# RECOMBINANT DNA TECHNOLOGY
## (BIOT 3103)

**Time Allotted : 2½ hrs** **Full Marks : 60**

***Figures out of the right margin indicate full marks.***

***Candidates are required to answer Group A and any 4 (four) from Group B to E, taking one from each group.***

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

## Group – A

1. Answer any twelve: **12 × 1 = 12**

*Choose the correct alternative for the following*

(i) Type II R-M system is most preferred because
(a) Their restriction and modification sites are same
(b) Their recognition and modification sites are same
(c) Their recognition and restriction sites are same
(d) Their restriction and modification sites are different

(ii) Sticky-end cutters produce
(a) Both 5' and 3' overhang
(b) Either 5' or 3' overhang
(c) Only 5' overhang
(d) Only 3' overhang

(iii) The following table provides information about four proteins. Which one of the following options correctly identifies the order of elution in size exclusion chromatography and the increasing order of mobility in SDS polyacrylamide gel?

| Protein | Native mol. wt. (Da) | pI | Type |
|---|---|---|---|
| P | 32000 | 6.4 | monomer |
| Q | 40000 | 8.5 | homodimer |
| R | 25000 | 4.9 | monomer |
| S | 45000 | 8.5 | homotrimer |

(a) Chromatrography: PRQS; Electrophoresis: PRQS
(b) Chromatrography: SQPR; Electrophoresis: PRQS
(c) Chromatrography: SQPR; Electrophoresis: RPQS
(d) Chromatrography: RPQS; Electrophoresis: SQPR

(iv) The benefit of using qPCR is given below. Which statements are correct?
(P) It shows increased fluorescence after every cycle.
(Q) It shows a continuous increase in the fluorescence.
(R) It can be used to determine the number of transcripts in a given sample.
(S) It overcomes the inherent bias of the end point PCR
(a) (P) and (S)
(b) (Q), (R) and (S)
(c) (P), (Q) and (S)
(d) (Q) and (R)

(v) Given below are two sets of terms related to various methods used in recombinant DNA technology

| Group-I | Group-II |
|---|---|
| (P) Streptavidin | (1) DNA-protein interaction |
| (Q) Southwestern blotting | (2) FAM |
| (R) IMAC | (3) Biotin |
| (S) TaqMan | (4) $Ni^{+2}$ |

Which the correct match between group-I and Group-II?

(a) P- (4); Q - (3); R - (1); S - (2) (b) P- (3); Q - (2); R - (4); S - (2)
(c) P- (1); Q - (2); R - (4); S - (3) (d) P- (3); Q - (1) R - (4); S - (2)

(vi) Thy enzyme used for 5' end labelling of DNA is

(a) Klenow fragment (b) DNA pol I
(c) Terminal transferase (d) Polynucleotide kinase.

(vii) While generating a gDNA library, the genomic DNA is digested with an octacutter enzyme. Theoretically the average size of the gDNA fragments will be

(a) 256 bp (b) 4096 bp (c) 65,536 bp (d) none of these.

(viii) Eukaryotic mRNA can be purified through affinity chromatography in oligo dT-cellulose column, because it contains

(a) poly A tail at the 3' end (b) poly A tail at the 5' end
(c) 7M guanosine cap at the 3' end (d) 7M guanosine cap at the 5' end.

(ix) In a qRT-PCR experiment for quantitation of unknown RNA from a COVID19 patient sample, the $\mathbf{C_T}$ value was 12. What was amount of RNA copies present in the unknow sample?

(a) 4.096 copies (b) 2048 copies,
(c) 8192 copies, (d) 4096 copies

(x) A researcher desires to clone a gene of a microorganism. Its genome size is 1.5x $10^4$kb. The average size of its library fragment is 5 kb. The genomic library was created in vectors that were transformed into bacterial cells. If there is a 95% probability of the transformation, how many recombinant bacterial colonies will have to be screened to find this particular gene?

(a) 7000 (b) 8000 (c) 9000 (d) 10000

*Fill in the blanks with the correct word*

(xi) T4 DNA ligase needs _____ as cofactor.

(xii) Klenow fragment lacks _____ exonuclease activity.

(xiii) In western blotting hybridization nature of the probe is _____.

(xiv) In Snager's dideoxy chain termination method of DNA sequencing, the ratio of dNTP: ddNTP is _____.

(xv) In SDS PAGE to reduce disulphide bond the chemical used, is _____.

## Group - B

2. (a) Discuss the design of pET vector. How overexpression of the cloned gene is controlled in it? *[(CO2)(Analyse/HOCQ)]*

   (b) Compare the suitability of T4 and T7 DNA polymerases in the following application:
   (i) Fill-in reaction (ii) DNA sequencing. *[(CO1)(Remember/LOCQ)]*

   (c) During DNA cloning, why alkaline phosphatase treatment of vector is often used before Vector-Insert ligation? *[(CO2)(Apply/IOCQ)]*

**4 + 4 + 4 = 12**

3. (a) Discuss radiolabelling of 5' termini of dsDNA with T4PNK. *[(CO2)(Analyse/IOCQ)]*

   (b) Which phosphate group of ATP must be radiolabelled for 5'-end radiolabelling of DNA and why? Discuss with a labelled diagram. *[(CO2)(Understand/HOCQ)]*

   (c) Suppose you have isolated a DNA fragment by BglII digestion. Can you ligate the same in a vector digested with BamHI without using any linker/adaptor? How? After ligation, can you further subclone the fragment into another expression vector by redigesting the hybrid site (BamHI-BglII) by any of the enzyme? Explain. *[(CO6)(Apply/IOCQ)]*

**3 + 3 + 6 = 12**

## Group - C

4. (a) Describe the principles and steps of pyrosequencing methods of DNA sequencing with labelled diagrams only. Why this method called pyrosequencing? *[(CO2)(Remember/LOCQ)]*

   (b) What are differences between Sanger dideoxy methods and pyrosequencing methods of DNA sequencing? *[(CO2)(Analyse/IOCQ)]*

   (c) Based on which important enzymatic reaction these above methods are developed? Write that reaction. *[(CO2)(Apply/IOCQ)]*

   (d) You are trying to restriction-map a plasmid. An BahIRI digest gives you a 15 kb fragment, and XhoI digest gives you a 2 kb and a 10 kb fragment, and the double digest by BamHI and SalI gives you fragments of 2, 4, and 6 kb. From these results, you deduce restriction map of the plasmid. *[(CO2)(Apply/HOCQ)]*

**4 + 3 + (1 + 2) + 2 = 12**

5. (a) Explain the principle, steps and purpose of Hot start PCR, with diagram. *[(CO3)(Analyse/HOCQ)]*

   (b) Explain the principle, steps and purpose of Nested PCR, with diagram. *[(CO3)(Remember/LOCQ)]*

   (c) You are trying to restriction-map a plasmid DNA. An *SalI* digest gives you a 12 kb fragment, and *EcoRI* digest gives you a 7 kb and 6 kb fragment, and the double digest by *SalI* and *EcoRI* gives you fragments of 3, 4, and 6 kb. From these results, you deduce restriction map of the plasmid. *[(CO6)(Apply/IOCQ)]*

   (d) Derive the theoretical equation for the kinetics of PCR and draw the graphical representation of the equation. This theoretical plot for the kinetics of PCR will be same or different from practical plot and explain why? *[(CO3)(Apply/IOCQ)]*

**3 + 3 + 3 + (2 + 1) = 12**

## Group - D

6. (a) Illustrate different methods for size fractionation of DNA for preparation of DNA fragment for DNA cloning. *[(CO4)(Illustrate/IOCQ)]*
   (b) What are linkers and adapters? Discuss with suitable example. *[(CO4)(Remember/IOCQ)]*
   (c) Describe briefly their application in gDNA library preparation. *[(CO4)(Apply/IOCQ)]*

**4 + 4 + 4 = 12**

7. (a) Discuss the steps of making a cDNA library with a flow diagram. *[(CO4)(Discuss/HOCQ)]*
   (b) How cDNA library is screened? Describe any one method. *[(CO4)(Remember/LOCQ)]*
   (c) Differentiate between gDNA and cDNA library. *[(CO2)(Apply/IOCQ)]*

**4 + 4 + 4 = 12**

## Group - E

8. (a) Explain the cloning of human TPA gene for the production of rh-tissue plasminogen activator, with labelled diagram. *[(CO5)(Analyse/IOCQ)]*
   (b) Describe principle and steps of the techniques of DNA finger printing to solve the parenting problem. *[(CO5)(Remember/LOCQ)]*
   (c) Explain the strategy of Human gene therapy which was used for therapeutic treatment of Ashanti DeSilva with diagram. *[(CO5)(Apply/IOCQ)]*

**4 + 4 + 4 = 12**

9. (a) What is DNA finger printing? Describe AFLP techniques with labelled diagram. *[(CO3)(Analyse/IOCQ)]*
   (b) What are advantages of AFLP over RFLP? What are different applications of DNA finger printing? *[(CO4)(Remember/LOCQ)]*
   (c) Write the names two techniques which can be used to study large scale analysis of gene expression at RNA level. Describe the principle and steps of anyone of those techniques you have mentioned. *[(CO2)(Apply/IOCQ)]*

**(1 + 3) + (2 + 2) + (1 + 3) = 12**

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| Cognition Level | LOCQ | IOCQ | HOCQ |
|---|---|---|---|
| Percentage distribution | 23.96 | 59.37 | 16.67 |