

KATHMANDU UNIVERSITY  
End Semester Examination  
February, 2025

Marks Scored:

Level : B.Tech.  
Year : III

Course : BIOT 306  
Semester : II

Exam Roll No. :

Time: 30 mins.

F. M. : 20

Registration No.:

Date : 14 FEB 2025

SECTION "A"

[20 Q. × 0.75 = 15 marks]

Choose and mark [X] the most appropriate option from each set of choices

- The bacteria with glycosylation activity.  
 *Campylobacter jejuni*                       *E. coli*  
 *Bacillus subtilis*                               *Agrobacterium tumefaciens*
- The plasmid carrying genes for ampicillin, kanamycin and tetracycline resistance.  
 RP4                       pBR322                       pUC8                       TOL
- In the genetic map of  $\lambda$  phage the gene responsible for integration and excision is?  
 cIII                       cI                       N                       xis
- Bacterial cell number is estimated by measurement of optical density (OD). A sample of the culture is placed in a glass cuvette and light with a wavelength of 600 nm shone through. The amount of light that passes through the culture is measured and the OD is calculated. The value of 1 OD for *E. coli* cells is equivalent to?  
  $0.8 \times 10^6$  cells/ml      $0.8 \times 10^7$  cells/ml      $0.8 \times 10^8$  cells/ml      $0.8 \times 10^9$  cells/ml
- The buoyant density of DNA in Ethidium bromide-caesium chloride density gradient centrifugation is?  
  $1.65 \text{ g/cm}^3$                         $1.70 \text{ g/cm}^3$                         $1.75 \text{ g/cm}^3$                         $1.80 \text{ g/cm}^3$
- Which of the following is **NOT** a pedigree of pBR322?  
 R1                       R6-5                       pMB1                       colE1
- The restriction enzyme that cuts at the SUP4 site in the vector pYAC3?  
 BamHI                       HindIII                       SnaBI                       AluI
- Which of the following is **NOT** an *in vitro* mutagenesis technique?  
 Restriction fragment deletion                       Nucleotide removal at restriction site  
 S1 nuclease treatment                       Insertion of an oligonucleotide
- Acetone and butanol are compounds produced by industrial scale culture of microorganisms. The microorganism used for its production is?  
 *Bacillus* spp.                       *Clostridium* spp.                       *Aspergillus* spp.                       *Streptomyces* spp.
- In an expression vector used to achieve expression of a foreign gene in *E. coli*, the gene of interest is cloned?  
 In between the promoter and ribosome binding site  
 In between the ribosome binding site and terminator  
 Before the promoter  
 After the terminator

11. Which of the following promoter is dependent on 3- $\beta$ -indoleacrylic acid for its transcription?  
 lac                       trp                       tac                       T7
12. Tissue plasminogen activator is used in the treatment of?  
 Cancer                       Diabetes                       Heart disease                       Infertility
13. A DNA sequence is having two ends, 5' and 3'. Which of the following statements is correct regarding the nature of the ends?  
 The 5' end is having hydroxyl group                       The 5' end is having phosphate group  
 The 3' end is having phosphate group                       Any group can be present at any end
14. The primers used for the amplification of human gene should be at least \_\_\_ nucleotides long.  
 15                       16                       17                       18
15. The insects belonging to order Coleoptera is poisoned by the \_\_\_\_\_ *B. thuringiensis*  $\delta$ -endotoxins.  
 Cry I                       Cry II                       Cry III                       Cry IV
16. Glyphosate competes with phosphoenol pyruvate in the EPSPS-catalyzed synthesis of enolpyruvylshikimate-3-phosphate, and hence inhibits synthesis of?  
 tryptophan                       lysine                       aspartic acid                       methionine
17. Bacteria protect themselves from viruses by fragmenting viral DNA upon entry with  
 Ligases                       Methylases                       Endonucleases                       Probes
18. A gene that codes for an enzyme that catalyses one of the step in the biosynthesis pathway of pyrimidine nucleotides and is used as selectable marker in yeasts.  
 LEU2                       URA3                       TRP1                       SUP4
19. The first breakthrough in mapping the human breast cancer susceptibility gene BRCA1 occurred in 1990 as a result of restriction fragment length polymorphism (RFLP) linkage analyses. This study showed that in families with a high incidence of breast cancer, a significant number of the women who suffered from the disease all possessed the same version of an RFLP called?  
 D17S1321                       D17S1325                       D17S60                       D17S74
20. Alkaline denaturation is carried out at a pH of?  
 10-10.5                       11-11.5                       12-12.5                       13-13.5

#### SECTION "B"

[10 Q.  $\times$  0.5 = 5 marks]

#### **Fill in the blanks**

21. \_\_\_\_\_ cleaves adjacent to arginine residues or factor Xa.
22. Conjugation and plasmid transfer are controlled by a set of \_\_\_\_\_ genes.

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23. Guanidinium thiocyanate is a \_\_\_\_\_ agent, and interferes with the hydrogen bonding that normally holds water molecules together.
24. In third-generation sequencing method a sophisticated optical system called a \_\_\_\_\_ is used to observe the copying of a single DNA template, with each nucleotide addition identified from its attached fluorescent label.
25. If restriction sites are included in the end-sequences of the gene, then treatment with the appropriate enzyme will produce sticky ends that allow the gene to be ligated into a cloning vector. This procedure is called \_\_\_\_\_ synthesis.
26. Expression vectors for *A. nidulans* usually carry the glucoamylase promoter which is induced by starch and repressed by \_\_\_\_\_.
27. If the recombinant protein is placed under the promoter of  $\beta$ -lactoglobulin gene, then the recombinant protein is secreted in the \_\_\_\_\_.
28. The targeted gene in delayed ripening of tomato using antisense RNA technique is \_\_\_\_\_ gene.
29. The activation of Cre recombinase in terminator technology is done by placing the seeds in \_\_\_\_\_ solution.
30. The current methodology of DNA profiling is called CODIS (Combined DNA Index System), makes use of \_\_\_\_\_ STRs with sufficient variability to give only a one in  $10^{15}$  chance that two individuals, other than identical twins, have the same profile.

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SECTION "C"

[3 Q. × 7 = 21 marks]

*Attempt ANY THREE questions.*

1. In a cloning experiment why recombinants need to be identified? Describe the basis of selection of recombinants by: i) Insertional inactivation of an antibiotic resistance gene and ii) Insertional inactivation of gene other than antibiotic resistance gene. [1+3+3]
2. Design a 20-mer forward and reverse primer for the following sequence of DNA starting from 5'-3' end. The sequence of the template DNA is as shown below:  
3' ATGCCCTATTGGTAGTGTACTGATAGCTTGCATGAATTCGCGTACGTCGTATC5'  
Place in order the following steps involved in PCR:
  - a. newly synthesized strands act as templates
  - b. temperature lowered; DNA primers and polymerase added
  - c. heat separates strands of target DNA
  - d. complementary base pairing between primers and template
  - e. DNA nucleotide bases added; new strand synthesized. [4+3]
3. What do you understand by host-controlled restriction? A linear DNA when digested with EcoRI produces fragment of size 1.9 kb, 1.4 kb and 2.2 kb. The same DNA when digested with BamHI produces fragments of size 3.0 kb, 1.5 kb and 1.0 kb. Double digestion with both the enzymes produces fragments of size 1.9 kb, 1.1 kb, 0.3 kb, 1.2 kb and 1.0 kb. Construct a restriction map on the basis of information provided. [2+5]
4. What are the strategies undertaken to identify a gene for a genetic disease? Describe the linkage analysis of the human breast cancer susceptibility gene BRCA1. [2+5]

SECTION "D"

5. Write short notes on (**ANY FOUR**): [4 Q. × 3.5 = 14]
  - a. CTAB method for the purification of plant DNA.
  - b. Blunt end ligation with a DNA topoisomerase
  - c. Transcript analysis by PCR.
  - d. Use of glyphosate N-acetyltransferase to generate plants that detoxify glyphosate.
  - e. P elements as cloning vectors for *Drosophila*.
6. Write down **TWO** differences between (**ANY FOUR**): [4 Q. × 2 = 8]
  - a.  $\lambda$  phage and M13 phage.
  - b. M9 media and LB media.
  - c. Binary vector strategy and Co-integration strategy.
  - d. Reversible terminator sequencing and Pyrosequencing.
  - e. Gel retardation of DNA-protein complexes and Footprinting with DNase I.

P.T.O.

7. Give reasons why/ how? (**ANY FOUR**): [4 Q. × 3 =12]
- a. RK2 plasmid is used as a shuttle vector.
  - b. Codon bias of the gene might prevent efficient expression of a foreign gene cloned in *E. coli*.
  - c. Amelogenin gene based method is preferred over Y chromosome based sex identification method.
  - d. Ion-exchange chromatography is used to purify DNA from a cell extract.
  - e. Cre recombinase enzyme is used for the excision of the kanamycin resistance gene from the plant DNA.