

## Avinashilingam Institute for Home Science and Higher Education for Women

Deemed to be University Estd. u/s 3 of UGC Act 1956, Category 'A' by MHRD (now MoE)

Re-accredited with 'A++' Grade by NAAC. CGPA 3.65/4, Category I by UGC

Coimbatore - 641 043, Tamil Nadu, India

### Continuous Internal Assessment Test I – February 2025

#### Semester-II

Class : I PG  
Major : Biotechnology

Time: 2 Hours  
Max. Marks: 60

#### 23MBTC10 – Recombinant DNA Technology

##### Course Outcomes

CO1: Students will be able to **demonstrate** the application of various techniques in developing a recombinant molecule

CO2: Students will be able to **explain** the process of gene cloning

CO3: Students will be able to **relate** the importance of genetically modified organisms for human welfare

CO4: Students will be able to **justify** the importance of genome projects and the potential application of recombinant techniques

CO5: Students will be able to critically **analyze** the various issue related to gene manipulation

##### Part – A

(6 x 1 =6)

Choose the correct answer

- Which among the following is not true about a phagemid (CO1 K1)
  - Contains  $\lambda$  att site
  - Can be propagated as phage only
  - Contains the ori of both plasmid and  $\lambda$  phage
  - Can be propagated both as plasmid and phage
- Which among the following selection markers is present in the plasmid pBR322 (CO1 K1)
  - Amp but not Tet
  - Tet but not Amp
  - Amp and Tet
  - Amp only
- What is the expansion of pBR in pBR322 (CO2 K1)
  - Plasmid Baltimore & Rodriguez
  - Plasmid Boliver & Rodriguez
  - Plasmid Berkley & Rodriguez
  - Plasmid Boyer & Rodriguez
- An expression library is the result of conversion of \_\_\_\_\_ which accounts for about 5% of total RNA population in the cell (CO2 K3)
  - miRNA
  - sn RNA
  - rRNA
  - mRNA
- A fragment of 100 – 300 Kb size can be cloned in \_\_\_\_\_ (CO2 K3)
  - Plasmids
  - BAC
  - Cosmid
  - Ti plasmid
- Which among the following enzymes is used for removing single stranded DNA (CO2 K3)
  - Polynucleotidyl kinase
  - S1 nuclease
  - Endonuclease
  - DNase

##### Part – B

(3 x 6 = 18)

Answer **ALL** questions.

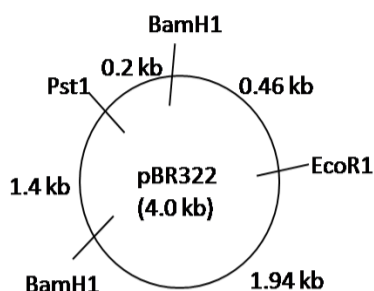
Each answer should not exceed 200 words or one page.

- (a) What does MCS stands for in a cloning vector. Explain its significance (CO1 K1)  
(Or)
- (b) Give an account of adaptors in cloning experiments (CO1 K4)
- (a) How do you perform agarose gel electrophoresis? What is the function of tracking dyes? How are nucleic acids visualized on the gel? (CO2 K2)  
(Or)
- (b) How do you differentiate a recombinant clone from a transformant. What is the significance of antibiotic selection marker (CO2 K3)

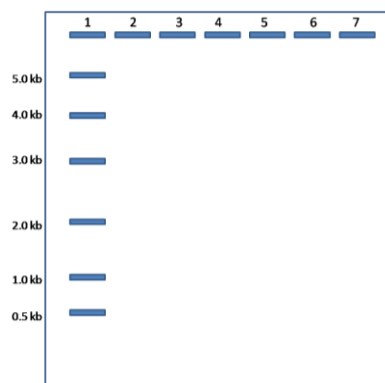
9 (a) The plasmid vector pBT2024 is digested with the enzymes PstI and PstI resulting in the following fragments : PstI: 20 Kb, PstI : 11Kb, 6Kb, 3Kb and a double digest gave 8Kb, 6Kb, 3Kb fragments. Construct the restriction map of the plasmid pBT2023 (CO2 K5)

(Or)

9 (b) Plasmid pBR322 is a 4.0 kb plasmid containing amp and tet markers with ori and restriction sites for EcoRI, BamHI and PstI. Given the restriction map for pBR322, show on the agarose gel picture below where the approximate position of restriction fragments generated from the given restriction digests would be located after carrying out AGE (CO2 K5)



Lane 1 – size standards  
 Lane 2 – BamHI digest  
 Lane 3 – EcoRI digest  
 Lane 4 – PstI digest  
 Lane 5 – EcoRI + BamHI  
 Lane 6 – EcoRI + PstI  
 Lane 7 – BamHI + PstI



**Part – C**

**(3 x 12 = 36)**

**Answers should not exceed 600 words or three pages**

10 (a) Explain in detail the CTAB method and Delaporta method used for isolating DNA from leaves of arabidopsis. Explain the difference between both (CO1 K2)

(Or)

10 (b) Explain in detail a method used for isolating RNA and purifying mRNA from callus tissues. (CO1 K2)

11 (a) Given the following DNA sequence (CO2 K6)

**5'aag gcc cag gaa ttc cta ccc ggg gtt aag tta aca cgg gaa tcc tta aat 3'**

- What is the action of DNA polymerase
- Find out the restriction sites of EcoRI, Mfe1 and Sma1 if present
- What is the action of the enzyme EcoRI on the dsDNA
- What will be the action of SI nuclease on the product of reaction (iii)
- Can the product (ii) be self - ligated after treatment with alkaline phosphatase
- Create a site for BamHI while maintaining the size of the given DNA fragment

Sma1 site – CCC^GGG, Mfe1 site – C^AATTG, EcoRI – G^AATTC, BamHI – G^GATCC

(Or)

11 (b) Plasmid pBT2025 is 7.0 kb in length and has single restriction sites for AgeI, EcoRI, and Hind3 enzymes. You have cloned a DNA fragment of 4.0kb size into the AgeI site.

- What will be the size of the recombinant plasmid.
- The ampicillin selection marker lies in the Age1 site, while lacZ is in EcoRI site. Can the recombinant plasmid be selected using amp resistance?
- Depict the agarose gel pattern of the uncut, recombinant and restricted recombinant plasmid
- From the data below, determine the restriction map of the resulting plasmid (CO2 K6)

Enzyme combinations	Size of Fragments obtained			
AgeI	7.0	4.0		
EcoRI	6.0	5.0		
AgeI + EcoRI	4.3	3.3	2.7	0.7

Can you clone the fragment using EcoRI site on the plasmid, Justify your answer

12 (a) Polymerase chain reaction has been considered as a revolutionary technology of the 20<sup>th</sup> century. Justify the statement (CO3 K2)

(Or)

12 (b) How will you transform a natural Ti plasmid into a plant transformation vector. (CO3 K3)