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**.

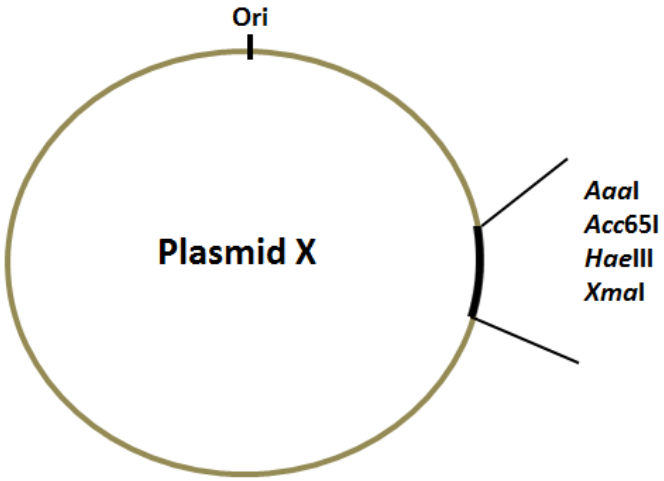


Fig. 1.1

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]

1. Restriction site is a specific DNA sequence consists of 4 to 6 base pairs ;
2. which is ***palindromic**** where reading from 5' to 3' (or 3' to 5') of each of the strands yield the same sequence

(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]

```
5′ C A G _ _ _ 3′
3′ _ _ _ _ _ _ 5′
```

(iii) Discuss the significance of having the multiple cloning site on plasmid **X**. [1]

1. Presence of MCS that has a number of UNIQUE different restriction sites on a plasmid allows the plasmid to be used to insert / contain a wide range of different foreign genes;

(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]

1. Yes the cut DNA by Acc65I can join to plasmid cut by AaaI
2. as they both produce complementary / same sticky ends with GTAC / CATG
3. that can form hydrogen bonds and ***anneal**** by ***complementary base pairing****
4. Adenine pair with thymine and cytosine with guanine

(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]

1. *Hae*III produces ***blunt ends****
2. Plasmid / Restriction fragments produced will not ***reanneal****.
3. Less specific , can ligate into any blunt-ended DNA fragments of interest (ref to wider range of plasmid / fragments to clone)
4. Ref to 4base cutter vs 6 base cutter – shorter fragments, easier to clone

Must have 1

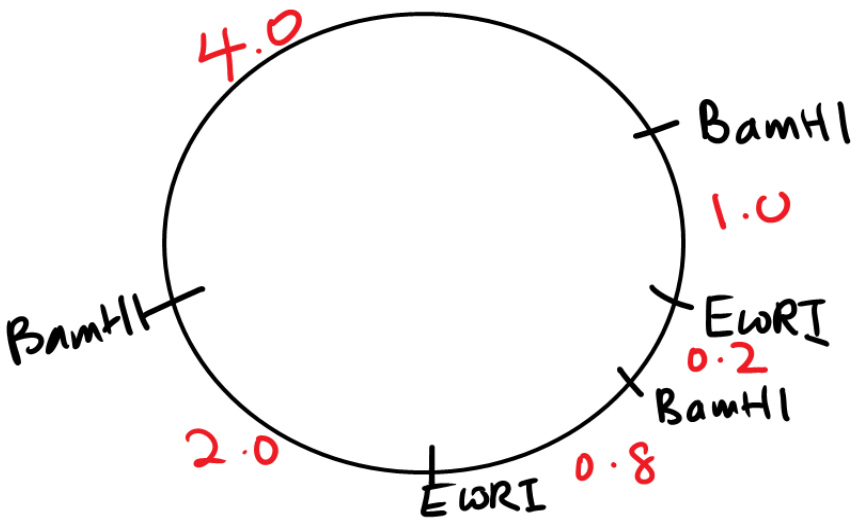
(c) Plasmid **Y** was digested with *Eco*RI, *Bam*HI, and both restriction enzymes together. All reactions are allowed to run to completion and **Table 1.2** shows the sizes of the resulting DNA fragments:

*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]

For Examiner's Use



Can be clockwise / anti clockwise

(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]

Identify optimum temp, where activity is the highest ;
so as to achieve complete digestion at the fastest rate / shorter time;.

.

[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]

1. Due to the presence of DNA polymorphisms/nucleotide differences in homologous regions/loci/marker of different individuals
2. Resulting in variations in number and/or location of restriction sites and
3. Varying number of tandemly repeated nucleotide sequences among individuals
4. when digested by ***restriction enzymes****, produce fragments of different length and/or number / unique banding pattern among individuals.

(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.

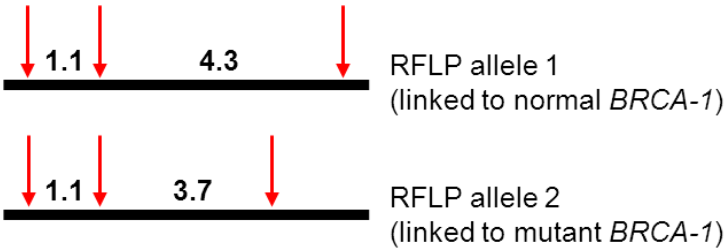


Fig. 2.1

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.

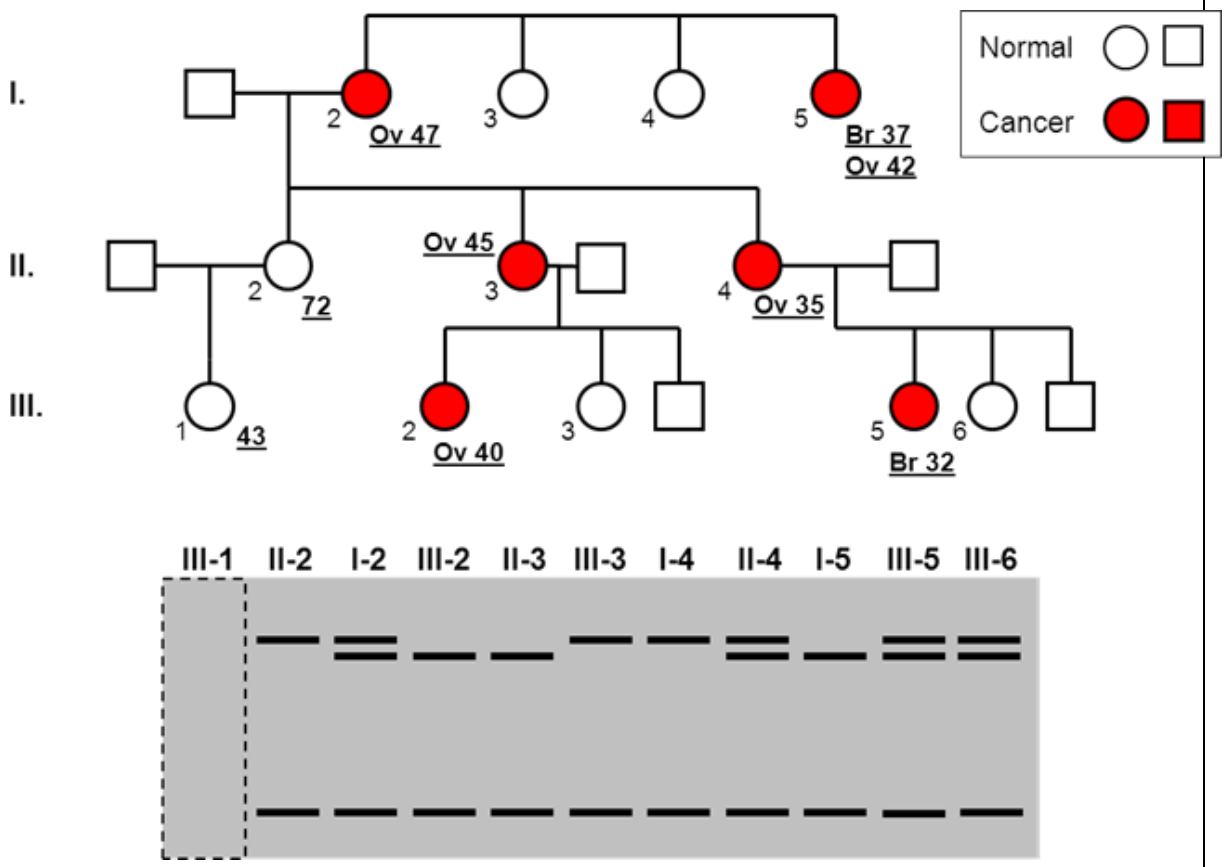
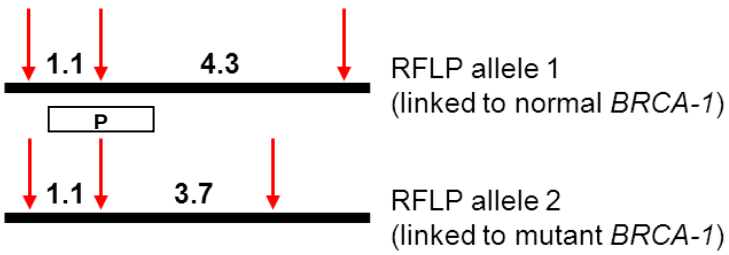


Fig. 2.2

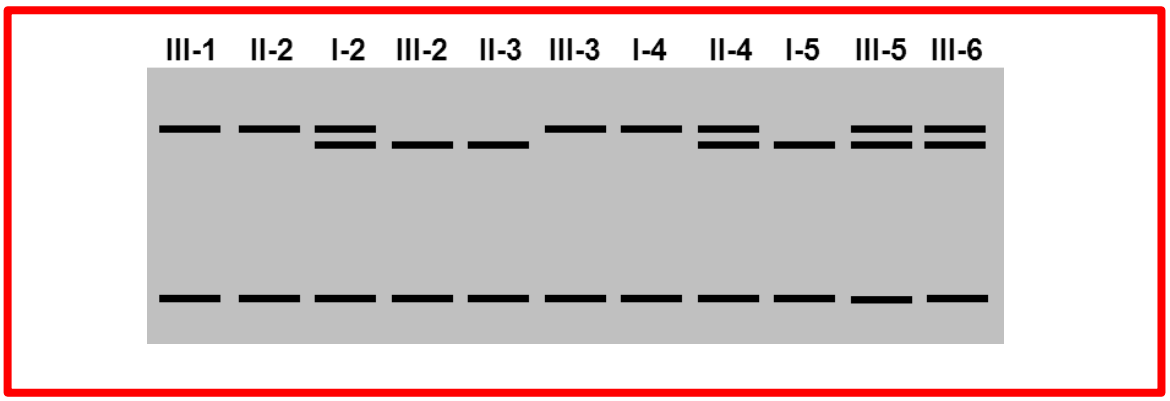
(i) Based on the Southern blot results shown in **Fig. 2.2,** indicate with a box on **Fig. 2.1**, the position where probe **P** will bind to the RFLP alleles. [1]



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

1. Dominant
2. Individuals I-2 / II-4 / III-5 [R: III-6] are heterozygotes (with a normal and a mutant *BRCA-1*), however they have cancer.
   or
   In individuals I-2 / II-4 / III-5 [R: III-6], the presence of just a single copy of mutant allele will mask the effect of the normal allele and result in cancer.

(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]

1. So that the RFLP allele and disease allele will have a higher chance of being inherited together;
   OR
2. To minimise chance of ***crossing over**** at the region between disease allele and marker allele so that linkage of RFLP allele to disease allele is not disrupted;

(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.

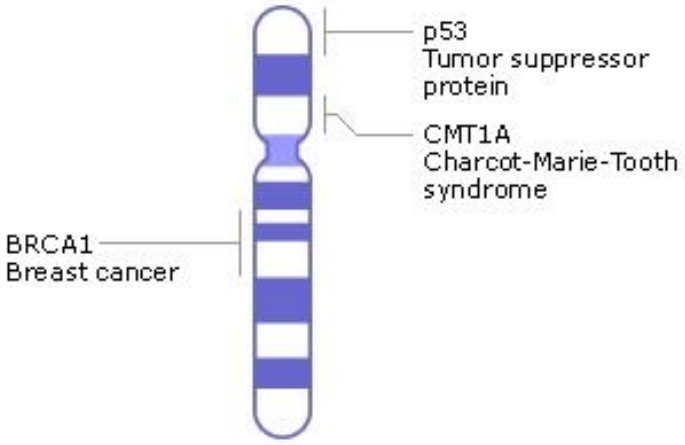


Fig. 2.3

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

A diagram showing arrangement / position / order /sequence of genes/genetic markers [reject: allele] and their relative distance on a chromosome.
[R: show linkage]

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

1. Identify at least 2 genes / genetic markers that can be detected as RFLPs / unique banding patterns
2. Parents with known genotype at two RFLP loci are crossed
3. To find recombinant frequency in offspring
4. Recombination frequency (%) of linked RFLPs indicates relative distance between 2 RFLP loci
   Or
   1% recombinant frequency = relative distance of 1 centiMorgan/cM
   Or
   If % recombination freq between 2 RFLPs on a chromosome is high, they are far apart / if % recombinant freq is low, they are close together.
5. Give formula: recombination freq = number of recombinants in progeny / total number of progeny x 100%

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]

A method of treating genetic diseases which involve introduction of a ***normal, functional**** allele into cells of an affected individual where it will express the normal protein.

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

1. ***recessive****
2. X-chromosome / sex-linked disease
3. Mutation in gene for common gamma chain / an interleukin receptor

Any 2 of 3

4. SCID is a result of a ***recessive**** mutation
5. on ***chromosome 20****
6. Mutation in gene coding for ***adenosine deaminase enzyme (ADA*)***;

Any 2 of 3

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.

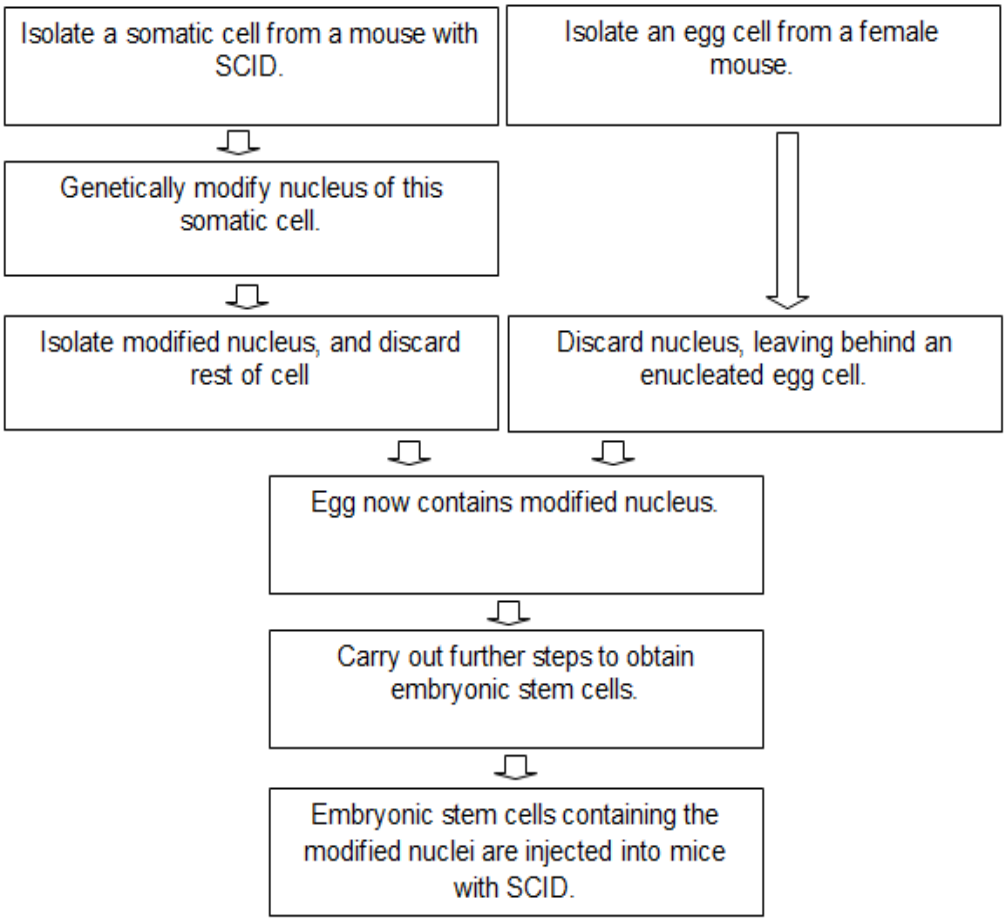


Fig. 3.1

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]

Diseased mouse is an adult and has no embryonic stem cells.

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

1. Grow egg cell in culture into a ***blastocyst****
2. isolate ***inner cell mass cells**** (of blastocyst) to get embryonic stem cells

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

May not ***differentiate**** into haematopoietic stem cells/ B lymphocytes /T lymphocytes;

(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**.

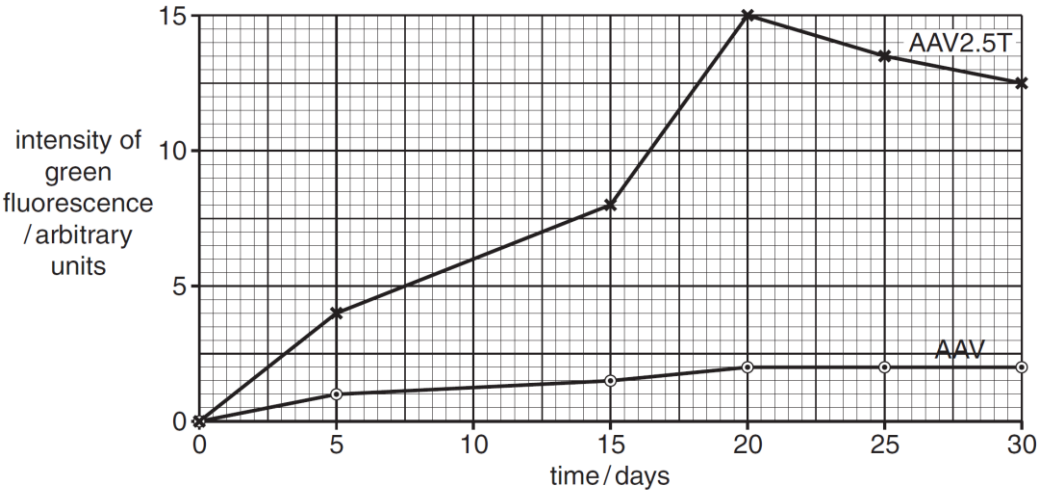


Fig. 3.2

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]

Similarity

1. Both infect blood stem cells;
2. data AAV2.5T increase from 0 to 15 a.u from Day 0-20 vs AAV increased from 0 to 2 a.u.;

Differences

3. AAV2.5T infects more cells than AAV;
4. AAV2.5T increased by 15 a.u from Day 0-20 vs AAV increased by only 2 a.u.;

OR

5. Ability to infect cells decreases after 20 days for AAV2.5T; (idea of peaking)
6. Proportion of cells infected by AAV2.5T falls from 15 a.u. to 12.5 a.u. while AAV remains steady at 2 a.u.;

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

1. Acts as a ***selectable marker****;
2. Able to identify infected cells with gene of interest/ recombinant plasmid which fluoresce in presence of luciferin;

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

Glycoprotein of AAV2.5T has an ***improved**** binding ability to haemotopoietic stem cells;

(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**.

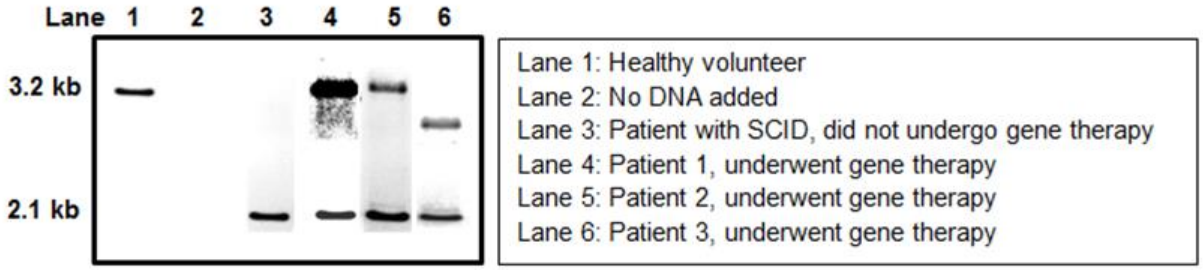


Fig. 3.3

With reference to **Fig. 3.3**,

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

1. Presence of ***normal functional allele****, **3.2 kb** fragment in patients 1 and 2 / ref to lane 4 or 5
2. Integrate into host chromosome;ensures stable gene expression;

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

Multiple copies of normal functional allele integrated into host chromosome;

[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.

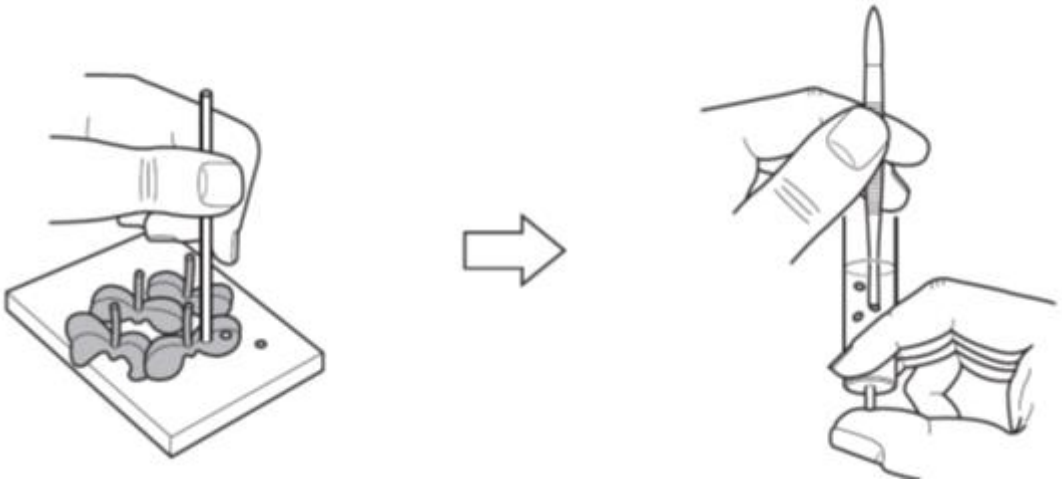


Fig. 5.1

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.

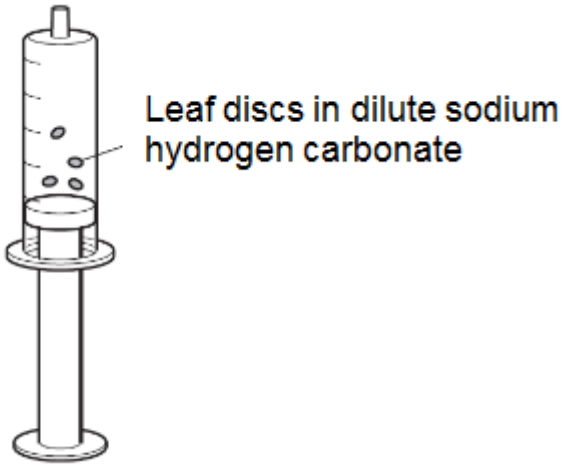


Fig. 5.2

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]

1. **A**im : To investigate the effect of light intensity on sun and shade leaves and to compare their light saturation values.

2. **T**heory [T1 to T7 - max 3]:

(Main theory) – 1 mark

[T1] Light reaction of photosynthesis results in photolysis/splitting of water to produce oxygen.

[T2] In light dependent reaction, light energy causes excitation / loss of e- from chlorohyll a /in the reaction centre.

[T3] Light saturation is when increasing light intensity the photosynthetic rate will not increase/ light is no longer limiting

(Measurable quantity) – 1 mark

[T4] Time taken for the disc to rise

→ Possible alternatives e.g. time for (all or a specified number of) discs to rise / specified time and count the number of discs floating / distance risen in stated time

Or

[T5]oxygen that collects in the intercellular spaces / decreases the density /increases the buoyancy of the leaf disc thus causing the it to float

(Predicted trend) – 1 mark

[T6] The higher the light intensity, the faster the rate of photosynthesis and the shorter time taken for the leaf disc to rise until light saturation is reached.

OR

[T7] Shade leaf has lower maximum photosynthetic rate than sun leaf at their respective light saturation point. ORA

[DV] **Dependent variable:** and rate of photosynthesis is measured as inverse of time taken for leaf disc to rise or 1/time taken [1]

[IV] **Independent variable:** light intensity at 60, 70, 80, 90, 100, 110 lux / varying distance of the lamp e.g. 20, 30, 40, 50 and 60cm

– at least 5 regular intervals (either the lux or distance) & method + use of ***photometer**** to determining the light intensity. [1]

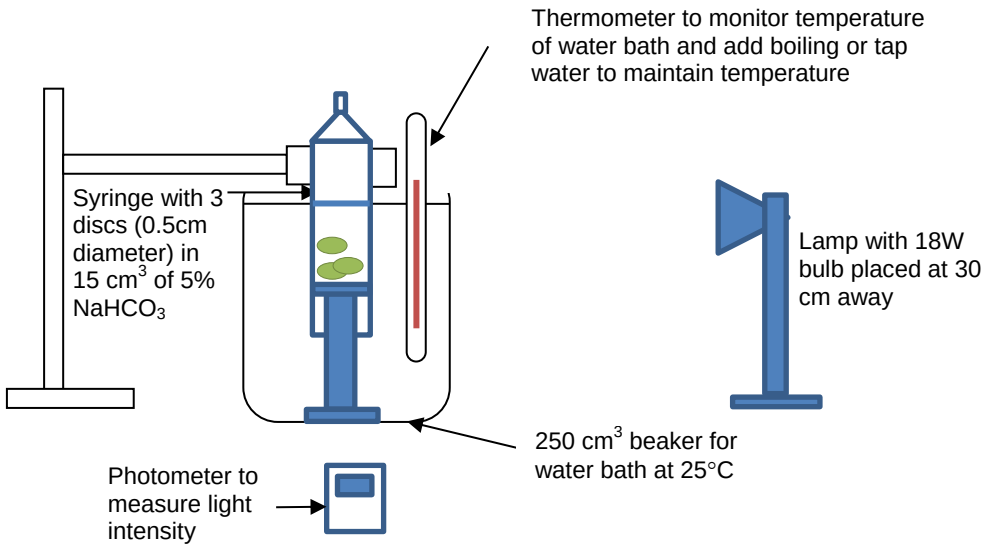
Note : 5 values can be marked from the table, but procedure must be mentioned somewhere

3. **P**rocedure (PAN CR)

a) [P] **P**ilot test*

Conduct a pilot experiment to determine suitability of apparatus, suitability of range of independent variable (e.g. light intensity), optimum conditions, amount of materials used.

b) **A**nnotated diagram



Constant variables: What, How (Why)

[CV1] what, how variable 1;

[CV2] what, how variable 2;

What variable	How	Why
Constant surface area/size of leaf disc	Use same ***straw**** (0.5 cm) to cut of leaf disc	(why) increases surface area increases the amount of chlorophyll /surface of leaf for photosynthesis
Number of leaf discs	use 3 leaf discs	More leaf discs may take longer time for all to float/ shading others through overlap
Same leaf/same location of same plant	Discs are from the same leaf/ same location such as from the shaded region for shade leaf and the exposed to light region for sun leaf	All cells have the same development stage and amount of pigment
Concentration of sodium hydrogen carbonate	e.g. 5% concentration	Source of carbon dioxide which is a limiting factor to photosynthesis.
Volume of sodium hydrogen carbonate	e.g 15 cm^3	larger volume has higher quantity of CO_2
Temperature	use a ***water bath**** and maintain the temperature at e.g. 25^0C by adding warm or cold water and monitor with a thermometer	Photosynthesis is an enzyme catalyzed reaction and thus rate is affected by temperature.

a) Numbered steps

1. Using a ***straw**** (diameter 0.5cm), cut out leaf discs from the leaf blade/ base of leaf from different sun and shade leaves. Ensures discs of same size. Cut 27 discs for each type of leaf.

2. Place 3 pieces of leaf discs from ***sun leaves**** in the ***25 cm^3 syringe**** using forceps.

3. Draw up 15 cm^3 of ***5% sodium hydrogen carbonate**** into the syringe *.

4. Force out the air from the leaf discs by creating a vacuum and ensure all the discs sink.

5. Place the syringe as shown in the water bath with $25^{\circ}C$ water. Add boiling or cool water, to maintain the temperature. Use thermometer to monitor temperature.

6. [E] Allow 2 min of equilibration time for the (why) temperature in the test tube to reach that of the water bath at 25ºC. [1]
   OR
   [E] A: expose to light for 2 min and then suck out the air in leaves before switching on the light… (why) photosynthesizing at steady rate at specific light intensity

7. Ensure there is no other light source by switching off all other lights/ carry experiment out in a dark room. [1] ref. to a method of eliminating other light sources;

8. Fix distance of ***lamp**** to beaker at 20 cm and on the light and use ***photometer**** to measure light intensity
   Or

use a photometer to measure a light intensity of 110 lux, adjust distance of lamp

9. Start the ***stop watch**** and stop once all the 3 leaf discs have just risen/lifted from the bottom of syringe. [1]
   NB: describes a way of producing precise results, e.g. time taken for, first / specific number of, leaf discs to, rise from the bottom of the syringe / reach the top of the syringe **;**

10. [RR1] Prepare another 2 sets by carrying out steps 1 to 9 to serve as replicates to check for anomaly / anomalous results.
    [A : replicates as - at least 3 separate syringes OR at least 3 discs in single syringe]
    [R : ensure no anomalous results]

11. Repeat steps 1 to 10 under different light intensities of 60, 70, 80, 90, 100 lux/ distance of the lamp of 30, 40, 50 and 60cm.

12. Carry out steps 1 to 11 / entire experiment described above using shade leaves. [1]

13. [RR2] Repeat the entire experiment twice to check for reproducibility. RR1 & RR2- [1]

c) [C] **C**ontrol [1]

- Set up 2 control experiments with one using sun leaf discs and another using shade leaf discs. All conditions remain the same EXCEPT there is no light source. (procedure)
- The disc remains at bottom of syringe in absence of light to show that light is required for plant to photosynthesize to give off oxygen / disc to be lifted. ORA (purpose)

4. **D**ata Recording and Processing
Record and process data as follows:
Table [1]

- Light intensity / lux or distance from light source / cm
- Time taken / s (or min)
- Rate / s^{-1} or min^{-1}

[T] Table showing light intensity on photosynthetic rate of sun / shade leaves

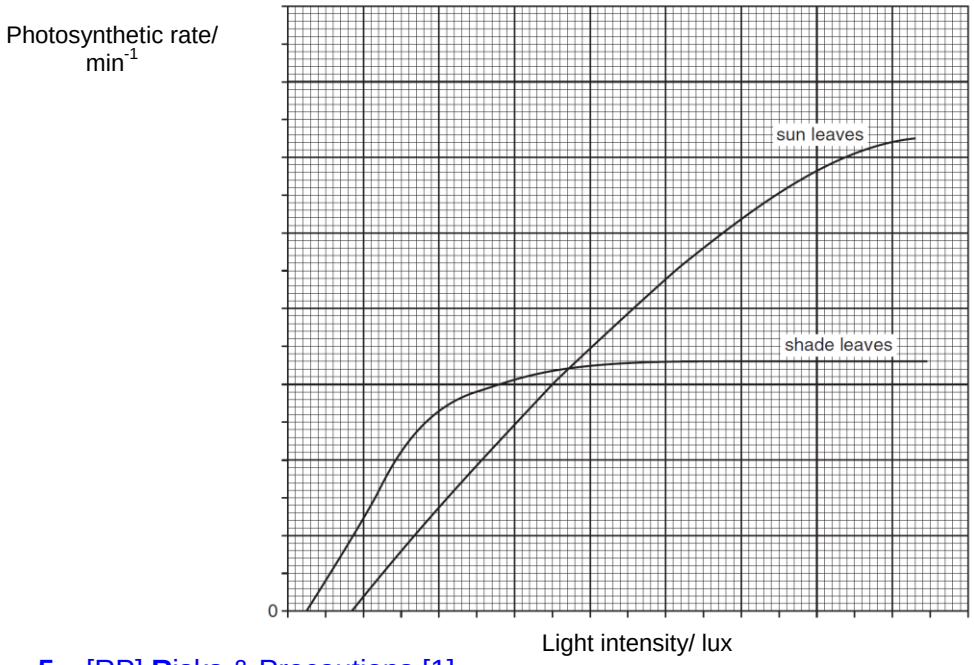
Light intensity/ lux	Time taken for leaf disc to float / s				Photosynthetic rate / min^{-1}
	Replicate 1	Replicate 2	Replicate 3	Average	
70					
80					
90					
100					
110					

[G] Graph showing intensity of light on the rate of photosynthesis of sun and shade leaves

Graph – 1 mark (3 points)
1. low light : gradient of sun should be less than shade
2. high light : max rate / platuea of sun leaf is higher than shade, at a higher light

saturation point.

3. Correct axes labelled with units following from the table



5. [RP] **R**isks & Precautions [1]

a. Wear gloves [P] when handling sodium hydrogen carbonate as skin may may be sensitive to the chemical [R]
(A: allergy to plant)

b. Dry your hands before switching the electric lamp on or off [P] to avoid being electrocuted [R].
[Reject : Wear insulating gloves [P] when handling the hot lamp to prevent burns, cos LED light emits low heat]

Section B

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

1. An example of a genetically modified aquatic animal is the genetically modified / transgenic Atlantic ***salmon****;
2. GM salmon carries a ***growth hormone gene**** from ***Chinook salmon**** coupled with an ***antifreeze promoter**** from the ***Ocean pout****;
3. Antifreeze promoter allows growth hormone gene to be expressed in the GM salmon even during winter;
4. As such, GM salmon is able to grow throughout the year,
5. faster maturation rate / reaching mature/marketable size earlier/twice as fast as compared to non-transgenic salmon;
6. GM salmon has an increased feed conversion efficiency;
7. With GM salmon, yield of salmon can be improved to meet the demand of the world / increase turnover rate;

(must have comparative statement to normal salmon in answer)

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

Exploitation (of animals) [3m]

- Genetically modifying animals may cause suffering or pain or distress in animals / welfare of animals is compromised in the process; (1 mark)

(Max **2** example)

- E.g. One of the aims of genetically modifying animals is to increase yield of animals and there seems to be little concern as to whether the animals are biologically capable of withstanding the additional stress of increased production of meat / growth rate / yield;

- E.g. Genetically modifying animals (oncomouse) for medical research whereby an oncogene was introduced into mouse to investigate the effect of the oncogene / creating a transgenic mouse with an introduced oncogene in cancer research;

- E.g. There can be unexpected undesirable outcomes / side effects in well-being of animals due to unpredictable interaction of the introduced DNA with host genes;

- E.g. Large numbers of animals are needed in genetic engineering as current techniques remain relatively inefficient (e.g. limitations in controlling the integration site of foreign DNA / unexpected outcomes) and many surplus animals are exposed to harmful procedures;

Eugenics [2m]

- Babies/individuals (no adults) may be produced by introducing/selecting favourable genes, using techniques from genetic modification [1 mark]

(Give **1** example)

- Ethical issue - because babies can't give informed consent (undermining respect for individual autonomy)
- Ethical issue - do not know long term safety effect
- Ethical issue - it is not fair because not everyone has equal access to this technology

Cloning [1 mark]

- One of the ethical concerns is that the techniques could possibly be applied to humans.

[R: Cloning of animals and violates the free will of animals]

Implications in food choices [1 mark]

- Some religious and ethnic groups avoid eating certain food → GM food may have genes from the prohibited type of food;
- e.g. Vegetarians and GM vegetables containing gene from animal / Muslims and GM food containing genes from pig;

Pose as plausible health risks to humans

- **Allergies and Disease**
  Potential to trigger allergies or disease in humans when a gene from an allergenic organism is placed into another one that typically does not cause allergies resulting in allergic reaction.

- **Antibiotic Resistance**

antibiotic resistant genes from GM plants/animals may be transferred to gut bacteria which can cause widespread antibiotic resistance in bacteria which harms humans.

- **Long term consumption**
  Long term consumption of GM plants/animals may lead to unknown health risks to humans. (e.g. through accumulation of toxins/metabolites)
  OR
  Secondary metabolites / by-products
  Consumption of GM food may produce unintended harmful secondary metabolites / by-products that lead to potential health risks.

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

Highlight = means increase yield

Meristem culture

1. Apical meristems / root or shoot tips which are disease-free are obtained for; (hence increased vigour)
   Or meristem which are responsive to plant growth regulators are obtained for;

Callus culture

2. ***callus**** formation is induced using intermediate concentrations of ***auxins**** and ***cytokinins****;
3. Callus are undifferentiated cells and undergo ***mitosis**** to give rise to ***genetically identical**** cells;
4. Callus cells are ***totipotent**** and each cell is capable of regenerating entire plant;
5. Callus can be sub-cultured and this allows for mass production of crop plants;

Protoplast culture

6. ***Cell wall**** of explant / callus can be removed to form ***protoplasts****;
7. Protoplast fusion/ colchicine can be used to obtain polyploid plants* which grow faster /grow larger/ have increased vigour and this allows for increase in yield of crop plants
   (e.g. polyploid potatoes are much larger than diploid wild varieties);
   A: using colchicine to produce polyploidy plants
8. Protoplast fusion can also allow genetic material of two different plant species to be combined/ formation of hybrid plants/ overcome reproductive barriers
9. Can combine good traits of two plant species therefore may have increased vigour and this allows for increase in yield of crop plants;
   (e.g. disease-resistant potato plants whereby virus-resistant gene from wild potato variety was transferred to commercial potato variety);

Transgenic plants

10. Transgenic plants introduced with a named foreign gene which confers favourable corresponding phenotype that can increase vigour/ yield can be obtained;
    (e.g. of named foreign gene - herbicide resistance gene / drought-resistance / copper tolerance / salt tolerance / Bt toxin
11. (delivery method) into protoplasts / callus via ***micro-injection / Agrobacterium / gene gun / electroporation****;
12. Give 1 mark for any relevant example.

13. Anther culture allows for cultivation of haploid plants, and plants with named desirable trait and therefore increased vigour can therefore be selected.

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