BITS, PILANI, DUBAI
Dubai International Academic city
I Semester 2010-2011
Comprehensive Examination (Closed Book)

Course Title: Genetic Engineering Techniques **Maximum marks: 20**
Course No. BIOT C418 **Date: 28.12.2010** **Duration: 2 Hours**

Note: a) Attempt all questions in the order
b) Answer to the point

Q1. What is site directed mutagenesis? Explain Dut- ung method and altered site based mutagenesis. (Point wise) [3]

Q2.Name the most widely expression hosts .What is the uniqueness of these hosts? Describe. (Point wise) [1]

Q3.What is understood by Real time PCR? Why is it preferred over normal PCR? What are the different approaches to do RT-PCR .Briefly describe the SYBR Green approach. [3]

Q4. List out
(i) Various DNA modifying enzymes studied by you. Write one major function for each of these (Draw a table and write)
(ii) Different parameters that you will consider in designing and handling the Forward and reverse Primers in lab for amplifying gene using PCR. [2+1]

Q5. Describe the technique of SDS – PAGE (Different steps, Point wise). How is SDS PAGE different from Agarose gel electrophoresis? Explain [2]

Q6.How is detection and analysis carried out using Western Blotting? Explain briefly [2]

Q7.Why restriction digestion of genomic DNA isolated from *E.Coli* with *Eco R*I show a smear on agrose gel electrophoresis? [1]

Q8.Show schematically how *in vivo* and *ex vivo* gene therapy is performed [2]

Q9.Differentiate between the following (Point Wise)
(i) Genomic and cDNA library
(ii) Plasmids and phages
(iii) Insertional inactivation and alpha complementation
(iv) Ethidium bromide and commassie staining [2]

Q10.How is the gene transfer carried out using Ti plasmid, explain Briefly. [1]

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FIRST SEMESTER 2010-2011
TEST -2 (OPEN BOOK)

COURSE NO. BIOT C418 11-12-10 MAXIMUM MARKS: 15
GENETIC ENGINEERING TECHNIQUES DURATION: 50 Min

Q1. In a cloning experiment you wish to clone a rare gene from Dinosaur, you digest a source DNA and Vector DNA .The restriction digestion yield the ends that are found to be incompatible, this experiment is very important for you, how will you fix these incompatible ends? Explain (2)

Q2. After a cloning experiment using vector gt10, some of the plaques derived from the vector were slightly turbid while the others were clear, why? Explain the underlying concept. (2)

Q3.(i) A circular bacterial plasmid containing a gene for tetracycline resistance was cut with restriction enzyme *Bgl*II. Electrophoresis showed one band of 14 kb. What can be deduced from this result? (1)

(ii)The plasmid was cut with *Eco*RV and electrophoresis produced two bands, of 2.5 and 11.5 kb. What can be deduced from this result? (1)

(iii)Plasmid DNA cut with *Bgl*II was mixed and ligated with donor DNA fragments also cut with *Bgl*II, to make recombinant DNA molecules. All recombinant clones proved to be tetracycline sensitive. What can be deduced from this result? Explain (2)

Q4. What is the purpose of using DNA polymerase from *Thermus aquaticus* for PCR rather than a DNA polymerase from a better-characterized bacterium such as *E.coli*? (2)

Q5. You are studying DNA synthesis using a biochemical assay. Your assay system contains everything , DNA polymerase needs to synthesize DNA.
The double-stranded DNA molecule used for your assay has the following sequence:
5' AAATTGGGCCATCATTTCGAGTATTCGACTCCCTAGATCC… 3'
3' TTTAACCCGGTAGTAAAGCTCATAAGCTGAGGGATCTAGG… 5'
You denature the above molecule by heating (i.e. separate the double helix into two single-stranded DNA molecules), and cool it down in the presence of the primer 5'GGGAGTCGAAT 3' which base pairs (hybridizes) to one of the two strands. To this mixture you now add DNA polymerase, dATP, dCTP, dGTP, and dTTP and a new DNA strand is synthesized.
Write the complete sequence of the new single strand of DNA (including the primer sequence) that will be synthesized in the above reaction. Label the 5' and 3' ends of this sequence. (3)

Q6. At Arizona State University scientists working on genetic modification of various traits in plants transformed a gene that code for a seed protein .How can you detect the tissue specific gene expression in such transgenic plant ? Explain (2)

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FIRST SEMESTER 20010-2011
TEST -1 (CLOSED BOOK)
24.10.10

COURSE TITLE: GENETIC ENGINEERING TECHNIQUES DURATION: 50MIN
COURSE NO. : BIOT C418 MAXIMUM MARKS: 15

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Answer all the questions in the given sequence only.

Q1.What is the common strategy used to produce crops with delayed ripening and longer shelf life of fruits? Explain (2)

Q2.Differentiate between (Not more than two lines)

(i) Insertion and replacement vectors
(ii) Binary and cointergrate vectors
(iii) Direct method and vector mediated gene transfer.
(iv) Degradive and virulence plasmids (2)

Q3. What are competent cells? Why you need to make cells competent and how you do so, explain? (2)

Q4List out

(i) The critical requirements of Tissue culture technique and the advantages of tissue culture (2)

(ii) The essential features of a vector? (1)

(iii) The factors which influence sepration of DNA through Agarose gel electrophoresis? (1)

Q5 (i) What is the basic principle involved in isolation of plasmid by alkaline lysis method? Briefly explain. (2)

(ii) What is the Utility of MCS (multiple cloning sites) in a vector? (1)

Q6. Write short note on YAC vectors, Phage vectors (2)

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FIRST SEMESTER 2010-2011
QUIZ-1 (CLOSED BOOK)

COURSE NO. BIOT C418 MAXIMUM MARKS: 10
COURSE TITLE: GENETIC ENGINEERING TECHNIQUES DURATION: 20 Min

1. What is the basic difference between Isocaudomers, Isoschizomers and neoschizomers? .

2. What do you mean by star activity of an enzyme?

3. What is meant by unit definition?

4. For a given restriction digestion experiment, what would be the order of addition of various components?

5. Once the reaction is complete, why it is required to inactivate the restriction enzyme & how is the restriction enzyme inactivated?

6. Eloborate the following abbreviations and show how a restriction enzyme is named?
Alu I , *Bam*HI , *Pvu*I ,*Eco*R1

7. A plasmid of 5.8kb is digested with Restriction endonucleases, *Eco*RI and *Sal*I, separately and together .Upon gel electrophoresis the following pattern of restriction fragments was obtained.
*Eco*RI – 2.8, 2.0, 1.0kb *Sal*I – 5.0 &0.8

With both *Eco*RI & *Sal*I – 2.5, 1.5, 1.0, 0.5, 0.3kbp. Construct a restriction map using the information given above. 2

8. The restriction enzyme *Eco*RI cuts DNA at the sequence GTTAAC, and the enzyme *Hae*III cuts DNA at the sequence GGCC. On average, how frequently will each enzyme cut double-stranded DNA? (In other words, what is the average spacing between restriction sites?) 2