M. Sc. (Biotechnology) Examination, 2023

(1st Year, 2nd Semester)

Recombinant DNA Technology

Paper – 236T

Time : Two hours Full Marks : 40

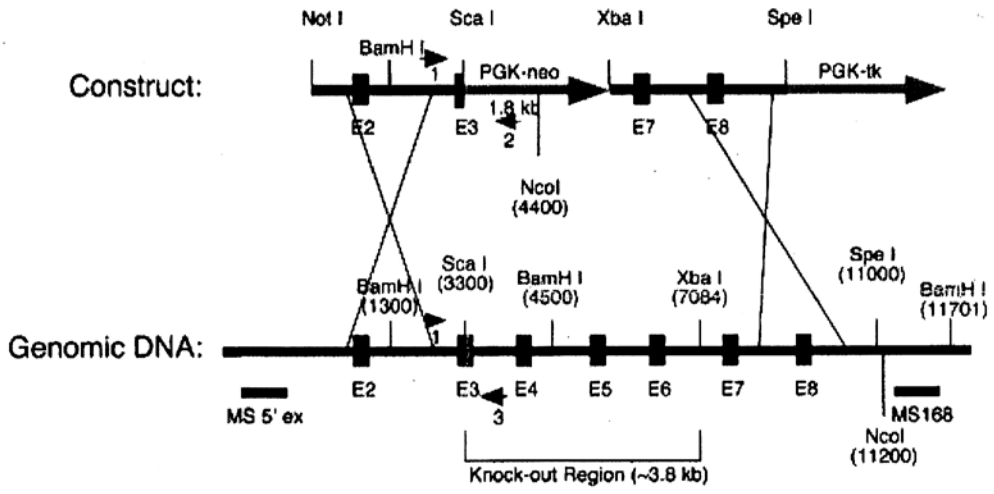
GROUP-A

Answer any TWO questions. (10 x 2= 20)

1. (A) How will you predict/locate miRNA in the genome by comparative genomics approach? (2)
(B) How will you detect the involvement of miRNA in a cell by reporter assay method? (2)
(C) Draw the amplification curves by plotting cycle numbers in X-axis and ΔRn in Y-axis (both in log view and linear view) that you would expect while performing a real time PCR. Mention the different phases of the real time PCR on the graph. (2 +2 = 4)
(D) At which phase of the PCR amplification the fluorescence is measured and why? (2)

2. (A) In a PCR reaction why do we keep the annealing temperature 4-5 °C lower than the melting temperature? (2)
(B) Explain the temporal regulation of gene expression in mouse by Tet-on and Tet-off system. (4)
(C) A 200 ul of PCR mix has 100 template DNA molecules and the reaction was performed for 10 cycles. How many molecules of amplicon will be generated? How many molecules of amplicon will be present in 0.1 ul of reaction mix? (3)
(D) What is the full form of CRISPR? (1)

3. Below is the diagram of a transgenic DNA construct which was used to make a knock-out mouse line of "X" gene by deleting the exon3 to exon7 of gene "X" from the genome.

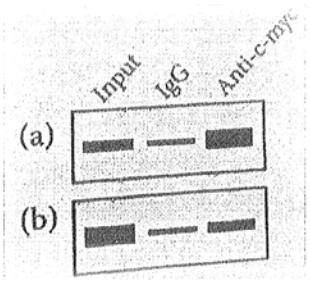


(A) What is the significance of including PGK-neo and PGK-tk construct in the transgenic construct? (4)

(B) In order to confirm the integration of transgenic construct in the genome you have performed Southern blot technique by using BamH1 enzyme to digest the DNA and used MS168 as probe (as indicated above in the diagram). Draw the blot after developing the membrane indicating the different bands that you expect in case of wild type and heterozygous animals with proper justification. (3)

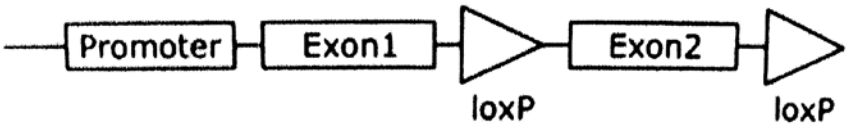
(C) "Dolly is not a transgenic lamb"- Justify the statement. (3)

4.(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+2=5)



(B). Briefly explain the principle of CRISPR-Cas system to regulate gene expression. (3)

(C) In a transgenic mouse line, loxP sites are introduced in the target gene A in the following manner.



This transgenic mouse line was mated with another transgenic mouse line where Cre recombinase is expressed only in cardiac cells (as indicated by the Promoter). What will be the expression profile of gene A in Cre/lox recombinant mouse and why? (2)

5 (A). What are the similarities and differences between siRNA and miRNA? (2)

(B). Explain in detail how will you calculate the fold change of p53 gene expression in your cancer cell line compare to normal cell by ΔCt method? (2)

(C). Restriction fragment length polymorphism (RFLP) is formerly used for DNA fingerprinting. Explain RFLP in brief and how it is used to identify suspect from the crime scene. (2+2=4)

(D) What are the main components of loading dye in agarose gel electrophoresis? Does the loading dye move along with the DNA in agarose gel? (2)

Group – B
Answer any one question

6. You are assigned to clone (i) a Bifidobacterium β-galactosidase (lactase) and (ii) an egg lysozyme into E. coli.
a) What basic steps are used to produce a genetically engineered E. coli to produce these prokaryotic and eukaryotic enzymes?
b) How can the bacteria that make the product of a particular cloned gene be identified after cloning?
c) What special problems must be considered if you clone eukaryotic genes into prokaryotic cell, E. coli?
d) If the expression level of a cloned enzyme in prokaryotic cell is very low, how can you improve gene expression on this enzyme

(3+2+3+2)

7. a) Starting with an isolated mRNA, one wishes to make a double stranded copy of the mRNA and insert it at the *Pst*I site of pBR322 via G-C homopolymer tailing. One then transforms *E. coli* with this recombinant plasmid, selecting for tetracycline resistance. What are the four enzymatic steps used in preparing the cDNA insert? Name the enzymes and describe the intermediates.
b) Which one you think the best system so far for expression of a foreign protein followed by the purification using the secretory pathway of the host. Justify your answer.

c) If you insert your gene of interest in the vir region of Ti plasmid, will you get a successful product? Explain.

(4+2+4)

8. a) You recently have made an interesting finding that two cell surface proteins namely β-integrin and ICAM interact together from co-immunoprecipitation experiments, which belong to cell surface adherent proteins. You want to further verify this interaction in vivo. What probable method you will choose to verify this? Briefly illustrate the rationale and strategy involved in the technique and just outline the steps involved in this.
b) How is *Bacillus thuringiensis* useful to the scientists?
c) A very determined graduate student set out to construct a restriction map for the plasmid pDA401 (total size=4kb). The restriction enzymes used were HindIII, BamHI and EcoRI. After carrying out the digestions, the resulting DNA fragments were electrophoresed and sized using a set of DNA size standards. The data obtained in each digestion are shown below.
From the data, construct a restriction map of pDA401 for the enzymes HindIII, BamHI and EcoRI.

Enzyme(s):	Segments observed after digestion
HindIII	3.82kb, 0.18kb
BamHI	2.35kb, 1.65kb
EcoRI	3.00kb, 1.00kb
HindIII+BamHI	2.35kb, 1.20kb, 0.27kb, 0.18kb
HindIII+EcoRI	1.87kb, 1.00kb, 0.95kb, 0.18kb
BamHI+EcoRI	1.60kb, 1.40kb, 0.75kb, 0.25kb

(4+2+4)

Group C

9. What is the purpose of performing Yeast Two Hybrid system? Briefly illustrate the process of construction of different plasmids for performing Yeast Two Hybrid system and how the product of the reporter gene is produced?

(1+4 = 5)

OR

10. Why copy number of natural plasmid needs to be maintained within bacterial cells? Explain in brief how Rep protein itself and antisense RNA of Cop A control copy number of R1 family of plasmid. (1+4 = 5)

11. What are the differences in basic principles between classical western and far western methods? Why do we use only native form of proteins in far western method? Probes can be used either directly to the gel or to the membrane after transfer. Is the statement correct? Justify your answer.

(2+2+1=5)

OR

12. What is the difference between jumping library and cloning library? Explain Fosmid Jump library. What are the possible limitations of chromosome jumping?

(2+1+2=5)