# P. P. SAVANI UNIVERSITY

**Fifth Semester of B.Sc. Examination**
**December-2021**
**SSBT3130–Recombinant DNA Technology I**

**11.12.2021, Saturday** **Time: 12:30 p.m. to 03:00 p.m.** **Maximum Marks: 60**

***Instructions:***

1. The question paper comprises of two sections.
2. Section I and II must be attempted in separate answer sheets.
3. Make suitable assumptions and draw neat figures wherever required.
4. Use of scientific calculator is allowed.

## Section-I (Total Marks - 30)

**Q.1 Short Questions** **[10]**

**1.1 Objectives** **[05]**

**1.1a** Following a transformation event using two separate plasmids (one carrying kanamycin resistance gene while the other carrying ampicillin resistance gene), the *E. coli* cells were plated on Kanamycin-Ampicillin containing plates. Which of the following type of cells will survive on the plate:

- **A** *E. coli* which take up only the plasmid carrying Ampicillin resistance gene
- **B** *E. coli* which take up only the plasmid carrying Kanamycin resistance gene
- **C** *E. coli* cells which take up both the plasmids one for kanamycin and the other for ampicillin
- **D** All of the above

**1.1b** Which of the following is a blunt cutter

- **A** Sca I
- **B** Sma I
- **C** Hind III
- **D** XbaI

**1.1c** High copy number of a low copy number plasmid can be obtained by____

- **A** plasmid amplification in presence of antibiotic resistance gene for Ampicillin
- **B** plasmid amplification in presence of antibiotic resistance gene for Chloramphenicol
- **C** plasmid amplification in the presence of a protein synthesis inhibitor such as chloramphenicol
- **D** All of the options are correct

**1.1d** Klenow fragment has

- **A** nuclease activity only
- **B** polymerase activity only
- **C** polymerase and ligase activity
- **D** polymerase and nuclease activity

**1.1e** After successful transformation which of the following ligated products will result in protein expression

- **A** Gene ligated in the forward direction to the promoter
- **B** Gene ligated in the reverse direction to the promoter

C Gene ligated within the promoter
D Vector self religation

**1.1f** The "*bla*" gene encodes β lactamase which provide _____ resistance
A Kanamycin
B Ampicillin
C Tetracycline
D Chloramphenicol

**1.1g** Insertion of new DNA into pBR322 that has been restricted with *PstI*, *PvuI*, or *ScaI*
A inactivates the *ampR* gene
B inactivates the *tetR* gene
C inactivates the *chlR* gene
D inactivates the *kanR* gene

**1.1h** Which of the following vector(s) have LacZ gene for visual screening
A pUC 18
B pUC 19
C λZAPII
D All of the above

**1.1i** Which of following metallic ion is used for making chemically competent cells
A $Mg^{2+}$
B $Ca^{2+}$
C $Fe^{3+}$
D $Na^{2+}$

**1.1j** For restriction mapping
A only single digestion is required
B only double digestion is required
C both single and double digestion are required
D only triple digestion is required

**1.2 Answer the Following: (MCQ/Short Question/Fill in the Blanks)** **[05]**

**1.2a** Ligation reaction requires energy (TRUE/FALSE)

**1.2b** Polymerases are enzymes that synthesize a new strand of DNA complementary to an existing DNA or RNA template (TRUE/FALSE)

**1.2c** Define 'Double digestion'

**1.2d** Unlike linkers, a/an _____is synthesized so that it already has one sticky end

**1.2e** *AluI* enzyme is an example of _____ cutter

**Q.2 Short Notes (Attempt any two)** **[06]**

**A** Bacterial transformation and selection

**B** Blunt end ligation using topoisomerase

**C** Adaptors

**Q.3 Explain in detail (Attempt any two)** **[14]**

**A** What are the key steps of gene cloning? Explain each step in detail?

**B** What are restriction endonucleases? Explain its different types in detail?

**C** A circular DNA plasmid, pDA102, has a size of 4.35 kb. When the plasmid DNA

digested with combination of restriction enzymes and the resulting fragments are electrophoresed, the following data is obtained.
Using these data, construct a restriction map of plasmid pDA102 for the restriction enzymes Sal I and Hha III

| | |
|---|---|
| Sal I | 2.30, 0.25, 1.80 Kb |
| Hha III | 2.10, 1.55, 0.70 Kb |
| Sal I + Hha III | 1.20, 1.10, 0.75, 0.70, 0.35, 0.25 Kb |

## Section-II (Total Marks - 30)

**Q.1 Short Questions** **[10]**

**1.1 Objectives** **[05]**

**1.1a** The *tra* gene is carried by

**A** R plasmid
**B** F plasmid
**C** Cryptic plasmid
**D** None of the options are true

**1.1b** M13 phage as cloning vector can be obtained in

**A** single stranded form
**B** both single and double stranded form
**C** double stranded form
**D** none of the options are correct

**1.1c** Phagemid was developed by combining

**A** M13 genome with plasmid DNA
**B** lambda phage genome with plasmid DNA
**C** YAC DNA with plasmid DNA
**D** BAC DNA with plasmid DNA

**1.1d** Which of the following is used for the analysis of large genomes

**A** Plasmid
**B** Phage
**C** BAC
**D** All of the options are correct

**1.1e** Region of Ti plasmid which is responsible for its integration to the host genome is

**A** Octopine breakdown region
**B** Virulence region
**C** T-DNA region
**D** Agropine breakdown region

**1.1f** Which of the following plasmids do not have any apparent effect on the phenotype of host

**A** R plasmid
**B** Cryptic
**C** Col
**D** Tryptic

**1.1g** Stuffer fragment

- **A** carries the selection marker
- **B** carries the cos site
- **C** carries the origin of replication
- **D** is the replaceable fragment

**1.1h** M13 vectors are useful for:

- **A** DNA sequencing
- **B** Mutagenesis study
- **C** probe generation
- **D** All of the above

**1.1i** In pEMBL8 vector, for visual screening of recombinant clones, MCS is present:

- **A** Outside the *lacZ* gene
- **B** within the *lacZ* gene
- **C** After the *lacZ* gene
- **D** Right before the *lacZ* gene

**1.1j** In YAC, the presence of the insert DNA in the vector can be visually checked by testing for insertional inactivation of:

- **A** URA3 gene
- **B** TRP1 gene
- **C** SUP4 gene
- **D** LEU2 gene

**1.2** **Answer the Following: (MCQ/Short Question/Fill in the Blanks)** **[05]**

**1.2a** Terminal deoxynucleotidyl transferase enzyme adds one or more deoxyribonucleotides onto the 3' terminus of a DNA molecule (T/F)

**1.2b** _____are hybrids between a phage Cos sequence and a bacterial plasmid

**1.2c** BsrFI recognizes **R**CCGG**Y**, where R=____ and Y= ____

**1.2d** If the first 323 amino acids of DNA pol I polypeptide is removed Nuclease activity will be lost (T/F)

**1.2e** In YEp13 vector FLP region codes for a protein that can convert A form to the B form (T/F)

**Q.2** **Short Notes (Attempt any two)** **[06]**

**A** Ti plasmid

**B** Shuttle vectors

**C** Phagemid

**Q.3** **Explain in detail (Attempt any two)** **[14]**

**A** Explain YAC in detail. Why three different selection markers are required for cloning in YAC?

**B** What are expression vectors explain one expression vector in detail?

**C** What are M13 vectors? Discuss the construction of M13mp2