HWA CHONG INSTITUTION (COLLEGE SECTION)
2015 JC2 H2 BIOLOGY
PRELIMINARY EXAMINTION PAPER 3 MARK SCHEME

Question 1

(a) Explain what is meant by the term *palindromic*. [2]

1. The base sequence of one strand reads the same as its complementary strand
2. When both strands are read in the 5' to 3' direction

(b)(i) Complete Table 1.1 to show the number of restriction sites in pUC and recombinant pUC. [1]

	in pUC	in recombinant pUC
number of *Eco*RI sites	1	2
Number of *Bam*HI sites	2	3

(b)(ii) Indicate the positions of *Eco*RI and *Bam*HI restriction sites on the recombinant pUC by drawing in Fig. 1.3. Show the size of each fragment in kb. [2]

1. Relative positions of *Bam*HI and *Eco*RI
2. Correct size of each fragments with appropriate units

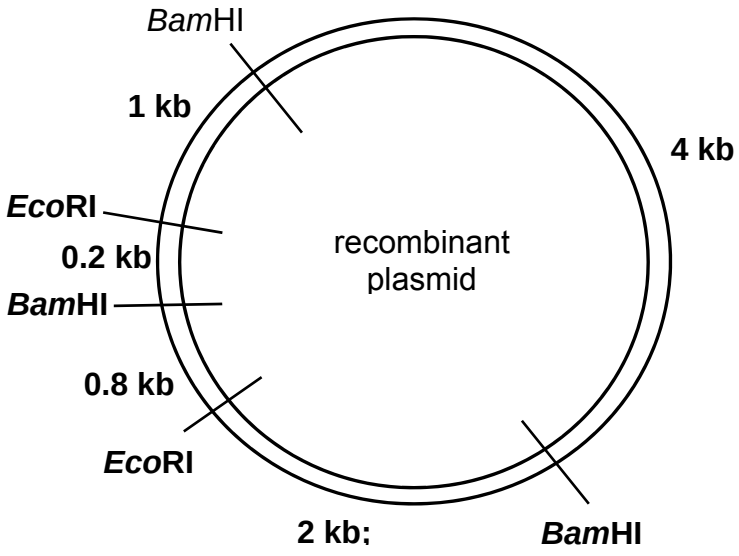

Fig. 1.3

(b)(iii) Identify which restriction enzyme, *Eco*RI or *Bam*HI, is suitable for use to clone gene X. Give a reason for your answer.
[2]

1. *Eco*RI

2. The restriction site occurs only once within the entire plasmid so that restriction digestion does not result in fragmentation of the plasmid / plasmid is linearised

(c)(i) Explain the significance of the use of mature mRNA in step 1. [3]

1. Mature mRNA does not contain intron, thus no need for additional step of removing introns
2. mRNA acts as a template for the synthesis of cDNA for the gene of interest
3. Continuous coding sequence to produce functional human insulin hormone

(c)(ii) Suggest why additional noncoding sequences are added in step 3. [1]

1. Linkers / adapters containing the desired restriction sites is cleaved to generate sticky ends for ligation

(c)(iii) Suggest an advantage of treating diabetics with human insulin produced by RDT. [1]

1. Identical to that produced by body. Hence, activity is the same / fast response / no immune response.
2. No need for animal insulin. For religious / ethical reasons.
3. Uncontaminated / pure. Thus, no risk of disease.
4. Production of insulin is very efficient / always available / mass production while extraction from animals is costly / complex / limited by supply of animals.

[Total: 12]

Question 2

(a) Outline a mutation that results in RPE65 deficiency. [2]

1. Ref. to base-pair substitutions / insertions / deletions
2. Ref. to changes in codon sequence
3. Ref. to changes in amino acid sequence and function of protein
   Accept: ref. to point mutation in promoter

(b) Explain why the normal *RPE65* allele was obtained from a genomic DNA library instead of a cDNA library. [2]

1. Photoreceptor cells are eukaryotic cells and introns can be spliced out
2. Ref. to *RPE65* genomic promoter to ensure specific expression

(c) AAV is a non-enveloped virus. Suggest how the normal *RPE65* allele enters the photoreceptor cell. [2]

1. Ref. to binding of glycoproteins on the capsid of AAV and receptor proteins on the cell membrane of photoreceptor cells
2. Ref. to receptor-mediated endocytosis

(d) Suggest why it is not necessary to include selectable markers in this study. [1]

1. Ref. to improved vision as a well-defined phenotype

(e) Suggest why one eye was left untreated. [2]

1. Ref. to differences in retinal sensitivity amongst participants
2. Ref. to baseline subtraction

(f) Describe and explain how the results differ between the two participants. [3]

1. Ref. to lower dose, no loci with significant improvement in retinal sensitivity throughout the 36 months
2. Ref. to higher dose, number of loci with significant improvement increased to a peak of 45 at 12 months before decreasing
3. Ref. to insufficient RPE65 protein produced to result in significant improvement in participant 1
4. Ref. to normal RPE65 allele not being integrated into the participants' chromosome / undergoing transient expression

(g) Suggest how stem cell therapy may be useful in the repair of the retina. [3]

1. Ref. to stem cell being capable of long-term self-renewal
2. Ref. to stem cell being unspecialised and can undergo differentiation
3. Ref. to photoreceptor cell being able to integrate with the host retina without rejection

[Total: 15]

Question 3

(a) Outline a procedure for growing orchids from cells in tissue culture. [4]

1. Establish cells under sterile / aseptic conditions in culture medium for callus formation.
2. Multiplication of microshoots by increasing cytokinin concentration in culture medium and subsequent subculturing to new multiplication media.
3. Root formation of microcuttings in test-tube containing in the presences of increased auxin and reduced cytokinin to form plantlets.
4. The microplant undergoes transplanting into a soil medium and acclimatization to the outdoor environment takes place.

(b) Contrast the processes of tissue culture to the traditional cultivation of orchids. [2]

Tissue culture	Traditional breeding
1a. Tissue culture is carried out in sterile environment and requires aseptic technique	**1b.** traditional breeding is carried out in non-sterile environment does not requires aseptic technique
2a. Any cells from the orchid / shoot meristem is the starting material	**2b.** Seeds from sexual reproduction is required to start the cultivation
3a. Soilless / Culture medium contains organic compounds / respiratory substrate	**3b.** Soil is used and no added organic compound / respiratory substrate
4a. Microcutting allows a callus to be divided into many pieces to give rise to many plants	**4b.** No microcutting / One seed gives rise to one plant eventually
5a. Acclimatisation required	**5b.** No acclimatisation required
6a. Shorter time is required	**6b.** Longer time is required

(c) Explain why this GM orchid is considered "true blue". [2]

1. Ref to permanent inheritance of the *flavonoid 3',5'-hydroxylase* (*FH*) gene.
2. The enzyme flavonoid 3',5'-hydroxylase can be continuously produced in the flowers and catalyse the formation of the blue pigment delphinidin.

(d) Suggest how PCR can be used to distinguish the "true blue" GM orchid in **(c)** from the dyed blue orchids. [2]

1. Design specific primers flanking part of *flavonoid 3',5'-hydroxylase gene*.
2. DNA only amplified for the "true blue" GM orchid in (g), but not the dyed orchid.

(e) Suggest why farmer **Y** might be concerned about the possibility of his crop being fertilised by pollen from farmer **X**'s crop. [1]

1. Farmer Y may lose some of his markets if he cannot state that his crops are GM free.

(f) Describe the relationship between cross-pollination and the gap between plots of land. [2]

1. The greater the gap between plots of land, the lower the chance of cross-pollination in general with supporting data.
2. Increasing distance does not decrease the chance of cross-pollination at the edge of non-GMO beyond 5 m. As the gap increases from 5 m to 7 m, the percentage of cross-pollination remains the same at 1 % at the edge of non-GM crop.

Question 4

1. Bacteriophages transfer bacterial genes / DNA between bacterial cells.
2. Successfully transduced *E. coli* cells will be resistant / able to survive when exposed to ampicillin to form colonies.
3. Using a micropipette tip, transfer 100 µL of *E. coli* cells into a microfuge tube.
4. Using another micropipette tip, add 10 µL of T4 phage suspension the tube with *E. coli* cells.
5. Incubate the tube for 15 minutes as timed using a stopwatch.
6. Using a micropipette tip, transfer 100 µL of the *E. coli* cell / phage suspension in the tube to the LB/amp plate.
7. Repeat Steps 3 – 6 for the LB plate.
8. Incubate both plates overnight at 37 $^{\circ}$C in the incubator oven.
9. Perform a control experiment except that the *E. coli* cells are not mixed with T4 phage suspension and plated on LB/amp plate.
10. Count the number of colonies on the LB/amp plate and LB plate.
11. Transduction efficiency is number of colonies on LB/amp plate divided by number of colonies on LB plate x 100%.
12. Wear disposable gloves throughout the experiment to prevent any infection from bacterial cultures.
13. AVP

Question 5

(a) Outline the process of nucleic acid hybridisation and explain how it can be used to detect and analyse restriction fragment length polymorphism (RFLP). [8]

A Nucleic Acid Hybridization Process

1. Alkali denaturation is carried out
2. causing DNA to become single stranded/ denatured
3. by disrupting the hydrogen bonds between complementary nucleotide bases
4. Fluorescently / radioactively labelled probes are then used to anneal to the ssDNA
5. via complementary base pairing (@ref complentary probes)
6. for visualisation via autoradiography (@ref visualisation using X-ray film)

B How it is used to detect and analyse RFLP

1. In RFLP analysis, DNA is first cut/ cleaved by a restriction enzyme
2. Gel electrophoresis is carried out to separate DNA fragments by size
3. And then transferred onto a nitrocellulose membrane via Southern blotting for visualisation
4. Due mutations which change RE sites/ fragment size
5. Thus bands of various sizes would appear
6. Resulting in a unique pattern characteristic of each RFLP allele
7. Resulting in a **unique pattern** characteristic of each RFLP allele

(b) Outline the process of nucleic acid hybridisation and explain how it can be used to detect and analyse restriction fragment length polymorphism (RFLP). [7]

Process of genetic fingerprinting

1. DNA is cut by restriction enzymes
2. on samples containing variable number tandem repeats (VNTR) alleles
3. DNA fragments are then separated by gel electrophoresis
4. and transferred onto nitrocellulose membrane via Southern blotting
5. for and bound to complementary radioactively labelled probes

Analysis of results

6. DNA is isolated from both parents and their child
7. and several VNTR alleles at multiple loci of interest are studied
8. A unique banding pattern of the parents and children can then be compared
9. The child will be related if some bands match those in the father
10. while the remaining bands match those found in the mother

(c) Suggest why some disease alleles can only be detected by linkage analysis. [5]

1a. When the nucleotide sequence of the disease-causing gene is not known

1b. When there are no existing restriction sites (RS) present in the wild-type / normal allele

2. Mutation of the normal allele to the disease allele causes no changes to the RS, ie it does not destroy / create RS / shift the position of RS, due to insertion and deletion mutations
3. Restriction digestion is unable to generate variation in lengths of restriction fragments. Thus, RFLP fragments from the disease allele cannot be distinguished from the normal alleles

4. A genetic marker with known location / highly polymorphic eg. RFLP, VNTR is located very close / tightly linked to the disease-causing gene locus
5. The disease-causing gene locus is almost always inherited together / co-inherited. Thus, presence of genetic marker indicates presence of diseased allele. However, linkage is not absolute, since crossing over can occur