15. i) Explain temporal regulation of gene expression in mouse by Tet-on and Tet-off system. 5

ii) How will you locate putative miRNA sequence in the genome of an organism by comparative genomics approach ? 3

iii) What are the requirements for setting up the multiplex PCR ? 2

16. i) What would you do to reduce non-specific PCR product in a PCR reaction ? 2

ii) What do you mean by baseline and cycle threshold in real-time PCR ? 2+2

iii) Explain in detail how will you calculate the fold change of p53 gene expression in your cancer cell line compared to normal cell by ΔCt method ? 4

M.Sc. (Biotechnology) Part II Examination, 2019

DNA Technology

Paper - 2/1

Time : Four hours Full Marks : 100

PART - I

Answer ***any six*** questions. (10 marks each)

1. a) How does lacZ staining work in blue white colony screening ? 2

b) Why some white colonies may not contain the desired recombinant plasmid? 1

c) It is possible that some blue colonies may contain the insert. Why ? 1

d) What is a reporter gene and how these are used in vitro promoter assay ? 2

e) Name 2 genes that might be used for this purpose. 1

f) It is possible to isolate plasmids from bacterial cells, alter their base sequence, insert additional foreign sequence and reincorporate them into bacterial cells. How might this be potentially useful ? 3

2. a) How do you prevent the self circularization of a vector during ligation step in a cloning procedure ? What enzyme is typically used for this reaction ? 2

   b) Distinguish between transient transfection and stable transfection ? What are the advantages/disadvantages of each of these two methods. 2

   c) What is a cosmid ? Mention what advantage it has over the plasmid vector ? 2

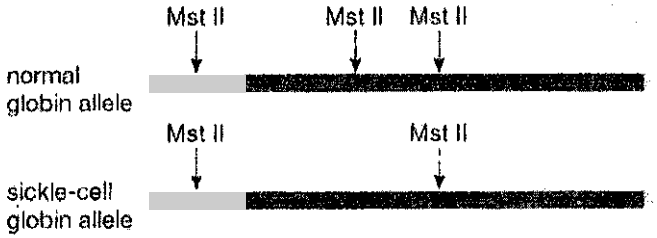
   d) How are Flavr Savr tomatoes different from regular tomatoes ? 2

   e) You are asked to make a deletion mutant of a protein with domain B deleted. The protein consists of three domains A, B and C (from N terminus to C terminus). How would you go about in constructing such a protein. 2

3. a) What type of plant molecules signal Agrobacterium to express vir genes ? Name one. 2

   b) What are opines and how do they benefit Agrobacterium? 2

   c) Starting with an isolated mRNA, one wishes to make a double stranded copy of the mRNA and insert it at the Pst1 site of pBR322 via G-C homopolymer tailing. One then transforms *E. coli* with this recombinant plasmid,



   iv) You have identified a gene X and demonstrated that the gene X is expressed only in spleen and liver by Northern blot analysis (as shown below). Can you definitely be certain that the gene X is not expessed in any other tissues apart from spleen and liver ? 2

13. i) What is the full form of CRISPR ? Briefly explain the principle of CRISPR-Cas system to edit genome of an organism. 4+1

   ii) What are iPS cells and how are they generated ? 2

   iii) Explain how would you use iPS cells therapeutically to treat type I diabetes ? 3

14. i) "Dolly is not a transgenic lamb" - Justify the statement. 2

   ii) Briefly explain the making of transgenic mouse by pronuclear microinjection method. 5

   iii) What are the similarities and differences between siRNA and miRNA ? 3

d) Explain why Dpnl is used in site directed mutagenesis. 2

6. a) What vector would you use to clone a fragment of length 2kb, 9kb, 40kb, 200kb ? 2

b) Explain the problem of inclusion body and how it is resolved. 2

c) What are the three basic steps in a DNA extraction method ? 3

d) Briefly explain what do you mean by negative selection. How is this selection useful in genetic analysis involving *Saccharomyces cerevisiae*. Which gene is popularly used for negative selection ? 3

7. a) Mention the use and regulation of the following promoters.

i) CMV

ii) Tetracycline

iii) Galactose 6

b) Following the standard procedure for genetic analysis you have identified a plasmid carrying a specific fragment from yeast genomic library which apparently complements the defect associated with the mutation. Suggest additional experiment to confirm that the plasmid truly contain the wild type gene corresponding to the mutation. Define a

ii) List two reasons why you should design your primers so that they only bind theflanking sequences and not the area with the repeated CGA sequences. 3

iii) What are the different ingredients required to perform PCR reaction ? What is the size of the PCR product, if you use the primers that you designed in (i) to amplify the above DNA sequence ? 2+1

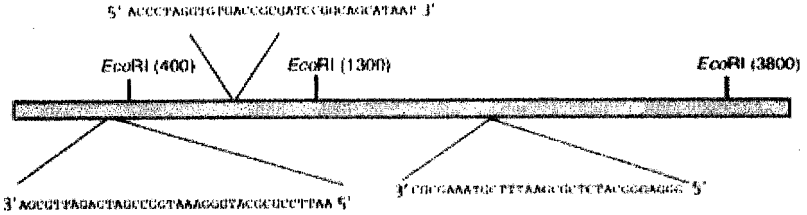
11. i) The following three pieces of sequences originate from the same DNA string and have been found by sequencing. What is the sequence of the full length DNA fragment ?

5' AGCGTTAG 3'

5' CCGGTAAA 3'

5' AGCCGGTA 3' 2

ii) The DNA fragment shown below is cleaved by the restriction enzyme EcoRI as indicated. The number in parenthesis shows the position of the cleavage site. The total length of the DNA fragment is 4000 bp. Small parts of the DNA sequence is shown.



strategy how you will be able to clone the wild type gene corresponding to the mutation. 2

c) Discuss the principle on which pET vectors are used to study gene expression. 2

8. a) What is the function of ARS and Centromeric sequences in a yCP vector ? 2

b) What is a binary vector ? Where do you find these vectors. 2

c) If you insert your gene of interest in the vir region of Ti plasmid, will you get a successful product ? Explain. 3

d) Which lambda expression vector is widely used for making cDNA libraries ? How is the cDNA library useful? What information can we gather from RACE experiments. 3

9. a) How is it possible that a bacterium that produces a restriction enzyme does not cut its own DNA? 2

b) Bacterial strains also harbor methylases. Why they are not desirable as cloning hosts. 2

c) What are the major advantages of using a pET expression system ? 4

d) Why is the presence of mini attTn7 necessary in pfast Bac vector. 2

PART - II

Answer ***any four*** questions.

(Each question contains 10 marks) 4×10=10

10. The DNA sequence shown below contains the sequence **CGA** repeated several times. Different individuals will have the sequence CGA repeated a varying number of times (suppose from 10 – approximately 100 times), While all individuals will have the same flanking sequences (marked in bold). The sequence does not encode protein.

5'**AGCTTTGCACATGGGCTATGCCTCAGTTTAAAATACATGCCTG**CGACGACGAC
GACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACG
ACGACGACCCGA**GAAAGAGTCTCTCTGTTGGATTCGCGC** 3'

i) Suppose, you want to amplify the sequence shown above using PCR in a way that will allow you compare the size of the PCR product that is produced when using DNA from a crime scene, with the size of the PCR product that is produced when using DNA from suspects. Design two primers that can be used for amplifying the area with the repeared sequences of CGA. The two primers should each consist of 20 nucleotides. Write the sequence of the two primers mentioning the 5' and 3' ends. Also mark where the two primers will bind on the sequence in the above figure. 2+2

After digestion with the restriction enzyme EcoRI, the gel is blotted onto a membrane and hybridized to the following labelled probe :

3' CCCTCCCGTAGAGCGCTTAAAGCATTTCGCG 5'

a) Briefly explain the methods to make probe by isotropic and non-isotropic labelling (one method for each type of labelling). 3+3

b) After hybridization, the membrane is put on a x-ray film. What size band will be apparent on the exposed film ? Explain with reasons. 2

12. i) Do eukaryotic cells have restriction endonuclease ? Justify your answer. 2

ii) How a reporter enzyme can be used to monitor transformation of host cells by foreign DNA in addition to a selectable marker ? 3

iii) Sickle-cell anaemia (SCA) is caused due to a mutation of a single nucleotide (A to T) of the β -globin gene which eliminates a MstII restriction site in the mutated allele (as shown below). How would you determine if a person is homozygous normal (wild type), heterozygous carrier or homozygous mutant for the the β -globin allele ? 3

selecting for tetracycline resistance. What are the four enzymatic steps used in preparing the cDNA insert ?

Name the enzymes and describe the intermediates. 4

d) 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. 2

4. a) Describe the mechanism by which DNA ligase functions. 3

b) How is a genomic library different from cDNA library? 2

c) What is a trc promoter ? What is the advantage of using this promoter ? 2

d) What is YAC vector ? Can you call it a shuttle vector? 3

5. a) i) What are Bacculoviruses ?

ii) What is their most popularly used host ?

iii) Outline with labeled diagram the major steps involved in use of bacculovirus as a heterologous expression system. 0·5+0·5+3

b) Name two popularly used cell lines used for expression in mammalian system. What is the most commonly used episomal vector used for mammalian cells. 2

c) What was the logic behind creating golden rice ? 2