Exam.Code:0910
Sub. Code: 6714

2062

B.E. (Biotechnology) Sixth Semester

BIO-611: Recombinant DNA Technology

Time allowed: 3 Hours Max. Marks: 50

NOTE: *Attempt five questions in all, including Question No. I which is compulsory and selecting two questions from each Unit. All questions carry equal marks.*

x-x-x

Q1. Answer the following:-

a. How does ampicillin inhibit the growth of bacteria? How does ampicillin resistance gene product confer resistance to bacteria against the ampicillin? Explain
b. Taq polymerase is known to add errors during DNA synthesis? Explain the reason.
c. Discuss the primer extension method of site directed mutagenesis.
d. Differentiate between Ti and Ri plasmids
e. Briefly discuss about RACE

(5x2)

UNIT - I

Q2. a. Discuss the principle and method of plasmid isolation by alkaline lysis method. Highlight the importance of each solution used. Why 2-3 bands of different sizes are observed for uncut plasmid on agarose gel, while only single band after digestion of same plasmid with unique restriction enzymes? Discuss

b. What is PCR? What for and how many minimum primers are used for amplification of a gene. While designing primers, what points should be kept in mind? Discuss the principle and procedure of multiplex PCR. When this is preferred over normal PCR? (4, 6)

Q3. a. Why is lambda phage called temperate phage? What determines lytic or lysogenic cycle? What is the advantage of lysogeny to the lambda phage? In what respect phage based vectors are better than plasmid based vectors? Discuss.

b. What types of selectable markers are used in vectors for animal cells? What is YAC vector? Discuss the methodology of gene cloning using YAC. Under which condition this vector is preferred? (5, 5)

Q4. a. What role do restriction endonucleases play in nature? What are isoschizomers and how these are used in gene cloning? How they play a role in gene cloning. Explain with help of example. For a restriction enzyme recognizing 6 bp, what is the probability of getting a restriction site in a 4 kb long DNA fragment? Discuss

b. Which modifying enzyme help to avoid self ligation of vector cut with single restriction enzyme? Discuss the process in detail. Shed light how it can be useful in gene cloning? (5, 5)

P.T.O.

UNIT - II

Q5. a How does a Western blot differ from Northern blotting? When is a Western blot/hybridization used in preference to a Northern hybridization? Discuss the steps of western blotting/hybridization in brief. .

b. Discuss the non radioactive end labeling of a DNA probes with help of example and its advantage over radioactive labeling. Why is it sometimes desirable to reduce the stringency of probe hybridization reactions? Explain. (5, 5)

Q6. a. Which electrophoresis technique is used for separation of chromosomal DNA of various sizes? Discuss the principle and method of technique. Why an agarose gel electrophoresis cannot be used for this purpose? Discuss.

b. What is the difference between a deoxyribonucleotide and a dideoxyribonucleotide? Why dideoxyribonucleotide is used in Sanger's method of DNA sequencing? What will happen if very high or very low amount of ddNTPs are used in Sanger's method of DNA sequencing? Discuss. (5, 5)

Q7. Write in brief about

a. Gel retardation
b. Phage display

(5, 5)

x-x-x