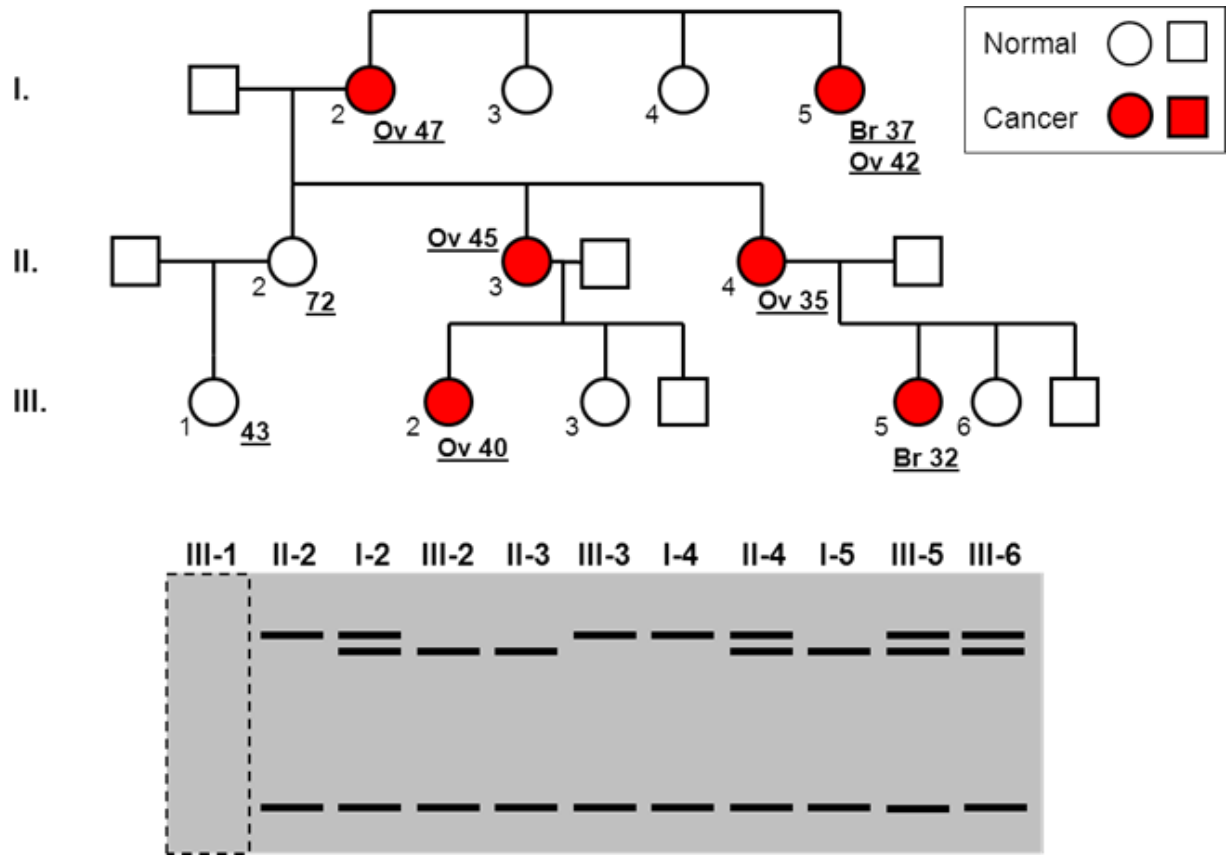

Fig. 2.2

(i) Based on the Southern blot results shown in **Fig. 2.2,** indicate with a box on **Fig. 2.1**, the position where probe **P** will bind to the RFLP alleles. [1]

(ii) Explain if the *BRAC-1* mutation is dominant or recessive. [2]

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(iii) Assuming that **III-1**'s father does not carry the mutation, indicate her banding pattern in the box in **Fig. 2.2**. [1]

(iv) For a RFLP allele to be useful in disease detection, it must be tightly linked to the gene locus of the disease allele. Explain. [1]

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(c) Before the genome of an organism is sequenced, preliminary mapping is usually first carried out. RFLP analysis is a useful technique in genome mapping. **Fig. 2.3** shows a chromosome map of a few of the 1600 genes on the human chromosome 17.

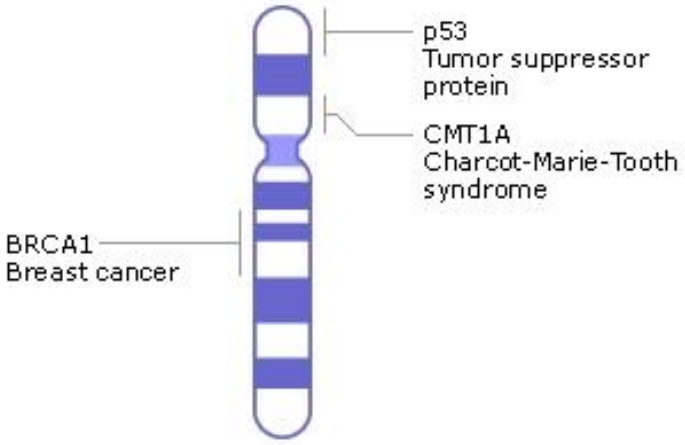

Fig. 2.3

(i) State what is meant by a chromosome map. [1]

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(ii) Explain how RFLP analysis can be used to construct a chromosome map. [4]

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[Total : 13]

For Examiner's Use

3 Patients with severe combined immunodeficiency disorder (SCID) cannot produce many types of white blood cells to fight infections. Children with SCID are vulnerable to serious infections and early death.

Early investigations focused on treating the disease via gene therapy on mouse models. The experiment involved adult mice which have a form of SCID similar to that in human beings.

(a) (i) Explain what is meant by gene therapy. [1]

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(ii) Describe the genetic basis of SCID in human beings. [2]

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Using the mouse model, a researcher attempted to treat SCID with embryonic stem cells. **Fig. 3.1** below shows how she used somatic cell nuclear transfer to obtain embryonic stem cells.

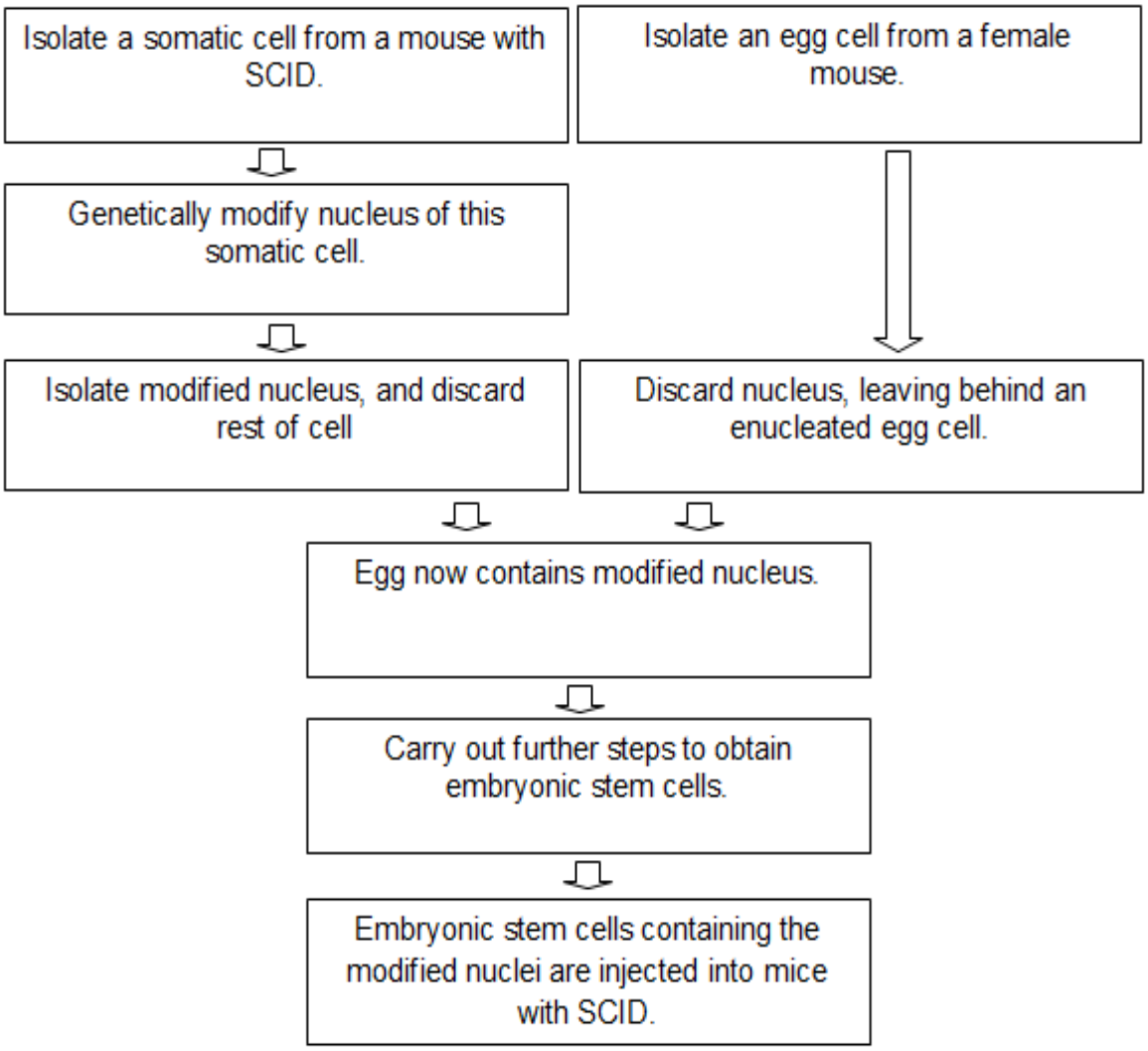

Fig. 3.1

With reference to **Fig. 3.1**,

(b) **(i)** explain why the isolation of embryonic stem cells from the mouse with SCID is not a feasible procedure; [1]

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(ii) list the steps needed to obtain the embryonic stem cells; and [2]

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For Examiner's Use

(iii) state why using embryonic stem cells may not guarantee success in treating SCID. [1]

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(c) Many attempts have been made to find different gene therapy methods to treat SCID.

One approach uses viruses to deliver therapeutic alleles into haemotopoietic stem cells. A team of researchers developed a new strain (AAV2.5T) from AAV, a non-pathogenic virus.

A gene to correct the SCID mutation is ligated to a gene that codes for an enzyme, luciferase. These were added to the DNA of the viruses. Luciferase produces a green fluorescence in the presence of luciferin.

The normal AAV strain and the AAV2.5T strain were added to cultures of white blood cells. After adding luciferin, the numbers of cells that had taken up the viral genes was estimated using the intensity of the green fluorescence which developed.

The results are shown in **Fig. 3.2**.

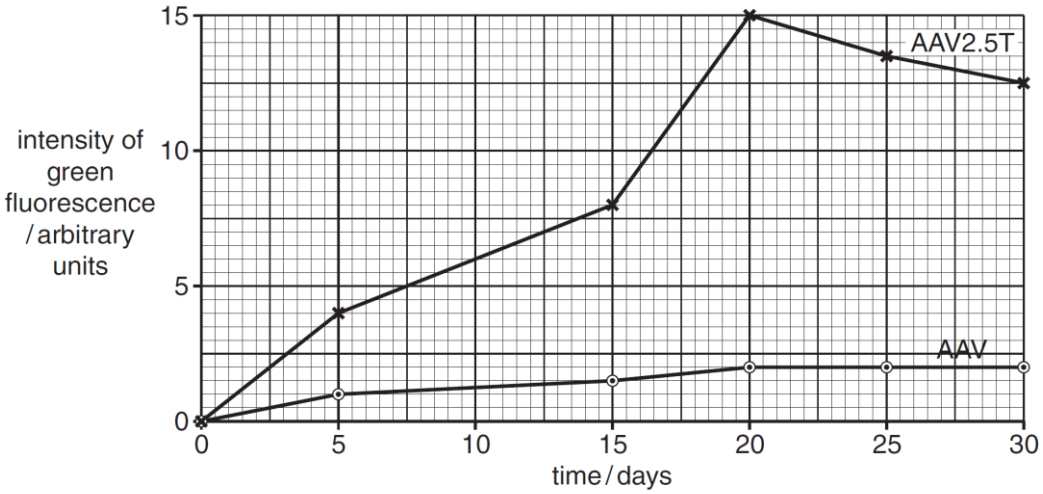

Fig. 3.2

With reference to **Fig. 3.2**,

(i) compare the ability of the two viral strains, AAV and AAV2.5T, to infect the stem cells; [2]

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(ii) explain why the researchers added a gene for luciferase to the viral DNA; [2]

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(iii) suggest the modification made to AAV in developing the new strain AAV2.5T. [1]

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(d) Clinical trials were conducted on a small number of patients with SCID using virus strain AAV. Blood cells were obtained from these patients, those with SCID that did not undergo gene therapy and healthy volunteers.

10 µg of extracted genomic DNA samples were digested with the same restriction enzyme, and subjected to Southern hyridisation using a specific probe.

The results of the blot are shown in **Fig. 3.3**.

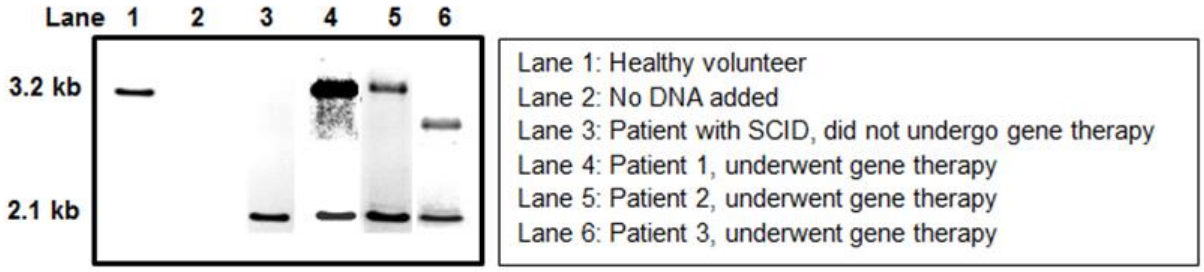

Fig. 3.3

With reference to **Fig. 3.3**,

(i) explain why the results show that this treatment may be a permanent solution to SCID; and [2]

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(ii) suggest why the 3.2 kb band in patient 1 is thicker. [1]

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[Total : 15]

4 Many plants produce two types of leaves. One type is produced where the leaves develop in full sunlight and are called 'sun leaves'. The other type is produced where the leaves develop in the shade and are called 'shade leaves'.

Leaf discs can be used to determine the effect of a variable on the rate of photosynthesis. The discs can be cut from the leaves and are then placed in a syringe. The syringe is slowly filled with water or very dilute sodium hydrogencarbonate ($NaHCO_3$) solution.

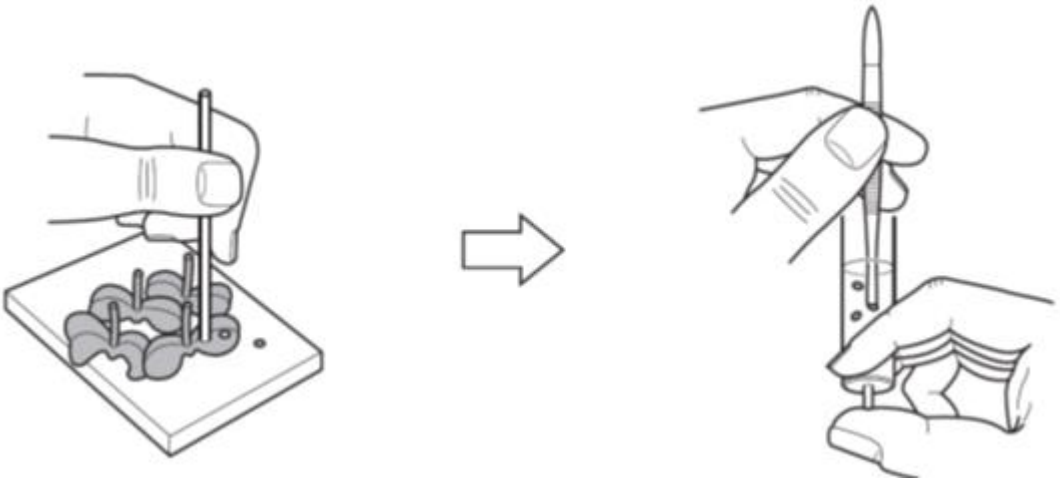

Fig. 5.1

Air inside the syringe is expelled by pushing in the plunger. Air is then drawn out of the intercellular air spaces of the leaf discs by applying the finger firmly to the nozzle and attempting to withdraw the plunger to create a vacuum. As the air is drawn out, the leaf discs sink and are then ready to use.

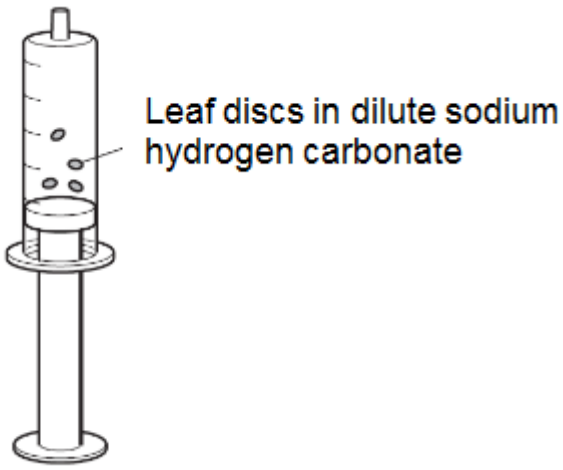

Fig. 5.2

Using the information and your own knowledge, design an experiment to investigate the effect of light intensity on the rate of photosynthesis in sun leaves and shade leaves and to compare their maximum photosynthetic rate at light saturation.

You must use the following:

- sun and shade leaves from *Miconia fallax*
- 25cm^3 syringe
- lamp (with 18W LED bulb, which emits very little heat)
- 5% sodium hydrogen carbonate
- straw (0.5 cm diameter)
- photometer
- stopwatch

You may select from the range of common laboratory apparatus e.g.:

- any normal laboratory glassware e.g. test-tubes, beakers, measuring cylinders, forceps, graduated pipettes, glass rods, etc.,
- bunsen burner
- thermometer
- meter ruler
- syringes
- etc

Your plan should have a clear and helpful structure to include:

- a description of the method used including the scientific reasoning behind the method;
- an annotated diagram, if necessary;
- an explanation of the dependent and independent variables involved;
- how you will record your results and ensure they are as accurate and reliable as possible;
- proposed layout of results tables and graphs with clear headings and labels;
- the correct use of technical and scientific terms and
- relevant risks and precautions taken.

[Total : 12]

Section B

Write your answers on the separate answer paper provided.
Your answers should be illustrated by large, clearly labeled diagrams, where appropriate.
Your answers must be in continuous prose, where appropriate.
Your answers must be set out in sections **(a)**, **(b)** etc., as indicated in the question.

5 **(a)** Using a named aquatic animal, explain the significance of genetically modified animals. [5]

(b) Discuss the ethical issues of genetically modified animals and plants. [6]

(c) Explain how different plant cloning techniques can help to increase the yield of crop plants. [9]

[Total: 20]

---- End of Paper ----