KOLEJ UNIVERSITI TUNKU ABDUL RAHMAN

FACULTY OF APPLIED SCIENCES AND COMPUTING

ACADEMIC YEAR 2014/2015

JANUARY EXAMINATION

BIOLOGY BABS3213
TECHNIQUES IN BIOTECHNOLOGY

MONDAY, 5 JANUARY 2015 TIME: 9.00 AM – 11.00 AM (2 HOURS)

BACHELOR OF SCIENCE (HONOURS) IN BIOSCIENCE WITH CHEMISTRY

Instructions to Candidates:

Answer **ALL** questions. All questions carry equal marks.

BABS3213 TECHNIQUES IN BIOTECHNOLOGY

Q1. Figures 1 to 5 show the maps of commonly used vectors for gene cloning. Identify the cloning vector or vectors based on the statements given in **Q1(a) – (g)**. For each case, justify and elaborate your answer by highlighting the details of specific features of the chosen cloning vector(s). Wherever applicable, include brief explanation of how cloning is done.

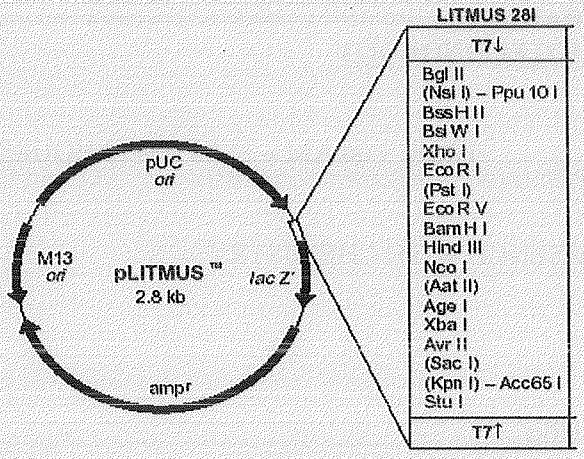


Figure 1

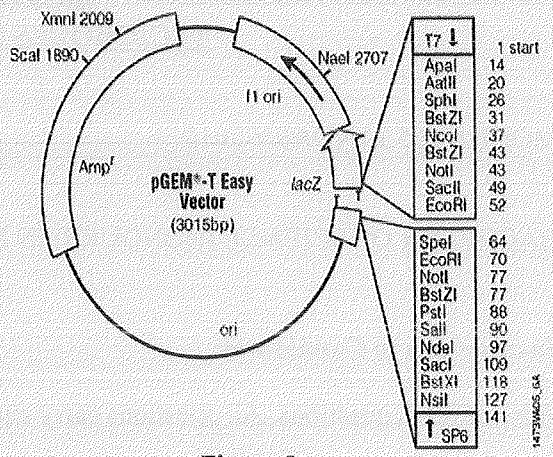


Figure 2

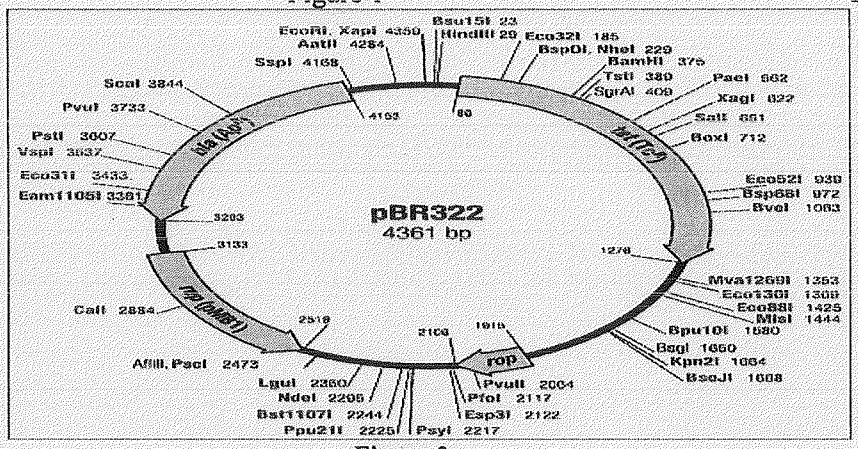


Figure 3

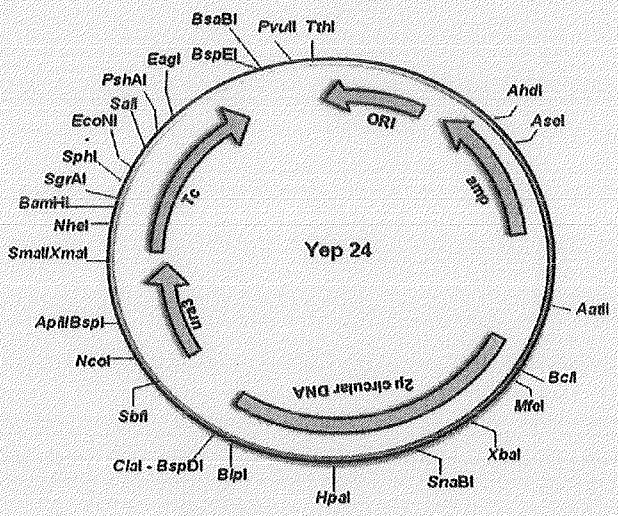


Figure 4

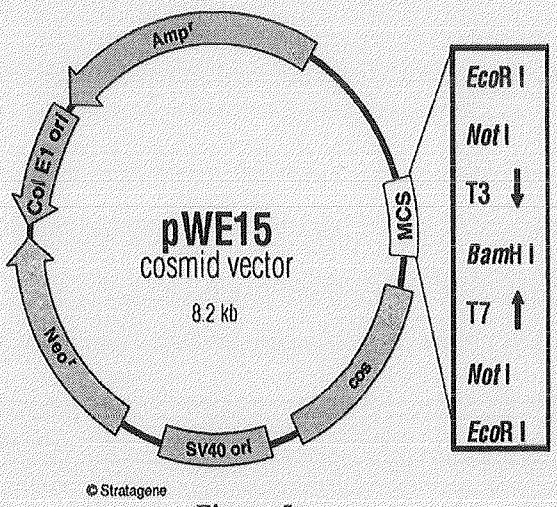


Figure 5

Q1.(Continued)

(a) Replica plating and negative selection is required for the selection of recombinants. (4 marks)

(b) Propagation of the cloned gene can be done in an eukaryotic host. (3 marks)

(c) RNA probe can be made. (4 marks)

(d) Single-stranded DNA needed as template for DNA sequencing can be obtained. (4 marks)

(e) The vector has been linearized (2 marks)

(f) Insert size of approximately 40 kb is possible to be cloned. (3 marks)

(g) This is a gene on the chromosome of *Sacchromyces cerevisiae*. (3 marks)

(h) Your coursemate cloned a fragment with vector pWE15 (Figure 5). After introducing into *E. coli* cells, she did not find plaques. Explain why is it so. (2 marks)

[Total: 25 marks]

Q2. (a) Natural products produced by plants and their synthetic derivatives are expected to play an important role in the development of innovative agents to inhibit the onset of cancer. A study was conducted to elucidate the antitumor activity of a mushroom, *Inonotus obliquus*, against human colon carcinoma HT-29 cells by the MTT assay and flow cytometric analysis.

(i) Briefly outline the principles of MTT assay and flow cytometric analysis. (8 marks)

(ii) Figure 6 shows the effects of hot water extract of *Inonotus obliquus* (IOWE) on the growth of HT-29 cells. Interpret the results. (2 marks)

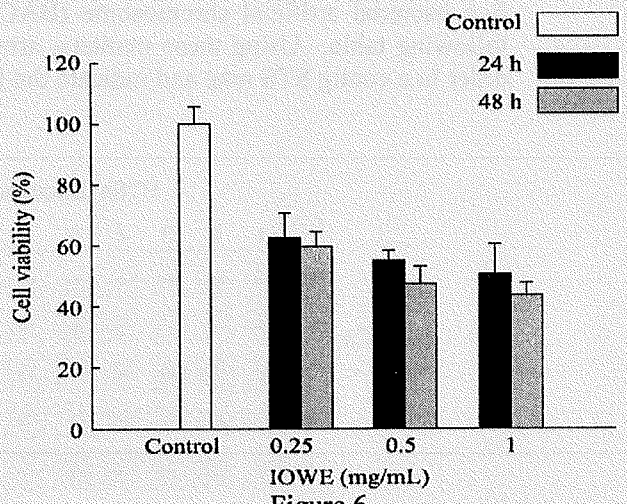


Figure 6

Q2(a).(Continued)

(iii) The levels of an antiapoptotic member, Bcl-2 and a proapoptotic member, Bax, was analyzed by western blot analysis (Figure 7). Summarize the results. Evaluate whether the mushroom has antitumour activity and suggest possible molecular mechanisms. (5 marks)

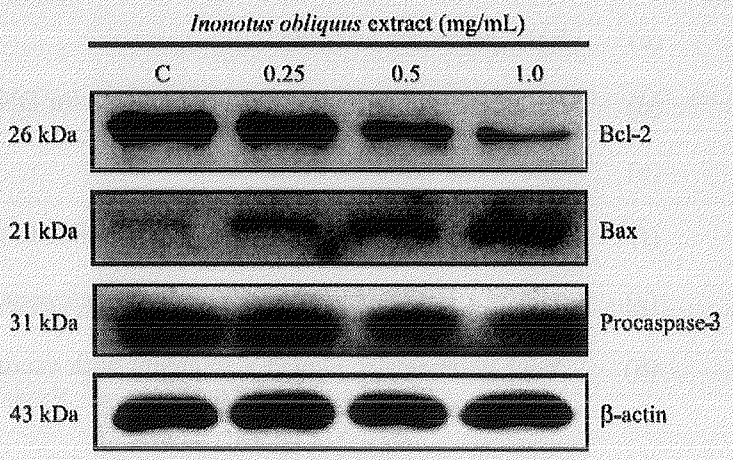


Figure 7

(iv) What is the role of β-actin in the Western blot? (4 marks)

(b) You read the following statement from a research paper: "The plasma levels of the secreted dengue-2 virus (D2V) nonstructural protein NS1 were determined by quantitative capture ELISA in children with dengue-2 virus infections." (Libraty *et al.*, The Journal of Infectious Diseases 2002; 1861165–8).
Explain the principle of capture ELISA and highlight its advantages. (6 marks)
[Total: 25 marks]

Q3. (a) (i) The presence (+) or absence (-) of twelve sequence-tagged sites (STSs) in each of five bacterial artificial chromosome (BAC) clones (A - E) is indicated in the following table. Using these markers, arrange the BAC clones in their correct order in a contig STS map and indicate the locations of the STS sites within them. (5 marks)

STSs (Sequence-tagged sites)

BACs	1	2	3	4	5	6	7	8	9	10	11	12
A	−	+	−	−	−	−	−	−	−	−	+	+
B	−	−	−	+	+	+	+	−	−	−	−	−
C	+	+	−	−	−	−	−	+	−	+	−	−
D	−	−	+	+	−	−	+	−	−	−	−	+
E	+	−	−	−	−	−	−	+	+	−	−	−

Q3(a).(Continued)

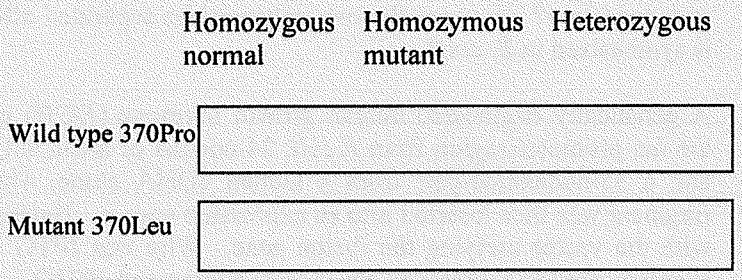
(ii) Suggest some reasons why a molecular biologist might wish to perform a pairwise sequence alignment. (5 marks)

(b) (i) DNA molecular testing using PCR and allele-specific oligonucleotide (ASO) hybridization to look for mutations of the open-anle glaucoma gene *GLC1A*. Open-angle glaucoma is by far the most common form of glaucoma,which if it is not diagnosed and treated, total blindness can occur. Sequences of the two allele-specific oligonucleotides (ASOs), one for the wild-type allele and one for the mutant allele are shown below.

Wild type 370Pro	5'GACAGTTCCCGTATTCTTG 3'
Mutant 370Leu	5'GACAGTTCCTGTATTCTTG3'

Based on the *GLC1A* gene sequence, primers were designed for PCR amplification of the region of the gene containing the mutation. The PCR product was dotted onto two membrane filters under conditions that denatured the DNA to single strands. Two ASOs (each 19 nt long) were made, one for the wild-type alle and one for the mutant allele. Each ASO was labeled and was then hybridized with the *GLC1A* DNA immobilized on one of the filters.

Copy the two membrane strips below into your answer book and draw the expected results of the dot blot autoradiograms for a homozygous normal, a homozymous mutant, and a heterozygous individual. (5 marks)



(ii) RNA was isolated from four different cell types and probed with radiolabeled cloned gene Y. The results are shown in Figure 8. Interpret the results of this experiment. (5 marks)

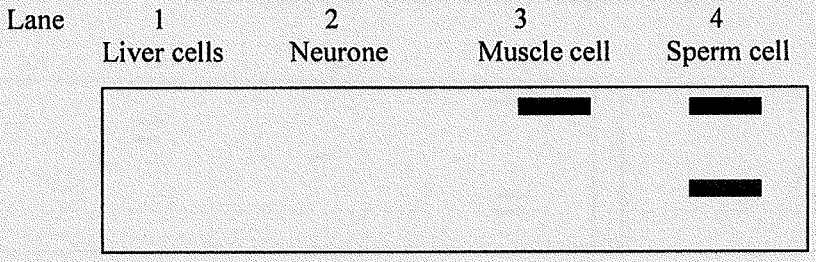


Figure 8

Q3(b).(Continued)

(iii) Figure 9 shows the maps of restriction sites (arrows) of a gene for student A and student B, respectively. The bar below the restriction map represents the probe.

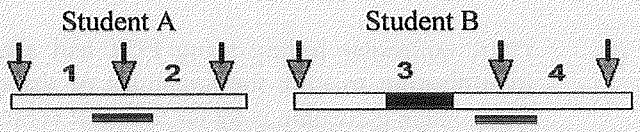


Figure 9

Draw the restriction fragment length polymorphism for students A and B to show the effect of insertion-deletion. (5 marks)

[Total: 25 marks]

Q4. (a) (i) Suggest a method for how somatostation (the growth hormone inhibiting hormone) can be cloned into a bacterial cells without making a cDNA. (2 marks)

(ii) Highlight some considerations that must be taken before we contruct an *E.coli* strain that will produce a mammalian protein such as human growth hormone. (9 marks)

(iii) To produce recombinant human insulin, the coding seuquences of the two polypeptide chains, the A and B chains, are placed next to the coding sequence of a native *E. coli* protein, β-galactosidase to create a fusion protein. Why is this step necessary? How are the two polypeptides separated after the fusion protein is synthesized in *E. coli*? (4 marks)

(iv) A genetically engineered human growth hormone (*HGH*) gene was made with the *lac* promoter region from *E.coli*, 24 codons of artificially synthesized DNA, and a 55-nucleotide-pair from a human cDNA clone. This engineered *HGH* fragment was then inserted into an expression vector. Bacteria were transformed with the vector carrying the fusion gene. Why was IPTG used in the medium supplemented to express the genetically engineered *HGH*? (3 marks)

(b) A student was given a short DNA fragment to sequence. He first cloned the fragment, isolated it and applied the Sanger sequencing method. The products of the reactions were separated by gel electrophoresis (Figure 10). Deduce the 5'to 3' base sequence of the original fragment based on the banding pattern. (4 marks)

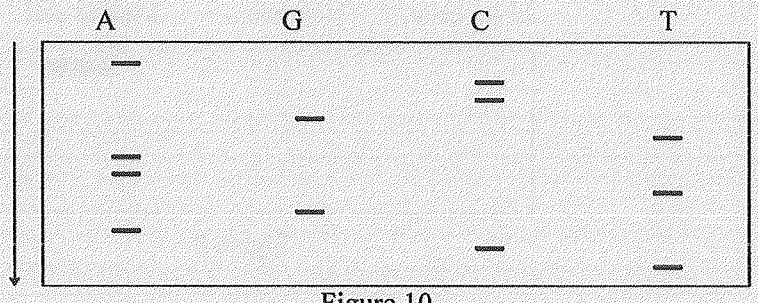


Figure 10

Q4.(Continued)

(c) Highlight three key issues which concern the safety of genetically modified foods produced through biotechnology. (3 marks)

[Total: 25 marks]