BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI – DUBAI CAMPUS
DUBAI INTERNATIONAL ACADEMIC CITY
FIRST SEMESTER 2013 – 2014
BIOT F241 GENETIC ENGINEERING TECHNIQUES
COMPREHENSIVE EXAMINATION (CLOSED BOOK)

Duration: 2h. **Date: 6.1.2014**
Weightage: 20% **Max. Marks: 20**

Note: a) Answer all the questions b) answer to the point and c) draw suitable diagrams.

1. Why RNase free reagents are required? Explain the different methods of preparation of RNase free reagents. [2.0]

2. Explain any one method of determination of the size of DNA? Give suitable diagram and the principle involved. [2.0]

3. Briefly explain on nucleic acid solubility, cationic shielding, phenol-chloroform extraction and nucleic acid precipitation. [2.0]

4. Explain the principle of bacteriophage T7 promoters based protein expression in *E. coli*? Give suitable diagram. [2.0]

5. Explain with suitable diagrams on any one method and applications of positive selection? [1.5]

6. Explain any two method and principles of affinity capture of fusion proteins. Give suitable diagrams. [2.0]

7. How high purity plasmid DNA is isolated using DEAE cellulose and silica matrices? Give suitable diagram. [3.0]

8. What are BAC and YAC and compare with plasmids. [1.5]

9. How restriction enzyme reaction is setup and inactivation is achieved and why? [1.5]

10. Explain any two applications of genetic engineering techniques in biotechnology? Give schematic diagram of the strategy followed. [1.0]

11. *E. coli* is most preferred host in molecular biology and compare it with *Agrobacterium tumefaciens*? Give suitable diagrams and mechanisms of approach for each. [1.5]

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BIOT F241 GENETIC ENGINEERING TECHNIQUES
TEST-II (OPEN BOOK)

Duration: 50 min. **Date: 8.12.2013** **Max. Marks: 15**

Note: (a) Answer all the questions
(b) Answer to the point

1. a) Why do you need a protein fusion in gene expression using recombinant protein production techology?

   b) What principle such tags are chosen and explain any three such tags with respect to large scale industrial application and high pure proteins for pharmaceutical application?

   c) What are the other uses of such tags associated with the target protein in genetic engineering technology? Provide schematic diagrams. [1+2+1=4.0]

2. Can you use shuttle vectors for plant cell transformation? If so briefly explain why do you need shuttle vector, and how will you transform a plant cell? Explain the strategies and methods with suitable diagrams. [3.0]

3. a) Why the genes controlling the expression of a protein from the plasmid vector is placed in the chromosomal DNA of the *E.coli* host? Explain in detail with suitable diagram.

   b) If you place such controlling element on the plasmid vector what are the consequences on target protein expression? [2+2=4.0]

4. a) 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. [2.0]

   b) What will you do if you want to make genomic DNA library with methylated DNA? [2.0]

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TEST-I (CLOSED BOOK)

Duration: 50 min. **Date: 13.10.2013** **Max. Marks: 15**

Note: (a) Answer all the questions
(b) Answer to the point

1. Why the selection of restriction enzymes in cloning methods is important and mention how it may impact your insert DNA if you wish to subclone (cut and paste) into any other plasmid vectors. Briefly explain with an example. [3.0]

2. What are the possible modifications of a plasmid by the host cell? Explain. [2.0]

3. How the size, shape of DNA and percentage gel affect the agarose gel electrophoresis and how it is significant in looking at DNA on a gel? Explain. [3.0]

4. What are PCR, RNA and tissure culture labs and explain in terms of genetic engineering techniques aspect? [3.0]

5. Why cationic shielding is required for DNA precipitation methods and mention the principle with suitable examples? [2.0]

6. Why the agarose gel are otherwise called as submarine gel electrophoresis and mention the most commonly used buffer systems with their advantages and disadvantages. [2.0]

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QUIZ-II (CLOSED BOOK)

Duration: 20 min. **Date: 19.12.2013** **Max. Marks: 5**

Note: (a) Answer all the questions
(b) Answer to the point

1. The type-II restriction endonuclease sites are palindromic in nature. Briefly explain with one example. [1.0]

2. HaeII and PhoI restriction enzymes are _____ because both digest DNA at the sequence GG/CC leaving a blunt end. [1.0]

3. EcoRI under wrong pH or in low ionic strength may recognize the more general N/AATTN sequence. This additional enzyme activity of EcoRI is known as _____. [1.0]

4. Define a unit restriction enzyme activity. [1.0]

5. Do you inactivate the restriction enzymes before cloning/ligation? If so how you do it (mention any one method). [1.0]

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QUIZ-I (CLOSED BOOK)

Duration: 20 min. **Date: 30.10.2013** **Max. Marks: 5**

Note: (a) Answer all the questions
(b) Answer to the point

1. What is the principle of silica and DEAE cellulose based plasmid DNA isolation and mention the applications of such DNA preparation? [1.0]

2. How the plasmid DNA isolation using CsCl centrifugation method is achieved and mention the methods to remove the salts and EtBr from the DNA? [1.5]

3. Mention any two most commonly used plasmid "Ori" and why such Ori is preferred in cloning vectors. [1.5]

4. What is the use of selection markers and MCS in plasmids and briefly outline their general properties? [1.0]