Ref. No. Ex/SC/BT/PG/Core/TH/331/2023

M. Sc. (Biotechnology) Examination, 2023

(2nd Year, 1st Smester)

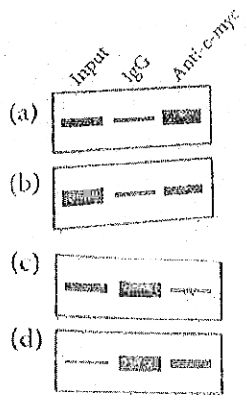
Subject : Recombinant DNA Technology

Paper : MSBT 331

Time : Two hours | Full Marks : 40

Group A
Answer any two questions

1. (A) A chromatin immunoprecipitation (ChIP) assay was performed to determine specific transcription factor binding sites on the promoter of a gene. Pull down was done using either IgG or antibodies against c-Myc. What do you understand by Input, IgG and anti-c-Myc shown in the picture below? Which one of the following PCR representations of DNA is correct (explain with proper justification)? (3+3=6)



(B) Suppose you have identified a novel gene "X" and found perfect homology in *C.elegans* and in mouse. Experimentally how would you show that gene "X" is both necessary and sufficient for cell proliferation. (2+2=4)

2.(A) DNA fragments from a gel are transferred to a nitrocellulose paper during the procedure called Southern Blotting. What is the purpose of transferring the DNA from a gel to a nitrocellulaose paper? (2)

(B) You have decided to perform an experiment to show that your favourite gene, the COOL gene is associated with the promoter sequence of a cardiac-specific gene "HEART". Explain briefly the experiment you would perform to establish your hypothesis. (1+2=3)

(C) Which salient features are used to distinguish between alleles in RFLP analysis? (2)
(D) "Dolly is not a transgenic lamb"- Justify the statement. (3)

3. (A) Explain temporal regulation of gene expression in mouse by Tet-on and Tet-off system. (4)

(B) A 200 ul of PCR mix has 100 DNA template DNA molecules and the reaction was performed for 10 cycles.

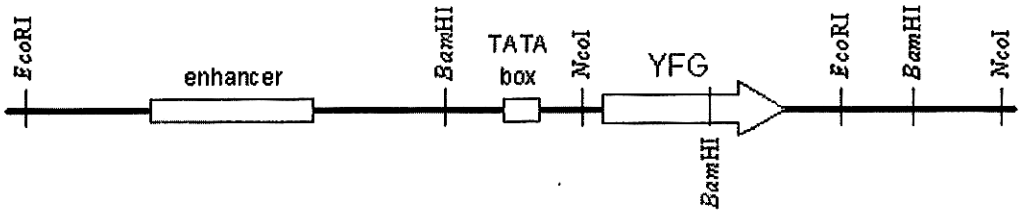
i) How many molecules of amplicon will be generated?
ii) How many molecules of amplicon will be present in 0.1 ul of reaction mix? (2+2=4)

(C) You are working with very large molecule of DNA fragments (which ranges between 450kb -50 kbs). How will you separate these large DNA molecules by using electrophoresis? (2)

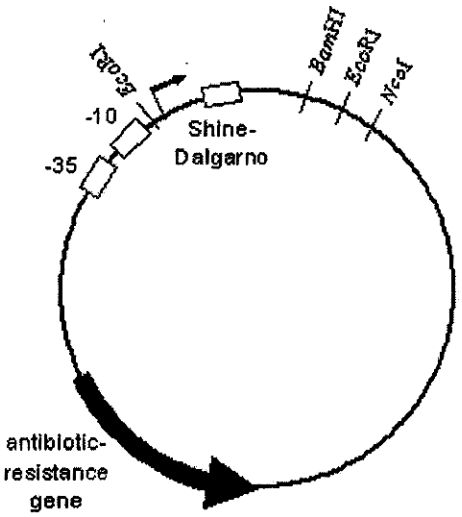
4. (A) You would like to make a transgenic mouse where gene "A" will be specifically deleted from brain. Explain the procedure to make the transgenic mouse in detail. (5)

(B) You have identified a novel tumor suppressor gene. How would you quantify the expression of that gene in a pancreatic tumor by SYBR green method? Explain the $\Delta\Delta$Ct method to calculate the fold change of that tumor suppressor gene between the pancreatic tumor cell and normal pancreatic cell? (2.5x2=5)

5. (i) The diagram below represents a section of the human genome. The coding sequence of a gene, YFG, is shown by an arrow, and boxes indicate the locations of some regulatory sequences. Locations of recognition sequences (cut sites) for three common restriction enzymes (EcoRI, BamHI, and NcoI) are also marked.



You would like to clone this gene in E. coli for further study. You have available the expression vector(plasmid) shown below:



a. Why is it important for this plasmid to be an expression vector? (2)
b. Why is it important for this plasmid to have an antibiotic-resistance gene? (2)
c. What restriction enzyme would you use to clone this gene?
Explain your choice. (3)

(ii) Which major promoter is used to express foreign gene in *Picchia pastoris*? What is the principal advantage of using this promoter? (2)

(iii) What is vertical and horizontal transmission? (1)

6. (i) What are bacculoviruses? What is their most popularly used host? Outline with labeled diagram the major steps involved in use of bacculovirus as a heterologous expression system. (1+1+2)

(ii) A student ligated a BamHI fragment containing a gene of interest to a pUC19 vector which was digested with BamHI, transformed E. coli with the mixture of ligation products and plated the cells on plates containing the antibiotic ampicillin and the chromogenic substrate X-gal.

a) Which colonies should the student pick and why to find the ones containing the recombinant plasmid (with the gene of interest in pUC)? (1+3)
b) How will the student further screen for false positive clones. (2)

7. (i) a) Name two most popularly used nutritional markers in yeast system?
b). What are the fundamental features of a shuttle vector? (1+1)

(ii) You are planning to express a foreign protein in mammalian cell.

a) Describe the essential features of a mammalian vector that are required for expression of a foreign gene in mammalian cell. (2)
b) How can you modify the foreign protein to facilitate its purification? (2)
c) What is the advantage of expressing a mammalian protein in mammalian cells versus bacteria? (2)

(iii) What is Bt cotton and golden rice? (2)

8. (i) The Restriction sites recognized by BamHI and Sau 3A are shown below:

BamHI	Sau3A
GGATCC	NGATCN
CCTAGG	NCTAGN

If two BamHI sites are ligated together, the resulting site can be cleaved with Sau3A. The same is true for Sau3A. Suppose you ligate a Sau3A end to a BamHI end. Can the Hybrid site be cut with BamHI? Can it be cut with Sau3A? Give reasons (2+2=4)

(ii) What are Terminator genes? How do they function? (2+2)

(iii) What is *A. tumefaciens* and what special feature does it have that has allowed it to become useful for genetic engineering? (2)