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JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT  
TEST -3 EXAMINATION- 2021

B.Tech Vth Semester

COURSE CODE: 18 B11 BT 512 / 16 B11 BT 513

MAX. MARKS: 35

COURSE NAME: Genetic Engineering

COURSE CREDITS: 03

MAX. TIME: 2 Hours

*Note: All questions are compulsory. Carrying of mobile phone during examinations will be treated as a case of unfair means.*

Q.1 Briefly answer any six of the following questions in not more than four lines:

- Mention specifically two methods used in DDRT- PCR to reduce the number of fragments to be analyzed on gel to identify differentially expressed genes?
- How ddNTPs used in sangers sequencing act as chain terminators?
- Figure out any two basic differences between cell based and PCR based gene cloning?
- Why do PCR products require special methods for cloning?
- Give two reasons to support the argument that genomic DNA libraries are good enough to isolate genes from a prokaryotic organism?
- In which of two steps and how poly A tail feature of eukaryotic mRNA is used in construction of cDNA library?
- How does GC content of transgene and host affect the level of protein expression?

Q.2

6x2=12

Suppose you are working on isolation human gene coding for the protein actin. You also know the amino acid sequence of human actin. You have available to you a human cDNA library, purified antibodies specific for the protein actin, and all the supplies and equipment needed to perform the techniques that we discussed in class. Outline two different experimental approaches to isolate the actin gene from cDNA library. 5

Q.3

- Let you are trying to express a recombinant protein in E.coli. During the experimentation you came to know that recombinant protein is accumulating in the form of inclusion bodies. Suggest and explain with examples three strategies which can be adopted to express this protein in *E. coli* in biological active form. 4
- What are linkage maps? Mention two specific points where linkage maps, information is used in whole genome sequencing via Hierarchical shotgun sequencing.  
or  
Give a list of gene transfer methods used to transfer genes to microbial, animal and plant cells. 2

P.T.O

Q.4

a. What are major differences between Sanger's original method of DNA sequencing and the fully automated version? Explain main modifications which lead to its automation. 4

b. Enlist the three universal steps of Next generation sequencing methods. How is the library amplified in the case of the 454 NGS platform? 3

Q.5

a. In context to cDNA library construction, diagrammatically outline a method to synthesize cDNA so that it can be cloned directionally in a cloning vector? 3

b. Calculate the minimum number of clones required in a gene library of *rice*? Given genome size  $5.7 \times 10^2$  MB, fragment size 20 KB, desired probability of finding the fragment 0.99. 2