2021

BIOCHEMISTRY — GENERAL

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) What is competent cell?

(b) What is the composition of LB broth?

(c) State two important characteristics of plasmid.

(d) Why is alkaline lysis method best for plasmid isolation from bacterial cells?

(e) State the use of Taq DNA polymerase in PCR.

(f) How can the size of plasmid DNA be determined by gel electrophoresis?

(g) Why do we aim to isolate single colony for microbiological experiments?

(h) 'PCR is a method for amplifying DNA *in vitro*'— Justify.

(i) What is meant by blue-white screening of recombinants?

(j) Which enzyme acts on the DNA after its entry into the cell? What is its function?

(k) What is the purpose of using isopropanol for plasmid isolation?

(l) Name any two restriction enzymes and mention their cut sites.

(m) What is klenow fragment?

(n) Which sterilization method will you choose for nutrient media and why?

(o) What is transformation? Mention any two factors that influence transformation.

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

(a) What is *in silico* cloning? Write down the work flow for *in silico* cloning. 2+3

(b) How will you prepare glycerol stock for bacterial strain? Why is it important to make glycerol stock? 3+2

(c) What are restriction endonucleases? Name the different types of restriction endonucleases. 2+3

(d) What is a vector? Explain with an example. Write down some properties for a good vector. 2+1+2

(e) What enzyme is used for DNA ligation? What co-factor does this enzyme need? Explain schematically how this enzyme ligates the ends of a vector and insert during cloning. 1+1+3

(f) (i) What is agar? What is its use?

(ii) What is the utility of glucose in plasmid DNA isolation?

(iii) Why is DNA viscous in solution? (1+2)+1+1

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

(a) What is plasmid? Explain the types of plasmid present in bacterial cell. What general properties a plasmid should have? Draw a simple diagrammatic representation of a plasmid. 2+3+2+3

(b) Write down the basic principle of PCR. What are the main components of the PCR reaction mixture? Explain with a labelled schematic diagram how DNA amplification occurs during PCR. 3+3+(2+2)

(c) What is meant by directional cloning? What is the advantage of this method over other cloning methods? Explain the workflow of directional cloning with labelled schematic diagram. 3+2+(2+3)

(d) (i) Explain the method for screening colonies with recombinants, during a cloning experiment, using a vector with Ampicillin resistance marker.

(ii) How can the purity of DNA be determined?

(iii) After transforming *E.coli* cells with 50 ng of transforming DNA, 150 colonies were obtained on the selection media plate. The final volume of the transformation mixture was 500 µl, of which 10 µl, was used for spreading on the plate. Calculate the transformation efficiency. 3+3+4

(e) (i) State the principle behind plasmid DNA isolation by Column Chromatography.

(ii) How can the size of DNA fragments be determined from gel?

(iii) Which type of DNA runs faster? —linear or circularized?

(iv) Why do we include the undigested plasmid while running the gel?

(v) Polymerase used for PCR is extracted from which species? At what temperature does denaturation of the double-helix take place? 2+2+2+2+(1+1)

(f) (i) Some cloning vectors have a lacZ gene. Explain how it helps during cloning.

(ii) State the difference between eGFP and GFP?

(iii) State three applications of PCR.

(iv) What would happen if you tried to use DNAP from *E.coli* in a PCR process?

(v) What is the purpose of heat shock during preparation of competent cells? 2+1+3+2+2