2021

BIOCHEMISTRY — HONOURS

Paper : SEC-B-2

(Recombinant DNA Technology)

Full Marks : 80

The figures in the margin indicate full marks.

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

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

(a) Differentiate between synthetic and complex media.

(b) Define : Mixed culture, Pure culture, Strain and Clone.

(c) Why is it important to adjust pH of a bacteriological medium?

(d) How can a single colony of *E.coli* be isolated?

(e) What is plasmid DNA? Give two examples.

(f) What is PCR? What is its importance?

(g) What is a selective marker?

(h) Why are type II restriction endonucleases important in Recombinant DNA Technology?

(i) Name any two enzymes that are essential for cloning and mention their function in cloning.

(j) What are the primary ingredients required in a medium for growth of bacteria?

(k) What is vector DNA ? What is its utility?

(l) Calculate the dilution factor if 5ml of bacterial culture is diluted with 45ml of LB broth.

2. Answer ***any four*** questions :

(a) What is a primer? What important characteristics of a primer should be ensured while designing it for PCR? 1+4

(b) What is the principle of plasmid DNA purification by the alkaline lysis method? Explain how the components of Solution II and Solution III help in this process. 2+3

(c) Distinguish between Spread plate and Pour plate Technique. 2½+2½

(d) Name 5 types of enzymes used in Recombinant DNA technology and write down their uses. 1×5

(e) How can bacterial cells be made competent artificially? What is the difference between a competent and a transformed cell? 3+2

(f) (i) Why does electrophoresis of undigested plasmid DNA give multiple bands, no bands or faint bands?

(ii) What are the three steps to PCR thermocycling? (1+1+1)+2

3. Answer ***any four*** questions :

(a) (i) Define slant culture and stab culture.

(ii) Distinguish between DNA pol I and klenow fragment.

(iii) What do you mean by ligation? Name the enzyme that can perform this function. (2+2)+(2+2)+2

(b) (i) What is the function of the restriction and modification system of bacteria? What are the different types of restriction and modification systems?

(ii) Name and mention the application of any two techniques that use the principle of nucleic acid hybridization. Briefly describe any one method and mention its application. 2+3+2+3

(c) (i) What is meant by star activity of a restriction enzyme? Give an example.

(ii) Write down the importance of blue white screening.

(iii) How would you distinguish between different forms of plasmid DNA by gel electrophoresis?

(iv) Explain why glycerol is added in the storage buffer of enzymes used in Recombinant DNA Technology. 2+3+3+2

(d) (i) How would you prepare Recombinant DNA by sticky end ligation and how would you select the recombinants ?

(ii) A 8 kb DNA fragment has been inserted into the plasmid pBR322 at a EcoRl site. The recombinant plasmid is cut with 3 different restriction enzymes. The fragments obtained following electrophoresis on agarose gels are

EcoRI : 8 and 6 kb

Hind III : 5. 5, 4.5 and 4.0 kb

Hind III + EcoRI : 4.0, 3.5, 3.0, 2.5 and 1 kb.

(A) Draw the restriction map of recombinant plasmid.

(B) If a Southern Blot is prepared from the gel, which fragments will hybridise to a probe of pBR322 plasmid DNA? Explain your answer. (2+3)+(3+2)

(e) (i) The restriction enzyme EcoRl cuts DNA at the sequence GAATTC and the enzyme Haelll cuts the DNA at the sequence GGCC. On average how frequently will these enzymes cut a double stranded DNA? Explain your answer.

(ii) While making transgenic mice, indicate how will you ascertain whether a particular transgene is :

(A) Integrated in genome

(B) Transcribed or

(C) Translated in the transgenic mice.

(iii) Name a reporter gene and explain how is it used in Recombinant DNA Technology. 3+(1½+1½+1½)+(1+1½)

(f) (i) Describe the principle of sterilisation by autoclaving.

(ii) What are the important constituents of complex media? Name common sources of Carbon, Nitrogen, Phosphates in minimal media.

(iii) Briefly describe how media can be decontaminated of spore producing bacteria?

(iv) What precautions should be taken while adding antibiotic to solid media before poring plates and why? 2+(2+3)+2+l