BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI – DUBAI CAMPUS
DUBAI INTERNATIONAL ACADEMIC CITY
FIRST SEMESTER 2012 – 2013
BIOT F241/BIOT C418 GENETIC ENGINEERING TECHNIQUES
COMPREHENSIVE EXAMINATION (CLOSED BOOK)

Duration: 2h. **Date: 8.1.2013**
Weightage: 20% **Max. Marks: 20**

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

1. What are RNase free reagents and how it is made? Mention why such reagents are required in molecular biology? [2.0]

2. How the size of an unknown DNA fragment is determined? Mention the role of agarose and polyacrylamide gel electrophoresis in DNA size determination? Explain with a suitable diagram. [2.0]

3. Write a short note on the following techniques and their use in standard protocols: [3.0]
   a. Nucleic acid solubility
   b. Cationic Shielding
   c. Phenol extraction
   d. Nucleic acid precipitation methods

4. Briefly explain with suitable diagrams on method and applications of positive selection and its applications? [1.5]

5. What is affinity matrix capture of fusion proteins? Explain the principle and application with a suitable diagram. [1.5]

6. What are phagemids? Differentiate with plasmids. [1.5]

7. Why restriction enzyme inactivation is required and mention the different methods used? [1.5]

8. What are polymerases? Briefly explain on any three types of polymerases and their applications. [2.5]

9. What are the applications of southern and northern blotting techniques? Briefly outline the principle and the method. [2.5]

10. Why *E. coli* is most preferred host in molecular biology laboratory protocols and list other microbes used in genetic engineering? Justify will you prefer *E. coli* or other bacterial host? If so what will you do to make the bacteria as your desired host for genetic engineering? Explain with suitable reasons and diagrams. [2.0]

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TEST-II (OPEN BOOK)

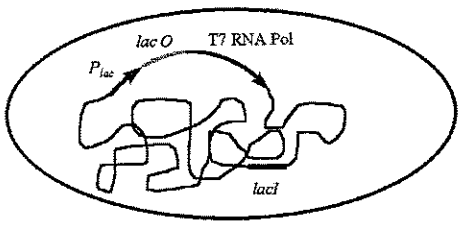
Duration: 50 min. **Date: 13.12.2012**
Weightage: 15% **Max. Marks: 15**

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

1. Plant transformation mediated by *Agrobacterium tumefaciens*, a soil plant pathogenic bacterium, has become the most widely used method for the introduction of foreign genes into plant cells and the subsequent regeneration of transgenic plants. *A. tumefaciens* naturally infects the wound sites in dicotyledonous plant causing the formation of the crown gall tumors. Virulent strains of *A. tumefaciens* when interacting with susceptible plant cells, induce diseases known as crow gall and hairy roots, respectively.

   a. List the different stages of *A. tumefaciens* based gene transfer to the plant cells with a schematic diagram. [1.5]
   b. Why the Virulent genes are required in gene transfer and briefly explain. Mention the application of non-virulent plasmids in *A. tumefaciens.* [1.0]
   c. Due to the climate change and water shortage, drought and shortage of rain fall affect severely the productivity in most of the rice growing regions in the world. Provide a strategy for transfer of a drought tolerance gene with a size of 3kb DNA sequence and essentially codes for the protein superoxide dismutase which confer both drought tolerance and free radical scavenging activity. The gene is originally isolated from a desert adapted plant species *Prosopis juliflora.* The target plant is rice which require high amount of water. Explain the mechanism of gene transfer and methods you will adopt to get a successful technique for large scale application. [2.5]

2. One of the most important parts of the pET Expression system involves the fact T7 RNA polymerase is present. Prokaryotic cells do not produce this type of RNA, and therefore the T7 RNA polymerase must be added. Usually the host cell for this expression system is a bacteria which has been genetically engineered to incorporate the gene for T7 RNA polymerase, the *lac* promoter and the *lac* operator in its genome. When lactose or a molecule similar to lactose is present inside the cell, transcription of the T7 RNA polymerase is activated. The diagram below shows the genome of the host cell.



BL(DE3) Host Chromosome

   a. Why the *lacI* is placed in the genome of the E. coli unlike other plasmids (eg., pUC19/pUC18) in which the *lacI* is constructed within the plasmid to be recognized by the host cells? [1.5]
   b. In case you wish to incorporate the T7 RNA Polymerase in the plasmid itself, how will you design the expression plasmid for recombinant protein expression? [2.0]
   c. How will you target the genes in the genome of *E. coli* without affecting the functional genes in the host genome? [1.5]

3. The methylation of cytosine bases in DNA provides a layer of epigenetic control in many eukaryotes that has important implications for normal biology and disease. Therefore, profiling DNA methylation across the genome is vital to understanding the influence of epigenetics. DNA methylation is an important regulator of gene transcription and a large body of evidence has demonstrated that genes with high levels of 5-methylcytosine in their promoter region are transcriptionally silent, and that DNA methylation gradually accumulates upon long-term gene silencing. DNA methylation is essential during embryonic development, and in somatic cells, patterns of DNA methylation are generally transmitted to daughter cells with a high fidelity. Aberrant DNA methylation patterns have been associated with a large number of human malignancies and found in two distinct forms: hypermethylation and hypomethylation compared to normal tissue. Hypermethylation typically occurs at CpG islands in the promoter region and is associated with gene inactivation. Global hypomethylation has also been implicated in the development and progression of cancer through different mechanisms.

   a. How will you select the genomic DNA for methylation analysis? Will you isolate from the cell line or from the *E. coli* genomic DNA library for methylation analysis? Briefly explain your decision and why? [1.5]
   b. How will you compare the methylation patterns? Briefly explain with a suitable diagram (assuming you have treated the DNA samples and run the gel). The patterns of separated fragments with respective enzymes on each *lanes*). [2.5]
   c. Why methylation analysis is required? Mention the applications. [1.0]

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TEST-I (CLOSED BOOK)

Duration: 50 min. **Date: 21.10.2012**
Weightage: 15% **Max. Marks: 15**

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

1. Why molecular biology enzymes, buffers, DNA and RNA are stored at low or ultra low temperatures and mostly in liquid conditions? List the components used the possible reasons. [2.0]

2. Why the DNA is acidified during alcohol precipitation and mention the desired molar concentration of salts used? Briefly explain the principle with suitable diagram. [2.0]

3. Why most reagents are prepared as stock solutions in genetic engineering experiments? What precautions which you will be taking with respect to stock solutions in the lab? [2.0]

4. Write a short note on the following:
   a. Why the plasmids undergo supercoiling? [1.0]

   b. Mention how the different forms of plasmid DNA is formed in the cell and appear as different plasmid bands in the agarose gel? [1.5]

   c. What will happen if the process of supercoiling is inhibited and mention the application. [0.5]

5. Explain the alkaline lysis method with respect to the role of each reagent component in the DNA isolation protocol. [2.0]

6. Briefly explain the following:
   a. Why do you need high pure DNA? [1.0]

   b. Two methods of DNA binding methods and principles involved with suitable diagrams. [2.0]

   c. Advantages and limitations of such methods over conventional DNA isolation protocols. [1.0]

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QUIZ-II (CLOSED BOOK)

Duration: 20 min. **Date: 19.12.2012**
Weightage: 5% **Max. Marks: 5**

Name:

ID No:

Note: a) answer all the questions and b) answer to the point

1. What are the applications of restriction mapping of plasmid DNA and Genomic DNA? [0.5]

2. What type of restriction enzyme you may select? Methylation sensitive or methylation insensitive? Justify your reasoning with suitable examples. [0.5]

3. You wanted to clone a newly isolated plasmid DNA from camel milk samples designated as pBIOT2020. The size of the plasmid is 7.0 kb in length and has single PstI, EcoRI, and BamHI sites. You have cut the plasmid with PstI and inserted a 4.0 kb fragment into the site for replication in *E. coli* for further analysis. From the data below, determine the restriction map of the resulting plasmid.

   PstI 7.0 4.0
   EcoRI 6.0 5.0
   BamHI 8.9 2.1
   PstI + EcoRI, 4.3 3.3 2.7 0.7
   PstI + BamHI 6.1 2.8 1.2 0.9
   EcoRI + BamHI 5.0 2.1 2.1 1.8

   a. How will you approach to solve the restriction analysis and mapping the plasmid DNA? [1.0]

b. What are the possible orientations you may select for positioning the restriction sites? [1.5]

c. Determine the restriction map with restriction enzyme position and size of each fragment on the DNA map. [1.5]

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QUIZ-I (CLOSED BOOK)

Duration: 20 min. **Date: 9.10.2012**
Weightage: 5% **Max. Marks: 5**

Name:

ID No:

Note: a) answer all the questions and b) answer to the point

1. What are plasmids and mention the basic characteristics of cloning and expression vectors? [0.5]

2. What is restriction enzyme incompatibility in a cloning experiment? Outline with any one example. [0.5]

3. Mention the different modifications of a plasmid by the host cell and what precautions will prevent such DNA modification. [0.5]

4. What are the possible routes of contamination in a cloning experiment? Mention any two in detail. [0.5]

5. What are the problems you may encounter if the DNA is not pure in a cloning experiment? [0.5]

6. What precautions will you observe when using micropipettes? List all possible steps for its handling and storage. [0.25]

7. What precautions will you undertake during pippetting small volumes using a 10 microliter/ 2microliter pipettes? [0.25]

8. What type of water is generally preferred in molecular biology experiments? Mention the type of water quality required and why? [0.25]

9. Why do you need to heat treat the RNase in plasmid DNA isolation? If not what will happen? [0.5]

10. What is smiling effect in agarose gel? Mention the different factors which cause this phenomenon. [0.5]

11. What is EEO and how this is overcome in experiments. [0.25]

12. TAE or TBE? Which one you will prefer and reason out with your justifications? [0.5]