

BITS PILANI, DUBAI CAMPUS  
SECOND SEMESTER 2011-2012  
COMPRE EXAM (Closed BOOK)

Course No.: BIOT C461

14.06.12

Max. Marks: 40

Course Title: Recombinant DNA Technology

Max. Time: 3Hrs

Note: Answer the questions in a sequence

- Q1a. Demonstrate how Site specific recombination allows precise manipulation of the genome in organisms where gene targeting is inefficient. (Explain and Draw a suitable diagram) [4]
- b. What are scorpion probes? (Draw a suitable diagram). [3]
- c. . Why animals are preferred models for transgenic technology? [3]
- Q2a. The mode of plasmid replication can affect the stability of the cloning vectors. Justify. [2]
- b. How can we obtain a double stranded DNA from an mRNA template? (Explain and Draw a suitable diagram) [3]
- c. Explain the CAPture method for full length cDNA cloning. (Explain and Draw a suitable diagram) [3]
- d. Give the principle of pyrosequencing. [2]
- Q3a. Explain the colony hybridization method for screening recombinant clones. (Explain and Draw a suitable diagram) [3]
- b. Plant cells are extremely pliable and can be interconverted and regenerated in a culture. Justify with a suitable diagram. [3]
- c. Differentiate between transient and stable transfection. [3]
- d. Discuss the significance of the following [2]  
i. alkaline phosphatase, ii. Linkers
- Q4a. Justify, "phage that survives one cycle of growth upon restrictive host can subsequently reinfect the same host efficiently". [3]
- b. Give the salient features of plasmid pBR322. [3]
- c Diagrammatically explain the antisense mechanism for regulating the plasmid copy number. [3]

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BITS PILANI DUBAI CAMPUS  
SECOND SEMESTER 2011-2012  
TEST- 2 (Open BOOK)

Course No.: BIOT C461  
Course Title: Recombinant DNA Technology

20.05.12

Max. Marks: 20  
Max. Time: 50 mins

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Note: Only the **prescribed text book** and **hand written notes** are allowed.

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- Q1a. How can DNA be transfected into an animal cell via the process of endocytosis? [2]
- b. Which is the most popular vector for cloning transgenes into plants? Can this vector be used to introduce genes in maize plants? Justify your answer. [3]
- c. Explain with examples how we can prevent a recombinant cell expressing a transgene from experiencing a 'metabolic drain'. [3]
- d. What are reporter proteins? Give examples of two reporter proteins that are used in analysis of a transgene introduced into transgenic animals. [3]
- Q2a. What is meant by transient transfection? Mention the advantages and disadvantages of the same. [3]
- b. What is somaclonal variation? Mention the advantages and disadvantages of the same. [3]
- c. Justify the significance of HAT medium. [3]

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BITS PILANI DUBAI CAMPUS  
SECOND SEMESTER 2011-2012  
TEST- I (CLOSED BOOK)

Course No.: BIOT C461                      01.04.12                      Max. Marks: 25  
Course Title: Recombinant DNA Technology                      Max. Time: 50 mins

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Answer all the questions in the given sequence.

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- Q1. Differentiate between [8]  
i. relaxed and stringent plasmids.  
ii. insertion and replacement vectors of  $\lambda$  phage
- Q2. Diagrammatically explain how LITMUS vectors are used for forming RNA probes. [3]
- Q3. Explain how RepA protein influences the copy number of plasmids. [4]
- Q4. What is the significance of M13 phage? How can we modify the phage DNA to construct a better vector? [3]
- Q5. Explain the CAPture method of cDNA cloning. [3]
- Q6. Explain the strategy for screening the cDNA clones on basis of biochemical or physiological activity of the gene product. [2]
- Q7. What are the two problems associated with gene manipulation? [2]

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BITS PILANI DUBAI CAMPUS  
SECOND SEMESTER 2011-2012  
QUIZ- 2 (CLOSED BOOK) ANSWERKEY

Course No.: BIOT C461                      1.05.12                      Max. Marks: 07  
Course Title: Recombinant DNA Technology                      Max. Time: 20 mins

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- 1. How can we enhance the sharpness of an autoradiographic image? [1]**  
The sharpness of the autoradiographic image is improved by replacing  $^{32}\text{P}$ -radiolabel by  $^{33}\text{P}$  or  $^{35}\text{S}$ -radiolabels. This is achieved by including an  $\alpha$ - $^{35}\text{S}$ -deoxynucleotide triphosphate in the sequencing reaction.
- 2. Justify why *E.coli* could fail as a host for gene cloning.**  
Use of *E.coli* is not always practicable because it lacks some auxiliary biochemical pathways that are essential for the phenotypic expression of certain functions, eg: degradation of aromatic compounds, antibiotic synthesis, pathogenicity, sporulation, etc.
- 3. What is polymerase slippage? [1]**  
Polymerase slippage is a term that refers to the inability of the *Taq* polymerase to incorporate the correct number of bases when copying runs of 12 or more identical bases.
- 4. Suggest an alternative DNA uptake mechanism for cells where transformation is difficult. What are the essential features of this mechanism?**  
Plasmid transformation is difficult in many bacteria and hence conjugation can be used as an alternate. The essential features for conjugation are the self transmissible plasmids with tra genes and conjugative apparatus.
- 5. How can we favour the integration of the plasmid vector into the host cell?**  
It is possible to favor integration by transferring a plasmid into a host in which it cannot replicate and selecting for a plasmid-born marker.
- 6. What is the significance of capillary array electrophoresis? [2]**  
Capillary electrophoresis is performed in high purity fused silica capillaries with an ID of 50  $\mu\text{m}$ . These capillaries are not prone to Joule Heating even when high electric fields are applied to obtain rapid separation of DNA fragments.  
They are flexible and can be easily incorporated into automated instruments and are available pre-filled with gel matrix.  
The product of a sequencing reaction is applied to the top of a capillary gel.  
The labeled DNA fragments migrate through the capillary in a vertical stream and are detected at the end.

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5. What is a major difference between the Type I and Type II R-M systems? [1]

6. What is the role of the methylase encoded by *dcm* gene (Dcm methylase)? [1]

7. What are adaptor molecules? [1]

8. Why are Lectins used in Western blotting? [1]