BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI
BITS PILANI – DUBAI CAMPUS, DIAC
FIRST SEMESTER 2011 – 2012 III YEAR B.E.(Hons.) BIOTECHNOLOGY
BIOT C418 GENETIC ENGINEERING TECHNIQUES
COMPREHENSIVE EXAMINATION (CLOSED BOOK)

Duration: 2hours **Date: 11.01.2012**
Weightage: 20% **Max. Marks: 20**

Note: a) Answer all questions, b) answer to the point and c) draw schematic diagram if required.

1. How the high level of protein expression is achieved with the help of T3 and T7 promoters in *E. coli*? Draw the mechanism with a suitable diagram. [2.0]
2. What are the requirements for setting up a restriction enzyme digestion? Mention the precautions to be taken. [1.0]
3. How the GST and His-tag fusion is used in the protein purification applications? Explain in detail with suitable diagram. [2.0]
4. Write a short note on any two methods of preparing RNase-free reagents. [2.0]
5. Explain DEAE cellulose and Silica matrices used for the isolation of plasmid DNA with suitable diagram. [3.0]
6. Write a short note on the following: [0.5+0.5+1=2.0]
   a. Why the plasmids undergo supercoiling in *E. coli*?
   b. Mention the enzymes involved in DNA supercoiling.
   c. How the supercoiling can affect the determination of molecular weight of plasmid DNA? Explain.
7. Why genomic DNA and plasmid DNA isolation show a smear on agarose gel? Justify your reasons. [1.0]
8. Explain the genome mapping with restriction enzymes with suitable diagram in detail. Mention the different applications. [2.0]
9. What are isozchizomers and neoschizomers? Mention any two enzymes for each. [2.0]
10. What are reverse transcriptases? [1.0]
11. Briefly explain on PCR and the requirements. [1.0]
12. What are the different applications of genetic engineering techniques in biotechnology? Give schematic diagram of the strategy followed. [1.0]

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TEST-II (OPEN BOOK)

Duration: 50 min. **Date: 18.12.2011**
Weightage: 15% **Max. Marks: 15**

Note: a) Answer all questions b) answer to the point and c) draw schematic diagram if required.

1. How will you clone a DNA fragment with a vector having different restriction sites? Explain at least any two types of cloning strategies with suitable diagram you may follow. [1+3=4.0]
2. How will you identify the methylation, and CpG methylation patterns in genomic DNA? Explain in terms of methylation, dcm+/-, dam+/- methylases and restriction enzymes and DNA stability. What will you do if you want to make a genomic DNA library with methylated DNA. [1+3=4.0]
3. How the restriction enzyme mapping is applied in identifying the gene of interest in a genomic DNA digested with restriction enzyme? Explain with a schematic representation of the strategy you follow. [4.0]
4. What are the different types of polymerases used in genetic engineering techniques? How the polymerases are applied in DNA cloning strateries of PCR products? Explain with schematic representations. [3.0]

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TEST-I (CLOSED BOOK)

Duration: 50 min. **Date: 23.10.2011**
Weightage: 15% **Max. Marks: 15**

Note: a) Answer all questions b) answer to the point and c) draw schematic diagram if required.

1. What are the advantages of submarine gel systems and mention how DNA and RNA can be separated? [1.5]
2. What are the difference between TAE and TBE buffer for DNA electrophoresis? [1.0]
3. What is cationic shielding of DNA and mention its applications in DNA isolation? Mention different reagents used. Draw a schematic diagram on the principle of shielding effect. [1.5]
4. Write a short note on plasmid DNA supercoiling? [1.0]
5. What are the importance of adding antibiotics to the growing cultures and mention the problems associated with over incubation (>18h) of *E. coli* with plasmid DNA. [1.0]
6. Describe the principle involved in alkaline lysis method in detail. [2.0]
7. Write a short note on the plasmid DNA isolation by Cesium chloride density gradient centrifugation. Draw schematic diagrams. [2.0]
8. What are the difference between the CsCl centrifugation and the DNA isolated by the other standard protocols. [2.0]
9. Write any two cloning vectors you are familiar with and general properties. [1.0]
10. Develop a method of gene cloning procedure for your desired gene (name the gene of interest) and mention the reason for cloning and its applications with the knowledge you have gained so far from the genetic engineering techniques. [2.0]

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QUIZ-II (CLOSED BOOK)

Duration: 20 min. **Date: 7.12.2011** **Max. Marks: 5**

Name:

ID No:

Note: Answer to the point

1. Name any two fusion proteins for protein expression and principle of protein isoloation associated with the fusion proteins. [1.0]

2. What is the use of alkaline phosphatase in cloning strategies? If alkaline phosphatase is not used, what will happen? [1.0]

3. How foreign DNA are introduced in plant cell? Give the principle. [1.0]

4. What is the principle of making Genomic DNA library? [1.0]

5. What are the basic requirements in expression vector for protein expression in *E. coli*? [1.0]

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QUIZ-I (CLOSED BOOK)

Duration: 20 min. **Date: 28.9.2011** **Max. Marks: 5**

Name:
ID No:

Note: Answer to the point

1. Write the ligation reaction with *Bam*HI and *Bgl*II with specific recognition sites and results. [1.0]

2. What are the possible contaminants in culture, DNA and cloning procedures and how it affects the recombinant clones. [1.0]

3. What are the different modification of a plasmid by the host cell and mention what precautions will prevent such DNA modification. [1.0]

4. What are the different techniques to inactivate the enzyme RNases for your laboratory reagents? [1.0]

5. How the DNA, RNA, protein samples and cells are stored? What precautions to be taken for long term applications. [1.0]