4. (a) What is an expression cassette? What are the signals that must be part of an expression vector such that it can efficiently produce foreign proteins in *E.coli?*

   (b) Draw the DNA sequencing profile (slab gel) obtained by Sanger's method, of the given strand of DNA: 5' ATG CCA TCC GAC TTT GAA CTA 3'.

   (c) Who invented PCR? What will be the number of molecules of an amplicon at the end of 25 cycles, if you start with one double-stranded DNA molecule? Does the amplicon form in the first cycle of PCR? (6,6,3)

5. (a) Outline the basic steps in making a genomic DNA library. How are these different from cDNA libraries? Which are the vectors that can be employed to make genomic DNA libraries?

   (b) What enables Agrobacterium tumefaciens to act like a natural genetic engineer? Explain in detail. (8,7)

6. Write short notes on the following:

   (i) Fusion tags in proteins

   (ii) Primer designing for PCR

   (iii) Pyrosequencing (5,5,5)

(100)

[This question paper contains 4 printed pages.]

Your Roll No.....

Sr. No. of Question Paper : 5043 **J**

Unique Paper Code : 2494000023

Name of the Paper : Tools for Genetic Engineering

Name of the Course : **B.Sc. (Hons.) Biochemistry (NEP)**

Semester : VI

Duration : 2 Hours Maximum Marks : 60

Instructions for Candidates

1. Write your Roll. No. on the top immediately on receipt of this question paper.
2. There are **6** questions.
3. Attempt **any 4** questions.
4. All questions carry equal marks.
5. **Question no. 1 is compulsory.**

P.T.O.

1. (a) Differentiate between the following:

   (i) Type I and Type II restriction enzymes

   (ii) Linkers and adaptors

   (iii) Natural promoters and hybrid promoters

   (iv) Neoschizomers and Isoschizomers

   (v) Transformation and Transduction

   (vi) Standard PCR and qRT-PCR

   (b) Explain briefly:

   (i) Digestion of a plasmid with a restriction enzyme might yield multiple bands on the agarose gel, even though the plasmid has only one site for that enzyme.

   (ii) pET vectors help in keeping a tight control over the expression of the gene of interest. (12,3)

2. (a) What are the features of a good cloning vector? Compare pBR322 with pUC vectors and discuss which features of pUC vectors make them more favourable for use in experimental work.

   (b) You are given a plasmid vector that has a unique site for BamHI enzyme (5' G/GATCC 3'). Cut this vector with BamHI. Now cut a gene of interest with the same enzyme and ligate them together to form a recombinant molecule. Show all the steps diagrammatically.

   (c) If you cut a DNA fragment with any 4-base cutter restriction enzyme, what size of fragments do you expect to see? In which conditions will you not get fragments of the expected size? (6, 5, 4)

3. (a) With the help of diagrams show how these enzymes are used in genetic engineering:

   (i) Terminal deoxynucleotidyl transferase

   (ii) DNase I

   (iii) DNA ligase

   (b) Discuss two methods of transforming bacterial cells, in detail.

   (c) What are the challenges faced while using E.coli as a host system while making mammalian proteins? (6,6,3)