HWA CHONG INSTITUTION
JC2 Preliminary Examinations
Higher 2

CANDIDATE NAME		**CT GROUP**	**14S ___**
CENTRE NUMBER		**INDEX NUMBER**	

BIOLOGY

9648 / 03

Paper 3 Applications Paper and Planning Question — **14 September 2015**

Additional Materials: Writing Paper — **2 hours**

INSTRUCTIONS TO CANDIDATES

There are **four** question booklets (**I** to **IV**) to this paper. Write your **name**, **CT group**, **Centre number** and **index number** in the spaces provided at the top of this cover page, and your **name** and **CT group** on the lines provided at the top of the cover page of Booklets **II, III and IV**.

STRUCTURED QUESTIONS

Answer **all** three questions.

Write your answers on the lines / in the spaces provided.

PLANNING QUESTION

Answer the question in booklet **IV**.

Write your answers on the lines / in the spaces provided.

FREE RESPONSE QUESTION

Answer the question.

Your answers must be in continuous prose, where appropriate.

Write your answers on the writing paper provided.

BEGIN EACH PART ON A FRESH SHEET OF WRITING PAPER.

A **NIL RETURN** is required for parts not answered.

INFORMATION FOR CANDIDATES

The number of marks is given in brackets [ ] at the end of each question or part question.

The use of an approved scientific calculator is expected, where appropriate. You may lose marks if you do not show your working or if you do not use appropriate units.

You are reminded of the need for good English and clear presentation in your answers.

For Examiners' Use	
Question	**Marks**
1	**/ 12**
2	**/ 15**
3	**/ 13**
4	**/ 12**
5	**/ 20**
Total	**/ 72**

BOOKLET I

STRUCTURED QUESTIONS

QUESTION 1

Restriction enzymes are used in recombinant DNA technology (RDT). Fig. 1.1 shows the palindromic recognition sequence of *Eco*RI, a restriction enzyme.

5' – N – N – **G** – **A** – **A** – **T** – **T** – **C** – N – N – 3'

3' – N – N – **C** – **T** – **T** – **A** – **A** – **G** – N – N – 5'

Fig. 1.1

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

The bacterial plasmid, pUC, is a useful cloning vector in RDT. A researcher is investigating the use of two restriction enzymes, *Eco*RI and *Bam*HI, to clone gene **X** into pUC. Fig. 1.2 shows the results when pUC and recombinant pUC are treated with either *Eco*RI, *Bam*HI or both.

pUC / kb			recombinant pUC / kb		
*Eco*RI	*Bam*HI	both	*Eco*RI	*Bam*HI	both
7.0	4.0	4.0	7.0	4.0	4.0
	3.0	2.0	1.0	2.8	2.0
		1.0		1.2	1.0
					0.8
					0.2

Fig. 1.2

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

Table 1.1

	in pUC	in recombinant pUC
number of *Eco*RI sites		
number of *Bam*HI sites		

(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]

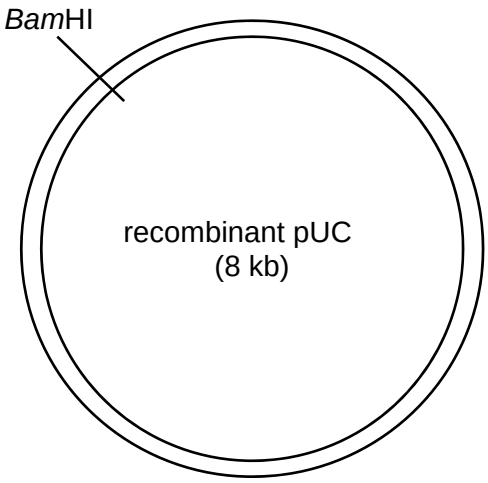


Fig. 1.3

(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]

Human insulin can be synthesised in *Escherichia coli* using RDT. Fig. 1.4 outlines how a gene coding for human insulin is produced.

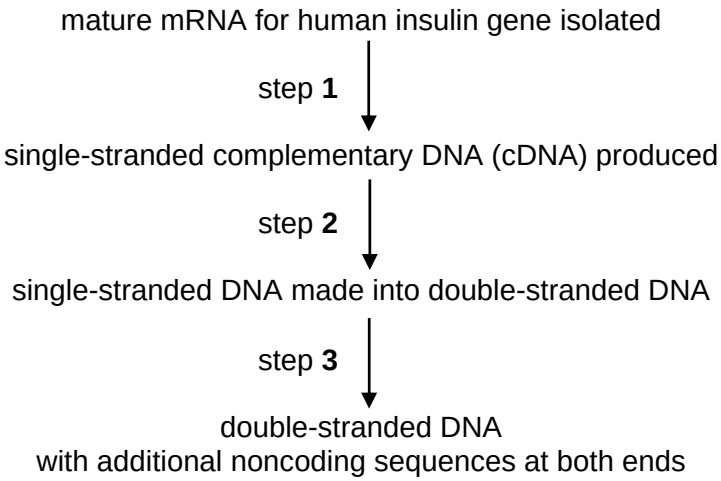


Fig. 1.4

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

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

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

[Total: 12]

Candidate Name: _____ **CT Group: 14S_____**

BOOKLET II

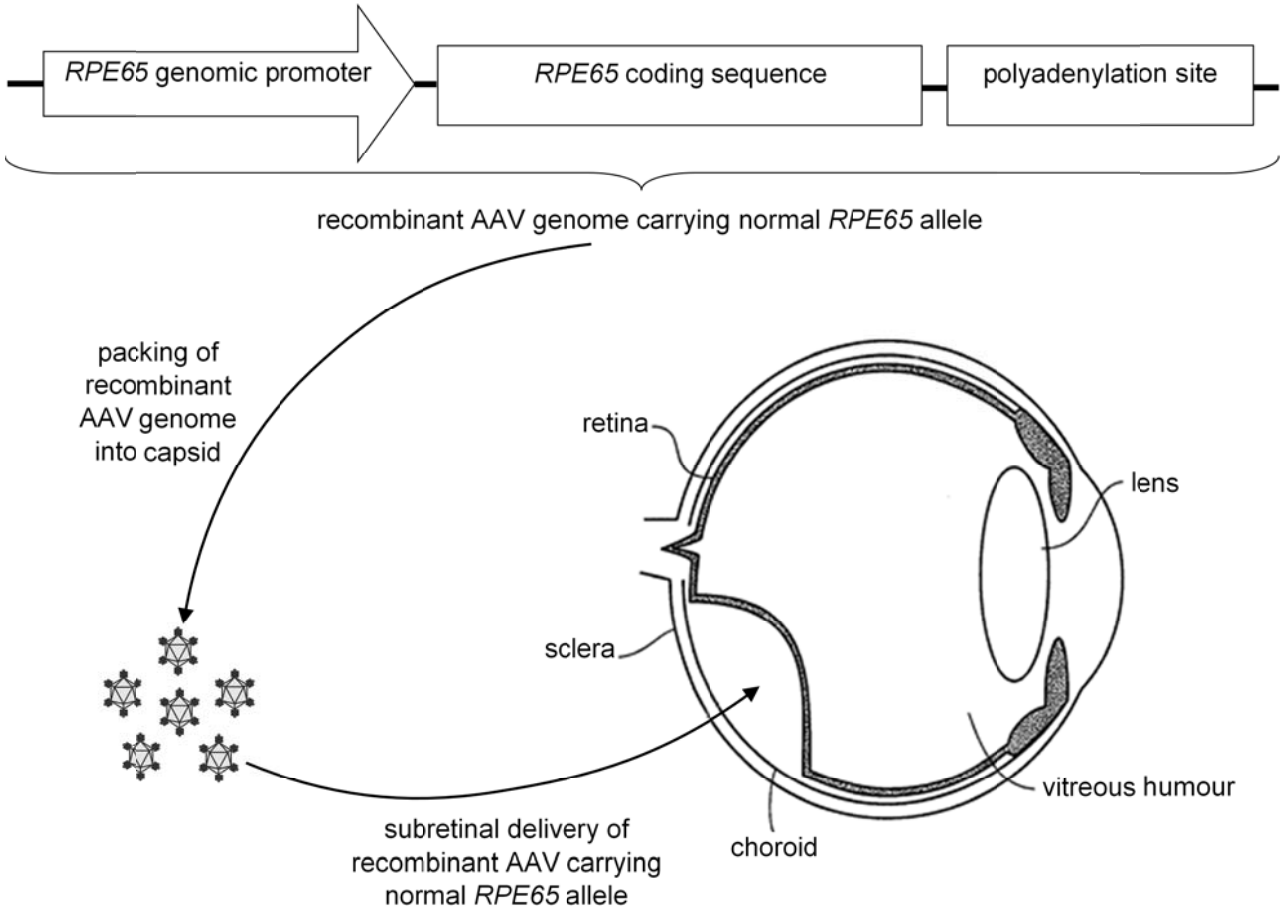
STRUCTURED QUESTIONS (CONTINUED)

[BLANK PAGE]

QUESTION 2

Leber's congenital amaurosis (LCA) is a group of inherited visual disorders caused by various point mutations in the *RPE65* gene. Such mutations result in RPE65 protein deficiency, which causes photoreceptor cell dysfunction and impaired vision from birth.

Between 2007 and 2010, a clinical trial to evaluate the efficacy of gene therapy using recombinant adeno-associated virus (AAV) was conducted on 12 human participants with LCA. Fig. 2.1 shows the insertion of the normal *RPE65* allele into an AAV vector, after which the recombinant vector was injected into the subretinal space of the retina.



* recombinant AAV genome, AAV and eye are not drawn to scale.

Fig. 2.1

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

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

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

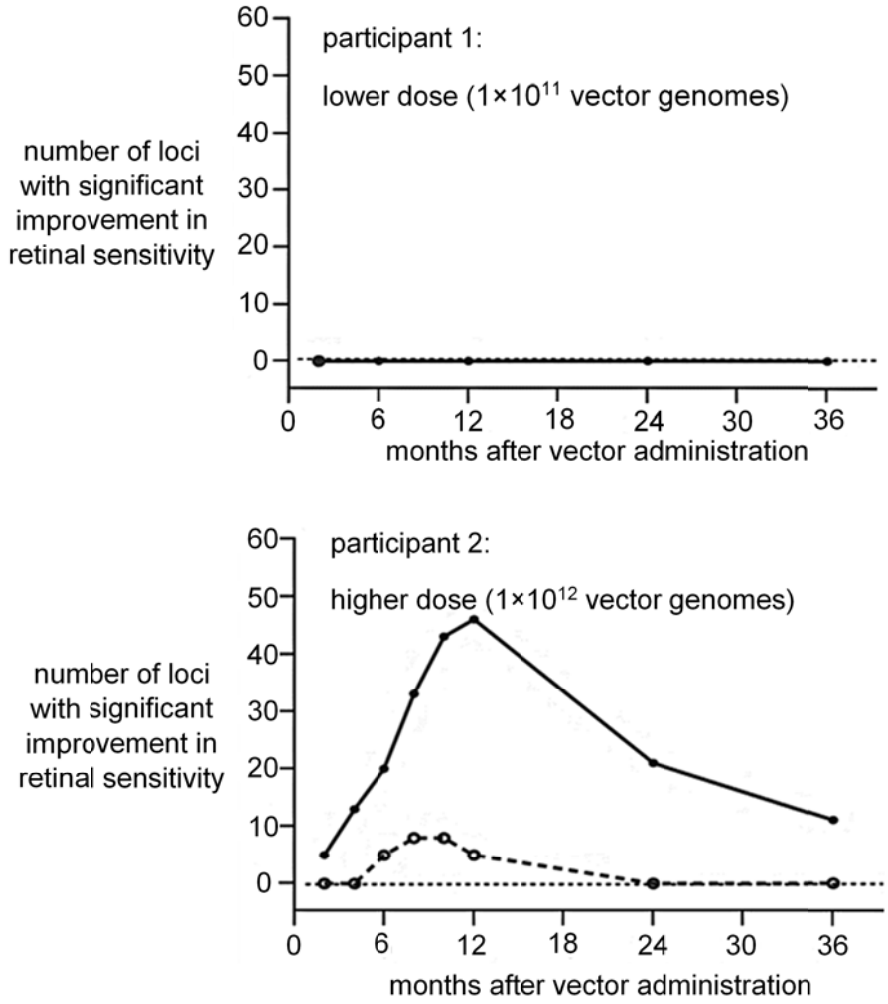
(d) Suggest why it is not necessary to include selectable markers in this study. [1]

For each participant in the clinical trial, an eye was selected as the study eye for treatment with the recombinant AAV vector. The other eye was left untreated. Retinal sensitivity was assessed in 76 loci (locations) on the retina of each eye.

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

The investigators examined the effect of the dosage of normal *RPE65* allele. Fig. 2.2 shows the retinal sensitivity of two participants whereby:

- participant 1 was administered a lower dose, and
- participant 2 was administered a higher dose of the recombinant AAV vector.



key
study eye: ——— (solid line) untreated eye: – – – – – – (dashed line)

Fig. 2.2

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

In the advanced stages of LCA, photoreceptor cells are lost. Hence, it is unlikely for gene therapy to be effective.

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

[Total: 15]

[BLANK PAGE]

Candidate Name: _____ **CT Group: 14S_____**

BOOKLET III

STRUCTURED QUESTIONS (CONTINUED)

[BLANK PAGE]

QUESTION 3

Orchids are popular in global floricultural trade. Orchids which are cultivated by traditional means from seeds take at least three to six years to flower. Tissue culture provides an alternative method to cultivate orchids.

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

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

A blue orchid has been the dream of many orchid hobbyists. It could be achieved by dyeing, but it is still not a "true blue" orchid.

With the advent of molecular biology in the last two decades, rapid production and selection of genetically modified (GM) orchids with desired traits has now become feasible. The *flavonoid 3',5'-hydroxylase* (*FH*) gene can be incorporated into the orchid genome. It is expressed in the flowers to produce delphinidin, a blue anthocyanin pigment, forming "true blue" orchids.

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

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

Genetic engineering has wide applications in crop plant growth.

The gene that confers herbicide resistance has been incorporated into some GM crop plants. This enables a farmer to spray his GM crop with a herbicide that will not harm the GM crop but does kill weed plants growing within the crop.

Two farmers have plots of land next to each other. They grow the same cereal crop.

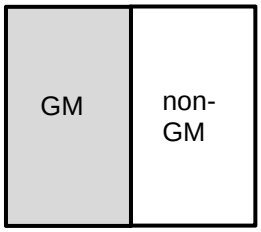
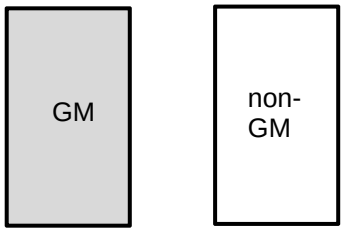
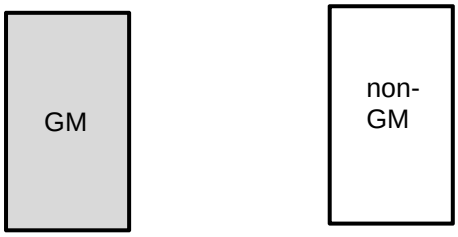
- Farmer **X** intends to grow GM crops that are resistant to herbicide.
- Farmer **Y** wishes to continue to grow non-GM crops.

Farmer **Y** was concerned and suggested to farmer **X** that pollen from the GM crop could fertilise the non-GM plants.

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

The farmers agreed to carry out field trials to establish whether leaving a gap between plots of land reduced the likelihood of cross-pollination. A number of trials were conducted so that the results of one trial did not interfere in any way with the results of another. The percentage of seeds produced at various positions as a result of cross-pollination was measured for each trial. The outline of these trials and the results gathered are shown in Table 3.1.

Table 3.1

	percentage of cross-pollination	
	at edge of non-GM crop	10 m into non-GM crop
trial 1  no gap between plots	10	2
trial 2  5 m between plots	1	0.5
trial 3  7 m between plots	1	0.3

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

[Total: 13]

[BLANK PAGE]

Candidate Name: _____ **CT Group: 14S_____**

BOOKLET IV

PLANNING QUESTION

[BLANK PAGE]

QUESTION 4

In transduction, bacteriophages carry bacterial genes from their first host cell to their second host cell due to aberrations in the phage reproductive cycle.

The T4 bacteriophage undergoes the lytic cycle and is thus capable of generalised transduction, where instead of viral DNA, a portion of bacterial DNA from host cells is accidentally packaged into the T4 capsid. The virus is fully capable of injecting DNA into a new bacterial host cell. When it does so, the new host cell receives that bacterial DNA instead of the T4 DNA, and thus does not get lysed.

Plan an investigation to measure the transduction efficiency of T4 phage particles in *Escherichia coli.*

You are provided with T4 phage particles grown on a plasmid-containing *E. coli* host strain. Hence the phage particles produced from that infection would contain plasmid DNA carrying the ampicillin resistance gene, amp^R.

Your planning must be based on the assumption that you have been provided with the following equipment and materials.

You must use:
- microfuge tubes of 10 μl T4 phage suspension
- microfuge tubes of 100 μl *E. coli* cells
- LB nutrient agar plates
- LB/amp agar plates
- incubator oven
- 70% ethanol

You may select from the following apparatus:
- microfuge tubes
- micropipettes and tips
- stopwatch
- glass beads
- parafilm sealing tapes

Your plan should have a clear and helpful structure to include:
- an explanation of the theory to support your practical procedure
- a description of the method used, including the scientific reasoning behind the method
- a risk and describe a precaution that is specific to the experiment
- the type of data generated by the experiment
- how the results will be analysed to determine the transduction efficiency.

[Total: 12]

FREE RESPONSE QUESTION

Your answers must be in continuous prose, where appropriate.
Your answers should be illustrated by large, clearly labelled diagrams, where appropriate.
Write your answers in the writing paper provided.
BEGIN EACH PART ON A FRESH SHEET OF WRITING PAPER.
A **NIL RETURN** is required.

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] #
#

(b) Describe how genetic fingerprinting can be used in paternity testing. [7]#
#

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

[Total: 20]

#

--- END OF PAPER---