2015

BIOCHEMISTRY

Paper – BCT – 203

(Recombinant DNA Technology)

Full Marks – 25

The figures in the margin indicate full marks

Candidates are required to give their answers in their own words as far as practicable

Group – A

Answer ***any three*** questions

1. A linear phage chromosome is labelled at both ends with 32p and digested with restriction enzymes. EcoRI produces fragments of sizes 2.9, 4.5, 6.2, 7.4 and 8.0 Kb. An autoradiogram developed from a southern blot of this digest shows radioactivity associated with the 6.2 and 8.0 Kb fragments BamHI cleaves the same molecule into fragments of sizes 6.0, 10.1 and 12.9 Kb and the label is associated with the 6.0 and 10.1 Kb fragments. When EcoRI and Bam HI are used together, fragments of sizes 1.0, 2.0, 2.9, 3.5, 6.0, 6.2 and 7.4 Kb are obtained

(a) Draw a restriction-enzyme target site map of this molecule, showing relative positions and distance apart.

(b) A radioactive probe made from a cloned phage gene X is added to southern blots of single-enzyme digest of phage DNA. The autoradiograms showed hybridizations associated with the 4.5, 10.1 and 12.9 Kb fragments. Draw in the approximate location of gene X on the β restriction map. $3\frac{1}{2}+1\frac{1}{2}$

2. (a) Give one example for each of the following enzymes : 1×2

(i) RNA dependent DNA polymerase

(ii) Template independent DNA polymerase.

(b) How will you incorporate two different restriction enzymes sites at two different ends of a C-DNA ? 2

(c) The restriction enzyme Hind III cuts DNA at the sequence 5′AAGCTT3′. On average how frequently will this enzyme cut double stranded DNA ? 1

3. (a) Distinguish between mode of action of T_4 DNA ligase and *E.Coli* DNA ligase.

(b) How many clones are needed to establish a gene library for human DNA (size 2.8×10^6 Kb) using fragments of average 20Kb, if one wishes 97% of human genes to be in the library ?

(c) You fused *dhfr* with the gene encoding tPA and cloned into an expression vector and transformed into $dhfr^-$ cells. How do you obtain high level expression of gene encoding tPA ? $1\frac{1}{2}+2+1\frac{1}{2}$

4. (a) Discuss briefly how you produce and select the variants of human growth hormone with increased affinity for growth hormone receptor.

(b) Give a strategy for the production of human growth hormone in the milk transgenic cow. $2\frac{1}{2}+2\frac{1}{2}$

5. (a) How do you transfer a bacterial enzyme expressed in cytoplasm of plant cells to chloroplasts of those cells ?

(b) What is meant by somatic embryogenesis ?

(c) Show schematically how you transfect plant cells by gene gun method.

(d) Write down the steps involved in liposome mediated transfection of plasmid DNA into BHK21 cells. $1+1+1\frac{1}{2}+1\frac{1}{2}$

6. (a) What properties must two different restriction enzyme possess if they yield identical patterns of band ?

(b) Distinguish between Ti-plasmid and T-DNA.

(c) Define restriction enzyme and state the characteristic features of type-II endonucleases. $1+1\frac{1}{2}+1+1\frac{1}{2}$

Group – B

Answer ***any two*** questions

7. Name the variables that are critical while designing PCR primers and mention contribution in a successful PCR reaction. Write down the steps one need to follow while making genomic DNA library using PCR. 3+2

8. What are the limitations of Sanger's dideoxy sequencing ? How several of these limitations may be resolved by the NextGen sequencing platforms ? Write your answer giving example of any one platform. 2+3

9. Write down the purpose and steps involved in following methods (***any two***) : $2\frac{1}{2}+2\frac{1}{2}$
   (a) S1 mapping
   (b) Nuclear Run-on transcription
   (c) Quantitative RT-PCR

10. Write down any one method that you would like to employ while studying DNA-protein interaction and protein-protein interaction. 2+3