5. (a) Write names of three hybrid vector. Describe the preparation of genomic library of a PCR product in any one the vector you mentioned with diagram.

   (b) Two DNA solution supplied to you, one is a 3.5 kb EcoRI/HinDIII insert DNA with concentration 1 bμg/10 bμl and another 4.31 kb EcoRI/HinDIII vector DNA with concentration 5 μg/10 μl. Now you calculate the amount of insert and vector DNA you will use for a ligation reaction by T4 DNA ligase (concentration 1U/μl), and also write ligase reaction set up for a final volume of 20 μl, with 10X ligase reaction buffer.

   (c) A pure protein was supplied to you. Describe the steps of cloning of gene X and selection of positive clone with diagram.

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

Group – D

6. (a) Describe the microinjection technique for gene delivery into animal cells.

   (b) Compare and contrast the following methods for raising transgenic plants and animals: (i) Electroporation (ii) Viral vector mediated.

6 + (3 + 3) = 12

7. (a) Define and give example of: shuttle vector.

   (b) What is T-DNA? Describe the two different vector systems designed based on Ti-plasmid.

4 + (2 + 3 + 3) = 12

Group – E

8. (a) (i) Write three names of genetic disorder and the names of corresponding genes. What is the name of techniques to cure genetic disorder?
   (ii) Explain the principle and steps of the method to cure any one of the genetic disorder you mentioned.

   (b) Write names thee biopharmaceuticals. Explain the cloning strategy of the gene for any one biopharmaceutical you mentioned.

   (c) Write the name of techniques used to identify the culprit of crime. Explain the principle of that technique.

(1 + 3) + (1 + 3) + (1 + 3) = 12

9. Write short notes about the following: **(4 × 3) = 12**
   (i) Genetically engineered vaccine.
   (ii) DNA based diagnosis of genetic diseases.
   (iii) Human genome project.

M.TECH/BT/1ST SEM/BIOT 5101/2019

ADVANCED GENETIC ENGINEERING
(BIOT 5101)

Time Allotted : 3 hrs **Full Marks : 70**

Figures out of the right margin indicate full marks.

Candidates are required to answer Group A and any 5 (five) from Group B to E, taking at least one from each group.

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

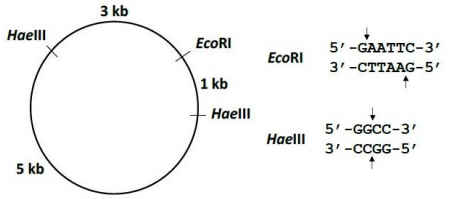
Group – A
(Multiple Choice Type Questions)

1. Choose the correct alternative for the following: **10 × 1 = 10**

   (i) The radioactive phosphate of ATP is transferred to the 5'-hydroxyl of DNA by T4 polynucleotide kinase is
   (a) alpha (b) beta (c) gamma (d) delta.

   (ii) Which of following enzyme does not require a template for their reaction?
   (a) Reverse transcriptase (b) *Taq* polymerase
   (c) Terminal deoxynucleotide transferase (d) DNA pol-I.

   (iii) The product of complete digestion of the plasmid shown below with EcoRI and HaeIII was purified and used as a template in a reaction containing Klenow fragment of DNA polymerase, dNTPs and [α-^{32}P]-dATP in a suitable reaction buffer. The product thus obtained was purified and subjected to gel electrophoresis followed by autoradiography. The number of bands that will appear on the X-ray film is



   (a) 4 (b) 5
   (c) 2 (d) 1.

   (iv) A dNTP master mix is prepared by combining 50 μl each of 10 mM dNTP stock. Two micro liters from this dNTP mix are added to the PCR master mix of 25 μl reaction volume. What is the total dNTP concentration in the PCR reaction?
   (a) 200 μM (b) 400 μM (c) 800 μM (d) 250 μM.

   (v) You are subcloning a fragment of genomic DNA into an *E. coli* plasmid vector. As a first step, you cut out the fragment from an existing clone using a restriction enzyme. You then ligate the fragment into a similarly-digested plasmid vector

carrying an amp^r gene. The site you ligate into is in the middle of the *lacZ* gene coding for β-galactosidase. After ligation, you transform *E. coli* with the ligated molecules using a $CaCl_2$ solution or electroporation and plate on IPTG and X-gal plates with ampicillin. Successful transformation is indicated by _____, while successful insertion of DNA into the vector restriction site is indicated by _____.
(a) amp^r colonies; white colonies (b) white colonies; amp^r colonies;
(c) amp^r colonies; blue colonies (d) no colonies; white colonies

(vi) A gene was cloned into a unique HindIII restriction site present in the ampicillin resistance gene of a vector that contains both ampicillin and kanamycin resistance genes. To select for only recombinant clones, the transformation mixture should be plated on which of the following plates?
(a) Ampicillin containing plate
(b) Ampicillin plus Kanamycin containing plate
(c) Ampicillin containing plate followed by replica-plating on kanamycin containing plate
(d) Kanamycin containing plate followed by replica-plating on ampicillin containing plate.

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

(viii) Diagnosis of influenza virus infections can be done using some of the following techniques:
A. Western blot and Southern blot B. Northern blot and western blot
C. ELISA and RT-PCR D. PCR and electron microscopy
Choose the combination of techniques that correctly lists the detection methods.
(a) A and B only (b) A and D only (c) B and C only (d) C and D only.

(ix) A polymerase reaction is carried out for 10 cycles in a volume of 1 ml with 5 molecules of template DNA. Assuming that the efficiency of the reaction is 100%, the number of molecules of DNA present in 100 μl at the end of the reaction is
(a) 512 (b) 215 (c) 521 (d) 125.

(x) *Luc, gfp, β-gluc* are genes well known as
(a) visible marker gene (b) reporter gene
(c) selectable marker gene (d) transgene.

Group – B

2. (a) Write only the mechanism of reaction with labelled diagram of the following enzymes in genetic engineering: (i) Topoisomerase-I (ii) E.coli DNA ligase (iii) BR and LR clonase.

(b) Describe the features of the following vectors with diagram
(i) YAC (ii) pET28a+.

(c) Three restriction endonucleases (Sal I, EcoR I and Hind III) are used to cut a plasmid DNA separately singly and in different combinations. From the agarose gel electrophoresis result, sizes of fragments (in kb) are listed in order of size. Determine the correct order of restriction sites, and draw the final restriction map of the DNA, with the intervals between sites labelled.

Sal I)	20;
Sal I x EcoRI)	7, 13;
Sal I x Hind III)	4, 5, 11;
SalI X EcoR I X HindIII)	3, 4, 5, 8.

(3 × 2) + 3 + 3 = 12

3. (a) What is the principle of separation of protein by SDS-PAGE?

(b) Write the names of four modern technique for gene editing. Explain the principle most popular technique for gene editing.

(c) Show the following conversion with all requirement and condition:
(i) ss RNA to ds DNA (ii) Blunt end DNA to staggered end DNA.

(d) An aliquot of template DNA containing 3×10^2 copies of target gene is placed into PCR reaction. If we expect amount of yield will 6×10^{10}. The reaction has a mean efficiency of 95%. Calculate the yield of reaction after 35 cycles.

2 + (2 + 3) + 3 + 2 = 12

Group – C

4. (a) Describe the cloning strategy of a DNA fragment without restriction enzyme and DNA ligase.

(b) Describe the cloning steps of a DNA fragment in BAC vector.

(c) Explain the principle of purification of eukaryotic mRNA

(d) The rarest mRNA in a cell of a particular type has a concentration of five molecules per cell. Each cell contains 40,000 mRNA molecules. A cDNA library is made from mRNA isolated from this tissue. How many clones will need to be screened to have a 89% probability of finding at least one recombinant containing a cDNA copy of rarest mRNA?

4 + 4 + 2 + 2 = 12