

**2021**

**BIOCHEMISTRY — HONOURS**

**Paper : CC-13**

**Recombinant DNA Technology and Genetic Engineering**

**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.*

1. Answer **any five** questions from the following: 2×5
- (a) Write down the key features of Type II restriction endonuclease.
  - (b) Which vectors will you use to clone four different fragments of size 2kb, 9kb, 40kb and 200kb?
  - (c) What is palindromic sequence of DNA. Illustrate with a suitable example.
  - (d) Why multiple bands are seen, when a plasmid DNA is run on an agarose gel?
  - (e) Write down the function of CaCl<sub>2</sub> used in preparing competent cells during artificial transformation of bacteria?
  - (f) List the reagents required to set up a Polymerase Chain Reaction?
  - (g) Define isoschizomer using appropriate example.
  - (h) Write down the full form of ARS. Mention its application in recombinant DNA technology.
  - (i) Illustrate the structure difference present between dNTP and ddNTP.
  - (j) Give full forms of X-gal and IPTG.
- Answer **any two** questions from the following.
2. (a) Outline the basic steps involved in cloning a particular gene.  
(b) How will you prevent self-ligation of a plasmid vector digested with a restriction endonuclease? 3+2
3. (a) Briefly mention what you know about Restriction-Modification system.  
(b) List the characteristics that must be present in a cloning vector. 3+2
4. (a) Briefly outline the principle of Southern Hybridization.  
(b) Find below three small pieces of DNA sequence originated from a larger DNA fragment by sequencing. Deduce the sequence of larger DNA fragment.  
(i) 5'-AGCGTTAG-3', (ii) 5'-CCGGTAAA-3' and (iii) 5'-AGCCGGTA-3' 2.5+2.5
5. (a) Distinguish between linker and adapter. Write down of one application of each.  
(b) How many copies of a double stranded DNA will be synthesized after 10 cycle of a Polymerase Chain Reaction if one starts with a single copy of a DNA template? (2+2)+1

**Please Turn Over**

Answer the following questions.

6. (a) Following fragments were obtained when a linear DNA was digested with the two restriction enzymes namely EcoRI and HindIII and resulting fragments were run on agarose gel.
- (i) EcoRI: 5.1kb, 5.4kb and 3.5kb
  - (ii) HindIII: 6.5kb, 1.8kb and 5.7kb
  - (iii) HindIII + EcoRI (double digestion): 1.9kb, 4.6kb, 0.8kb, 3.2kb, 2.5kb and 1.0kb

Draw the restriction map of the DNA using the above data.

- (b) Mention any one application of each of the following enzymes:

- (i) Taq DNA Polymerase
- (ii) Klenow fragment
- (iii) Reverse Transcriptase
- (iv) Polynucleotide Kinase
- (v) T4 DNA Ligase

5+(1×5)

OR

7. (a) List the salient features of a Cosmid vector and discuss their significance. Mention what advantage it has over a Plasmid vector.
- (b) A double stranded DNA molecule is  $5 \times 10^6$  bp long and has a GC content equals to 50%. Calculate how many times on an average HpaII (with recognition sequence 5'-CCGG - 3') sites are likely to be present in this DNA.
- (c) What is meant by "Marker rescue"? Discuss with appropriate example. (4+1)+2+3
8. (a) The size of the human genome of an organism is  $2.8 \times 10^6$  kb. If you want to prepare a genomic DNA library with an average cloned fragment size of 20kb, how many clones are required to represent the above genome with a probability of 95%?
- (b) Define the principle underlying blue-white selection method of recombinants.
- (c) How does a genomic library differ from a cDNA library?
- (d) What special problems may be encountered if you want to clone eukaryotic genes in *E. coli* host?

3+3+2+2

OR

9. (a) Write down the fundamental principle of Polymerase Chain Reaction. Use schematic representation.
- (b) State the advantages and limitations of PCR.
- (c) What is meant by threshold cycle of quantitative PCR?
- (d) Which factors are important in designing effective primers PCR experiment? 4+2+2+2

10. (a) Write down the basic principle of Sanger's method of DNA sequencing.
- (b) Draw the pattern of bands you expect to find in a DNA sequencing gel if you anneal the primer of sequence 5'-CTAGG-3' to the following single stranded DNA fragment and carry out a dideoxy sequencing experiment. Assume that the ddNTPs are radioactively labeled.  
3'-GATCCAAGTCTACGTATAGGCC-5'
- (c) Mention one application each for PCR and reverse transcriptase PCR (RT-PCR). 3+5+2

OR

11. (a) Give a brief outline of the strategy used for the production of human insulin in *E. coli* using Recombinant DNA Technology.
- (b) Describe how herbicide resistant plants may be developed using Genetic Engineering.
- (c) Retroviral vectors are often chosen for gene therapy. Write down the advantages and limitations of using retro-virus mediated gene therapy. 4+4+2
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