Nov 2013 H2 Bio Paper 3

N13P3Q1

(a)

State two other ways in which RFLP can be used as a biological tool. [2]

RFLP can be used in the analysis of

1. **detection of disease** e.g. sickle cell anaemia;
2. ***DNA fingerprinting**** in **forensics** / **paternity testing**

(b) (i) Complete Table 1.1 to show the number of restriction sites and the size of the fragments resulting from digestion of each 15kb DNA molecule, for the three samples. [2]

Table 1.1

	*Bam*HI	*Eco*RI	*Bam*HI and *Eco*RI
Number of restriction sites	1	**2**	**3**
Size of fragments / kb	4,11	**2,3,10**	**1,2,3,9**

(ii) Using the information from Fig. 1.1 and Table 1.1, it is possible to map the 15kb length of DNA for *Bam*HI and *Eco*RI.

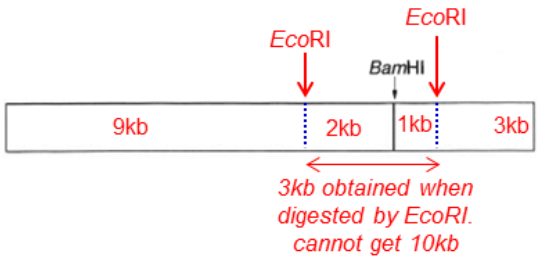
The restriction map for BamHI is shown in Fig. 1.2.

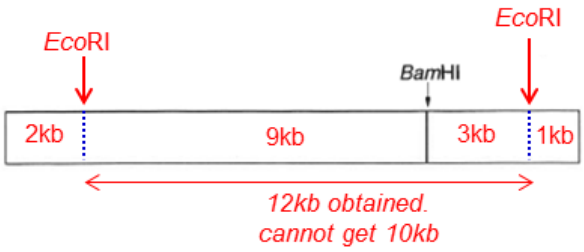
Complete the restriction map in Fig. 1.3 for both *Bam*HI and *Eco*RI, by adding the positions of the *Eco*RI restriction sites. Indicate the size, in kb, of each fragment. [2]

Fig. 1.3

Teacher's comments:

The 2 answers below cannot get the 10kb fragment, hence incorrect.





(c) Outline why gel electrophoresis separates DNA fragments. [4]

1. ***Negatively-charged DNA****;
2. migrates towards the positive electrode/anode when subjected to an electric field / current;
3. Fragments migrate through agarose gel matrix, made up of a meshwork of polysaccharides; which impedes movement of longer fragments more than shorter fragments;
4. Longer fragments migrate slower compared to shorter fragments;

(d) Outline the process of DNA hybridization that allows the RFLP pattern for a particular gene to be visualized [5]

To detect the RFLP pattern of a gene, after the DNA from is digested with restriction enzyme and the digested DNA separate using gel electrophoresis

1. ds DNA is denatured / made single-stranded and by alkaline / NaOH solution and transferred to a nitrocellulose membrane; exactly the same position as they were in the gel
2. The nitrocellulose membrane incubated with a ***radioactive single stranded DNA probe*****,** that is ***complementary in sequence**** to part of the target sequence / gene.
3. DNA fragments containing this part of the target sequence will hybridise to the probe **by *complementary base-pairing*;**
4. After hybridisation, membrane is washed to remove any unhybridised probes.
5. Using ***Autoradiography/X-ray film**** over the membrane, the banding pattern can be visualised. (The radioactivity of the bound probes exposes the film to form an image corresponding to the bands that have base-paired to the probe.)

N13P3Q2

2

(a) (i) Cytosine is a pyrimidine. Name the other pyrimidine. [1]

Thymine*

(ii) Suggest how methylation of cytosine nucleotides prevents the DNA of a prokaryote from being cut by its own restriction enzymes. [2]

1. Methylation of cytosine in prokaryotic DNA changes the conformation of the DNA at the ***restriction site**** such that it is no longer ***complementary in shape and charge**** to the restriction enzyme's ***active site****.
2. Hence, the restriction enzyme will be unable to recognise and cleave prokaryotes' own DNA.

(b) **(OUT OF SYLLABUS)**

(c) Another restriction enzyme, BspLI, has the same restriction site

Where N can be any nucleotide.

Using Table 2.1, a scientist predicted that BspLI would cut at more than 20 sites in the standard DNA.

Suggest why the scientist made this prediction. [2]

1. There are 4 possible nucleotides (adenosine triphosphate/ Guanosine triphosphate / cytosine triphosphate / thymine triphosphate),
2. Since BspLI restriction site is less specific than of *Sfo*I, there will be more than 20 cut sites;

N13P3Q3

(a) (i) Explain why the gene has at least 200 000 base pairs, but the protein only has 1480 amino acids. [3]

1. Besides the 4 440 bases which make up exons, code for the 1480 amino acids;
2. there are non-coding DNA such as introns;
3. e.g. promoter, stop codon, UTR (reject control elements such as enhancers/ silencers);

(ii) **(OUT OF SYLLABUS)**

(iii) Suggest, with reasons, why other mutations of the *CFTR* gene vary in the extent to which they cause symptoms of cystic fibrosis. [3]

1. Single base substitutions which lead to coding of another amino acid with similar R-group properties will cause little effect on symptoms of cystic fibrosis as it can still fold to similar conformation as original protein;
2. Mutations such as single base insertion or single base deletion within exons
3. can lead to frameshift mutation leading to an incorrect sequence of amino acid formed with different R-groups which cannot fold to form a functional protein leading to serious complications.
4. Base substitutions which lead to a premature stop codon can lead to a truncated protein which is non-functional will lead to have severe symptoms of cystic fibrosis.

(b) (OUT OF SYLLABUS)

N13P3Q4

Planning Question

1. **A**im : To investigate the lowest concentration of copper sulfate solution that has an effect on the leakage of pigment from beetroot cells.

2. **T**heory

(Main theory) [Max 1m]

[T1] Betacyanin is found in beetroot cells is prevented from leaking out of cells by membranes (vacuole & plasma membrane) which are made up of phospholipid bilayer embedded with proteins

[T2] The betacyanin are too large to pass through the transient pores in the phospholipid bilayer /The betacyanin are charged/polar and are unable to pass through the non-polar hydrophobic hydrocarbon core of the phospholipid bilayer.

[T3] Copper ions in the copper sulphate causes denaturation of the membrane proteins embedded in the phospholipid bilayer. The loss of 3D conformation of the membrane proteins increases the permeability of the membrane.
Accept when make reference to copper ions disrupting ionic interactions in tertiary structures.

(Measurable quantity)[1]

[T4] the degree of permeability of the membrane will be reflected by the amount of betacyanin pigment leaking out of cells. The permeability is determined by measuring the time taken for the colour of the bathing solution to match the colour standard.

(Predicted trend) [1]

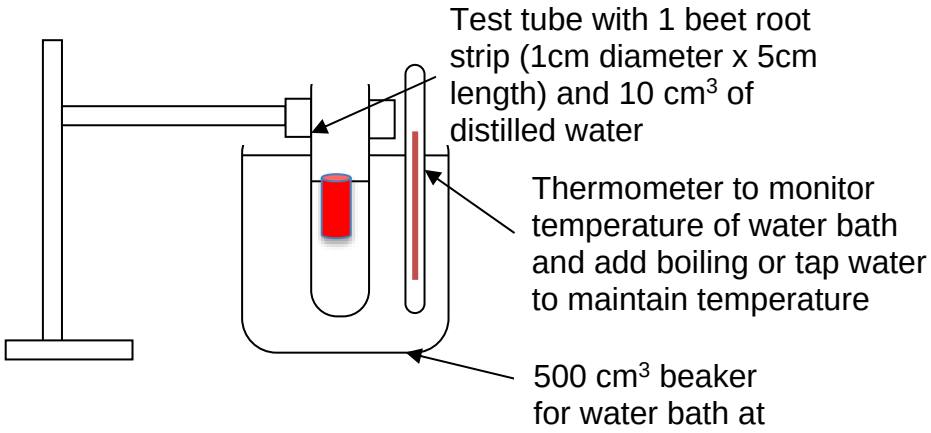
[T5] Increasing concentration of copper sulfate increases the membrane permeability which results in a shorter time taken for the colour of the bathing solution to match the colour standard.

[T6] Calculate rate of leaking and extrapolating lowest concentration that has an effect from the graph

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. concentration of copper sulfate solutions), optimum conditions, amount of materials used (number of pieces of beetroot cylinders, volume of distilled water) [1]

b) **A**nnotated diagram



c) Variables

[DV] **Dependent variable AND description of how it is measured :** Time taken/s for solution to match the colour standard [2]

Comment: Dependent variable → Rate of leakage of pigment/ s^{-1} How is this measured → 1/time taken

[IV] **Independent variable (at least 5 concentrations at equal interval) AND description of how it is controlled :** 0.2%, 0.4%, 0.6%, 0.8% and 1.0% copper sulfate solution [2]

[CV] **Controlled variable and description of how it is controlled:** [Max 2m]

- Temperature
- Type/source/ species of beetroot used
- Length /size of beetrootstrip used
- Volume of copper sulfate solution used
- Any valid point

d) **N**umbered steps

1. Using a ***cork borer**** (diameter 1cm), cut out cylinders of ***beetroot**** . Using a ruler and scapel, cut the cylinders of beetroot to a length of 5cm each. (dimension of the beetroot should be sensible, 10cm x 10cm x 10cm is really too BIG!) Prepare at least 55 beetroot cylinder. (number of beetroot cylinder require should be sufficient for setting up both the repeats and replicates)

2. Rinse the beetroot cylinders in running water until the water is colourless.

3. To prepare colour standard, place 1 beetroot cylinder in 10cm^3 of distilled water in a test tube and incubate in a water bath of 80^{o}C. After 5 minutes, remove the beetroot cylinder from the test tube using a pair of forceps and discard it. The red solution left in the test tube will be the colour standard. [1]

4. Prepare a 30^{o}C water bath by mixing hot and cold water in a 500cm^3 beaker. Use a thermometer to measure the temperature and adjust the temperature to 30^{o}C by mixing hot and cold water.

5. Prepare 10 cm^3 0.2%, 0.4%, 0.6%, 0.8% and 1.0% Copper sulfate solution by mixing appropriate volumes of 1.0% copper sulfate solution and distilled water in a test-tube as shown in the table below. Use a 10cm^3 syringe to measure the volume stated in the table.

Concentration of copper sulfate solutions /%	Volume of 1% copper sulfate solutions used /cm^3	Volume of distilled water used /cm^3	Total volume /cm^3
0.2	2	8	10
0.4	4	6	10
0.6	6	4	10
0.8	8	2	10
1.0	10	0	10

6. Place the test tubes contain various concentrations of copper sulfate solutions in the 30°C water bath. [E] Allow 2 min of equilibration time for the temperature in the test tube to reach 30°C. [1]

   Comment: Many students equilibrate by putting the beetroot in the copper sulfate solution. This is not acceptable. By doing so, the reaction would have started and the proteins in the beetroot cell membrane are likely to be disrupted within the equilibration time.

7. Place 1 beetroot cylinder into the test-tube containing 0.2 % copper sulfate solution. Start the stop watch.

8. Stop the stop-watch and record the time taken for the colour of the copper sulfate solution to matches the colour standard. Use the white cardboard as a background when comparing the colour of the test-tube and colour standard.

9. [R] Repeat step 5 to 8 using 2 more test tubes of copper sulfate solution with beetroot cylinder to serve as replicates, to check for anomalous results.[1]

10. [RR] Repeat the entire experiment twice to check for reproducibility of results. [1]

e) [C] **C**ontrol [1]
Keeping all other variable such as volume and temperature constant, place 1 beetroot cylinder in $10cm^3$ of distilled water and measure the time taken for the colour of the distilled water to match the colour standard. This is to show that any change in the permeability is due to the copper sulfate solution.

Comment: Some students mentioned using glass beads to substitute for the beetroot to show that red pigments come from beetroot cells. This is not acceptable for this experiment since the aim of this experiment is to investigate the effects of metal ions on membrane permeability. Some students wrongly used boiled and cooled beetroots in their control tube. Boiling disrupts membrane integrity and pigments will leak from the cells.

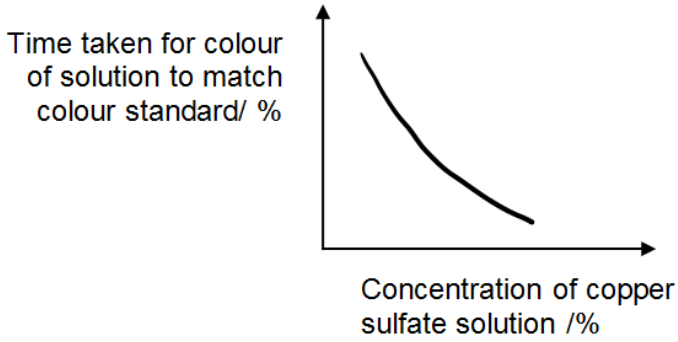
4. **D**ata Recording and Processing

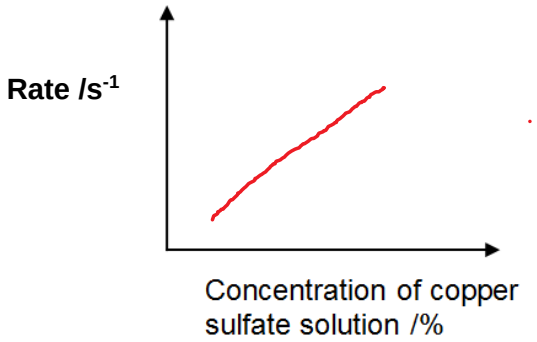
Record and process data as follows:

[T] Table showing time taken for the colour of solution to match colour standard [1]

Concentration of copper sulfate solutions /%	Time taken for colour of solution to match colour standard /s				Rate of leakage of pigments /s^{-1}
	Replicate 1	Replicate 2	Replicate 3	Average	
0.2					
0.4					
0.6					
0.8					
1.0					

[G] Graph showing the effects of concentration of copper sulfate solution on the time taken for the colour of solution to match colour standard [1]





[1] To obtain the lowest concentration of copper sulfate solution that has an effect on the leakage of pigment from beetroot cells, extrapolate the graph to obtain the concentration when graph cuts x-axis.

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

a. When using scalpel, cut the beetroot away from the hand to avoid getting cut.
b. When using the cork borer, use the sharp end on the beetroot to avoid accidentally cut your hand.
c. Avoid touching the hot Bunsen burner. Only handle it after it has cooled to prevent burns.
d. Use insulating gloves when handling the hot water to prevent scalding.

N13P3Q5

5 (a) Describe features of zygotic stem cells and embryonic stem cells that distinguish them from each other. [5]

Point of comparison	Zygotic stem cells	Embryonic stem cells
Differentiation potential	1a.They are ***totipotent*;***	1b. They are ***pluripotent*;***
Characteristics	2a. They have the ability to ***differentiate**** **into all of the cell types** that make up an organism **including the *extra-embryonic tissue**** such as the ***placenta****, which nourishes the embryo; 3a. Hence zygotic stem cells are able to form the entire organism;	2b. They have the ability to ***differentiate**** **into all of the cell types** that make up an organism **except the *extraembryonic tissue**** such as the ***placenta****; 3b.Embryonic stem cells alone **cannot form the entire organism** as extraembryonic tissues such as the placenta is required for foetal nourishment and development.
Examples and sources	4a. They are derived from a fertilised egg which forms the zygote. Cells that are **produced within the first 3 division (*8 cell stage**)** after the egg is fertilized;	4b. They are derived from **cells of the *inner cell mass of blastocyst**** at about 4 to 5 days post fertilization;

(b) Describe the features of blood stem cells and explain their normal functions. [8]

1. Blood stem cells are adult stems cells can be found in the ***bone marrow**** and the umbilical cord;
2. They undergo ***self-renewal ** by mitotic division to ensure a constant pool of blood stem cells;
3. As the life span of blood cells are short, blood stem cells replace those lost through normal cell death.
4. Blood stem cells are ***multipotent*;***
5. They have the ability to ***differentiate**** **into several related cell types** but is restricted to blood cells only;
6. They can divide asymmetrically after stimulation by molecular signals;
7. This produces stem cells for the maintenance of the stem cell pool and progenitor cells to increase or renew the population of specialized blood cells.
8. B and T lymphocytes which are derived from the lymphoid progenitor cell;
9. Red blood cells and platelet producer cells which are derived from the myeloid progenitor cell;

(c) **(OUT OF SYYLABUS)**