2021

MICROBIOLOGY — HONOURS

Paper : CC-10

(Recombinant DNA Technology)

Full Marks : 50

The figures in the margin indicate full marks.

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

Answer ***question no.* 1** and ***any three*** from the rest.

1. Answer ***any ten*** questions : 2×10

(a) Which type of restriction endonucleases are used in RDT and why?

(b) What are shuttle vectors? Explain with a suitable example.

(c) Why blocking step is performed in blotting experiments prior to hybridization with probe?

(d) Why T4 DNA ligase is preferred to *E.coli* DNA ligase in cloning of gene?

(e) Why genomic DNA is partially digested during genomic library preparation?

(f) What are recombinant subunit vaccines? Give an example.

(g) Which bacterium carries Ti plasmid? What disease do they cause in plants?

(h) Give an example of baculovirus based vector and mammalian SV-40 based expression vector.

(i) What features should be present in an expression vector?

(j) Why denaturation of DNA is required before setting up transfer in southern Blot?

(k) Write two differences between agarose gel electrophoresis and SDS-PAGE.

(l) Give two differences between YEP and YIP vectors.

(m) Why heat shock is performed in artificial transformation?

(n) What would be the expected effect on a PCR reaction, if the primers used are too long?

(o) Differentiate between a probe and a primer.

2. (a) A 6kb plasmid DNA molecule is digested with different restriction enzymes either singly or in combinations. Construct the restriction map and label the size of DNA fragments between the restriction sites. The digested products are —
EcoRI → 6kb
BamHI → 6kb
Hind III → 1·5kb and 4·5kb
EcoRI + BamHI → 2·5kb and 3·5kb
EcoRI + HindIII → 1kb, 0·5kb and 4·5kb
BamHI + HindIII → 2·5kb, 2kb, 1·5kb

(b) Blunt end ligations are difficult to perform. How can you convert blunt ends of an insert to sticky ends for efficient ligation during gene cloning?

(c) What is codon bias? How can it have any effect on production of recombinant proteins? 4+3+(1+2)

3. (a) Explain "alpha-complementation" and how it helps to identify a true recombinant clone.

(b) What is Bt cotton? Explain how they have been engineered.

(c) With a proper diagram, explain the basic steps of PCR. Mention the conditions.

(d) Name the two antibiotic resistance genes present in pBR322 vector. 3+(1+2)+3+1

4. Write short notes on (***any four***) : 2½×4

(a) Shotgun sequencing

(b) Gene therapy

(c) Chromosome walking

(d) Colony PCR

(e) Biolistic method of gene delivery

(f) Dot-blot.

5. (a) What are isoschizomers and neoschizomers? Give examples.

(b) Explain how linkers and adaptors help in the cloning of inserts in any vector.

(c) Explain why Sanger's sequencing method is called 'chain termination method'.

(d) Give an example of restriction enzyme that produces— (i) 5′ overhang and (ii) 3′ overhang. Mention the overhangs they generate. (1+1)+(1½+1½)+3+(1+1)

6. (a) Why cosmids are preferred to lambda vectors for genomic library preparation?

(b) How can you minimize non-specific hybridization in a Southern blotting experiment?

(c) State the role of following reagents in SDS-PAGE :

(i) Glycine (ii) SDS (iii) APS.

(d) How can you measure the purity of isolated plasmid DNA? 3+3+(1×3)+1

7. (a) Describe the process of production of 'Recombinant Insulin', an enzyme of high therapeutic interest.

(b) Mention the advantage(s) of 'Microarray analysis' over 'Northern Blot analysis'.

(c) Schematically present the synthesis of c-DNA library of any eukaryotic cell.

(d) Which host should be used for production of recombinant protein, if the gene has been cloned in a vector having T7 promoter? 3+2+3+2