Nov 2014 H2 Bio Paper 3

N14P3Q1

1(a) (i) Name one genetic disease which has been treated with stem cell transplantation. [1]

Bone marrow haematopoietic stem cells transplants from normal healthy bone marrow donors to leukaemia patients
Or
Neural stem cell transplant for Parkinson's disease/multiple sclerosis by introducing adult neural stem cells into damaged tissue.

(ii) Explain why stem cell is suitable for this purpose. [3]

1. Adult stem cell is multipotent that ***differentiates**** into the respective ***specialized**** cell type, thus restoring function of damaged or diseased tissue.
2. ***Self-renewing**** nature of stem cells ensures that transplanted stem cells constantly ***replicate**** in the patient to maintain a constant pool of stem cells
3. As the 'healthy' stem cell carries the normal and functional allele thus they can produce normal levels of functional protein and be used to treat genetic diseases

b(i) Using the letter **R**, label Fig. 1.1 to identify a feature that allows the virus to bind to cells. [1]

Note: R can be any one of the 2 types of glycoproteins, one arrow is sufficient.

(ii) With reference to your knowledge of retroviruses, explain how expression of an inserted gene (transgene) is brought about following infection of host cells with the lentiviral vector. [3]

1. Once inside the host cell, retroviruses create double stranded DNA copies of their RNA genomes via ***reverse transcriptase****.
2. Viral genome together with the transgene are integrated randomly into host chromosomes via integrase.
3. Transgene will undergo transcription and translation by host enzymes to produce normal, functional protein.

(iii) **(OUT OF SYLLABUS)**

(c) **(OUT OF SYLLABUS)**

[Total : 12]

N14P3Q2 (OUT OF SYLLABUS)

N14P3Q3

(a) Describe the limitations of PCR. [3]

Feature	Limitations
Taq polymerase **lacks 3' to 5' proofreading ability**	**Errors** occurring early in the PCR reaction will get compounded with each replication cycle and all daughter molecules resulting from this early error will be exponentially affected.
Synthesis of PCR primers depends on **sequence information** from target region	Success of PCR **requires knowledge of sequences** flanking target region to be amplified. If the flanking sequences of a gene of interest are unknown, no proper primers can be synthesised to amplify the target DNA sequence. If primers are designed incorrectly, no amplification occurs / wrong DNA fragment(s) may be amplified.
Limit to size of DNA fragment to be amplified	DNA fragments to be amplified are **limited to about 3 kb[1].** Further increase in length of target sequence decreases efficiency of amplification. This is because the polymerase tends to 'fall off' DNA template before chain extension is complete.
Exponential amplification of **contaminant DNA**	It is possible to contaminate a fresh PCR reaction with **minute amounts** of contaminant DNA due to poor laboratory skills. Such unwanted DNA sequences may be amplified to significant amounts, alongside the target DNA sequences.

(b) (i) Describe the role of the buffer solution in the gel electrophoresis protocol. [2]

1. Buffers contain ions which allows conduction of electric current
2. Thus allowing the negatively charged DNA molecules to move from the negative electrode to the positive electrode

(b) (ii) Describe the role of the loading or tracking dye in the gel electrophoresis protocol. [3]

1. Contains glycerol which makes the DNA sample denser than buffer so that DNA sample can sink to the bottom of the well.
2. DNA is invisible, so the dyes colour the DNA sample showing if it has been loaded correctly into well.
3. 2 coloured dyes act as visual markers to show the progress of migration of DNA fragments in the gel.
4. One dye typically (moves at speed corresponding to 100bp DNA fragment which) runs ahead of sample and another (moves at speed of 1100bp DNA fragment which) runs after sample.

(c) (i) Outline the process of genetic fingerprinting using RFLP that could be used to test this seized ivory. [4]

1. Genomic DNA is extracted from the soft tissue/dried blood and cut with ***same restriction enzyme**** to obtain different-sized DNA fragments.
2. DNA is separated according to size in gel electrophoresis where ***negatively-charged DNA**** migrates towards the positive electrode/anode when subjected to an electric field / current;
3. Meshwork of agarose fibres impedes movement of longer fragments more than shorter fragments resulting in smallest fragments moving furthest/largest fragments least far from well
4. ds DNA is denatured / made single-stranded and by alkaline / NaOH solution and transferred to a nitrocellulose membrane
5. Carry out Southern blotting/nucleic acid hybridisation by incubating membrane with single strand ***radioactive probe**** which will hybridise with DNA fragment through complementary base pairing
6. Using ***autoradiography/X-ray film**** over the membrane, the banding pattern can be visualised.

(ii) Explain how the genetic fingerprints of the seized ivory could be used to confirm that it originated from elephants in Malawi. [4]

1. Genetic fingerprint is due to different alleles/markers producing different bands in gel resulting in the unique banding pattern in individuals
2. Different bands arise due to polymorphic nature of DNA in different individuals, there will be variations in number and location of restriction sites and number of tandemly repeated nucleotide sequence among individuals.
3. Genetic fingerprint of animals that provided ivory can be compared against fingerprint of elephants from Malawi to see how closely related they are.
4. If fingerprint pattern is similar to pattern to that of Malawi elephants, then ivory haul from Malawi.

N14P3Q4

4 Planning

[Total: 12]

Suggested answer scheme:

Part 1: Aim

To investigate effect of temperature and pH on rate of sucrase activity of 2 enzymes P and Q.

Part 2: Theory

(Main Theory): **[T1: 1 mark for any 2 points from 1 to 5]**

1. Active site of sucrase has a specific conformation, complementary in shape and charge to substrate, sucrose.
2. Conformation of active site, hence rate of sucrase activity, is affected by temperature and pH.
3. Excess $[H^+]$ or $[OH^-]$ ions disrupt ionic, hydrogen bonds, which determine tertiary/quarternary structure of enzyme hence its any conformation.
4. Increasing temperature (up to denaturation) increases kinetic energy of molecules, increasing rate of effective collisions between enzymes and substrate to form enzyme-substrate complex.
5. At optimum temperature and pH of each enzyme, rate of production of reducing sugars is maximum.

(Measurable variable):

6. Mass of brick red precipitate formed when products (glucose, fructose) obtained from hydrolysis of substrate (sucrose) are tested with Benedict's solution. **[T2: 1 mark]**

Dependent variable: rate of sucrase activity (P and Q) as indicated by rate of brick red precipitate formed

Independent variable: temperature

(to ascertain optimum temperature)

Part 3: Procedure

a. Pilot Test

Conduct a pilot experiment to determine suitable range of independent variables used, suitability of apparatus, concentration of substrate (sucrose). If substrate is too concentrated, it may be diluted with ***distilled water**** (we can't think of where else to use the distilled water!) **[P: 1 mark]**

b. Annotated diagram

Set-up simple, diagram probably not needed.

c. Numbered steps in procedure

1. Fill 3 test tubes with 5 cm^3 of ***5% sucrose**** buffered at pH 7. Use a pH probe, digital meter to measure and monitor pH, and syringes to measure volumes.
2. Fill another 3 test tubes with 1 cm^3 of ***2% sucrase P**** . These serve as **replicates** to check that no anomalies are present. **[R1: 1 mark for both replicates and repeats, including how they are carried out and why they are carried out]**
   Each sample will follow this procedure:
3. **Constant variables** include:
   - volume of substrate - kept constant 5 cm^3 because extent of enzymatic reaction is affected by substrate quantity,
   - duration of heating in boiling water bath - kept constant 3 minutes during Benedict's test as excessive heating converts sucrose into fructose and glucose.

   [CV1 and CV2: 1 mark each for 2 constant variables, including what variables, details on how they are kept constant and why it is necessary to keep variables constant]
4. Place all tubes in a water bath. Keep temperature constant at 30°C. Maintain temperature using ***thermostatically-controlled water bath**** and monitor temperature with a ***thermometer**** .
5. Allow substrate (step 1) and enzyme tubes (step 2) to acclimatize separately for 2 minutes to reach set temperature. Add sucrase to each of the sucrose solutions. Use ***stop watch**** to check starting time. **[E: 1 mark for correct acclimatization with reason]**
6. After reaction has proceeded for 5 minutes, add 6 cm^3 ***Benedict's solution**** to each tube, place in a boiling water bath over ***Bunsen burner**** for 3 minutes.
7. Pre-weigh each filter paper. After the 3 minutes filter the precipitate. Dry precipitate in a desiccator till a constant weight. Weigh mass of precipitate.
8. Calculate dependent variable which is rate at which sucrase P hydrolyses sucrose to fructose and glucose:

   average mass of brick red precipitate / time.

   [DV: 1 mark – show how to obtain dependent variable including calculation, method of obtaining must be scientifically sound]
9. Repeat steps 1-8 using buffered sucrose solutions at 20°C, 40°C. 50°C, 60°C. **[IV1: 1 mark - independent variable, with total of 5 temp values of equal intervals. Include how the temperature is created and maintained.]**
10. **Repeat** steps 1-9 twice more to check for reproducibility.
    [R1: 1 mark for both replicates and repeats, including how they are carried out and why they are carried out]

d. Control

Keep all variables constant. Set up control experiment at each of the five

temperatures using boiled and cooled sucrase.
No precipitate formed for Benedict's test – shows hydrolysis of sucrose to monosaccharides is an enzyme-catalysed reaction.
[Co: 1 mark for either control, including how it is carried out and reason why it is performed]

Part 4: Data recording and processing:

Table showing rate of sucrase P activity at pH 7

[T1: 1 mark for any full table including correct units - either for enzyme P or Q / either pH or temperature]

Temperature / ^{o}C	Mass of precipitate formed in 5 min / g				Rate of sucrase P activity / g min $^{-1}$
	Replicate 1	Replicate 2	Replicate 3	Average	
20					
30					
40					
50					
60					

11. Repeat Parts 3 and 4 using sucrase Q to replace sucrase P to determine optimum temperature of sucrase Q.

Independent variable: pH

Once optimum temperatures for sucrase P and Q are obtained, proceed to identify the optimum pH of sucrase P and Q.
Repeat Parts 3 and 4 – with the following changes:

Part 5: Procedure

c. Numbered steps in procedure

1. Fill 3 test tubes with 5 cm^3 of 5% sucrose buffered at pH 7.
4. Place all tubes in a water bath, keep temperature constant at optimum temperature of ***2% sucrase P**** determined in Parts 3, 4.
10. Repeat steps 1-9 using buffered sucrose solutions of pH 5, 6, 8 and 9.

[IV2: 1 mark - independent variable, with total of 5 H values of equal intervals. Include how the pH is maintained.]

d. Control

Keep all variables constant. Set up control experiment at each of the five pH using boiled and cooled sucrase.
No precipitate formed for Benedict's test as in Part 3.
[Co: 1 mark for either control, including how it is carried out and reason why it is performed]

Part 6: Data recording and processing:

Table showing rate of sucrase P activity, at optimum temp.

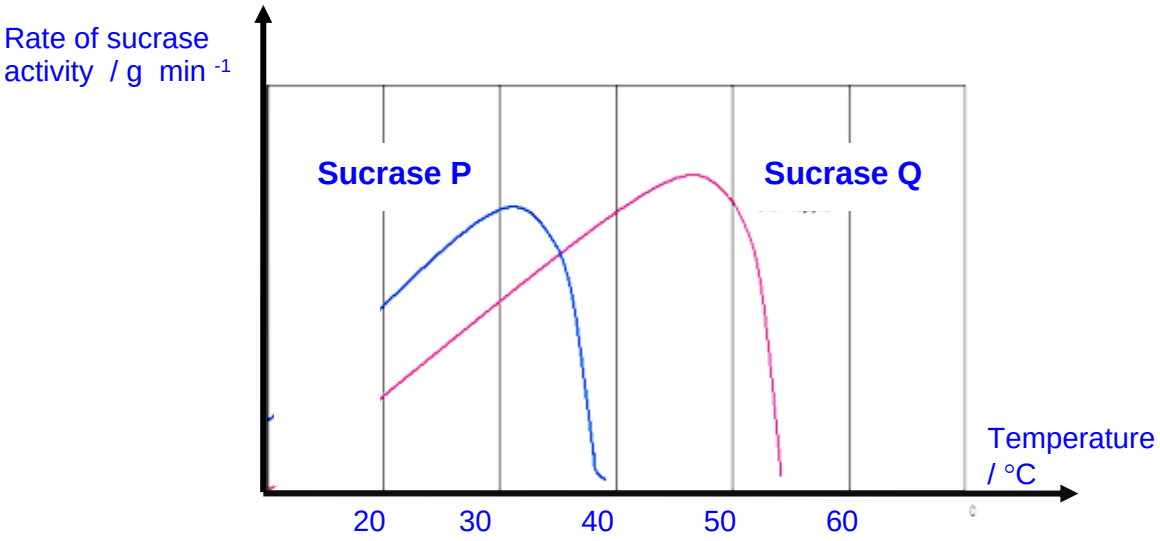
pH	Mass of precipitate formed in 5 min / g				Rate of sucrase P activity / g min $^{-1}$
	Replicate 1	Replicate 2	Replicate 3	Average	
5					
6					
7					
8					
9					

11. Repeat Parts 5 and 6 using sucrase Q replacing sucrase P.

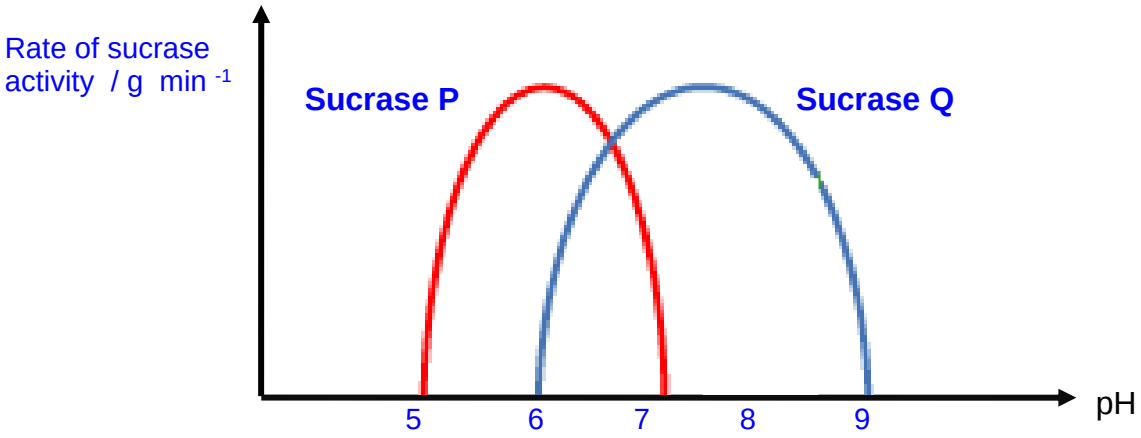
[Fa: 1 mark for doing experiment 1 variable at a time]

[M: 1 mark for using all compulsory reagents and apparatus marked with *]

Graph showing rate of sucrase activity at pH 7, of enzymes P, Q



Graph showing rate of sucrase activity, at the respective optimum temp. of sucrases P, Q



[G: 1 mark for either graph with trend and labels]

10. Risks and precautions [1 mark]

(i) Use a boiling tube holder to remove boiling tubes from boiling water bath, in order to prevent scalding.

(ii) Wear gloves when measuring enzymes P, Q (or use of acid/alkali) as they are irritants.

N14P3Q5

(a) and (b) (OUT OF SYLLABUS)

(c) Eukaryotic genes cannot be expressed directly in the bacterial plasmids because of difference between prokaryotes and eukaryotes, including the presence of introns. [7]

Outline these problems and explain how they are overcome in order to allow expression of eukaryotic genes in plasmids within *E. coli* cells.

1. Pre-mRNA produced from transcription of eukaryotic genes have introns and exons;
2. During ***splicing****** in eukaryotic cells, introns are excised and exons joined together by ***spliceosomes****** to form ***mature mRNA****;
3. Unlike eukaryotic cells, prokaryotic cells do not have ***spliceosomes****** and are unable to carry out splicing to form mature mRNA;
4. In turn, translation of pre-mRNA in prokaryotes results in non-functional protein produced;
5. To overcome this problem, ***reverse transcriptase****** can be used to reverse transcribe ***mature mRNA****** from eukaryotes to form ***complementary DNA****;

6. ***RNA polymerase****** in prokaryotes are unable to recognize and bind to eukaryotic ***promoters****** to express eukaryotic genes;
7. This can be overcome by inserting the eukaryotic gene just downstream of a prokaryotic ***promoter****** in the plasmid;

8. Prokaryotes are unable to carry out post-translational-modification to form functional eukaryotic proteins;
9. e.g. functional human insulin is formed following cleavage of the C chain and formation of disulfide bonds between the A and B chains;
10. A and B chains can be purified separately from different bacteria cultures and formation of disulfide bonds between them chemically induced;