BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI (RAJASTHAN)

First Semester 2023-2024

11-October-2023	**BIO F418 Genetic Engineering Techniques**	Max marks: 60 (30%)
Closed book type	**Mid-Semester Exam**	Time: 90 minutes

Provide justification, calculations and diagrams wherever necessary.

Name: **ID Number:**

Student's signature **Invigilator's signature**

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Q1. (a) What is the reason of satellite colonies formation during cloning. Write a proper justification. **[4M]**

Ans.

(b) You are asked to inoculate a colony of DH5α strain of *E. coli* in 50 ml LB media for preparation of competent cells. How much 100 mg/ml ampicillin will be required in this volume of LB media. Give proper justification. **[3M]**

Ans.

Q2. Write a short note on the content and importance of the following: **[2 x 5 = 10M]**

(a) 1 Kb Ladder

Ans.

(b) 6X Loading Dye

Ans.

(c) TAE and TBE buffer

Ans.

(d) TE buffer

Ans.

(e) Solution II in alkaline lysis method

Ans.

Q3. Give a proper justification for the following processes during different steps of plasmid DNA isolation. **[3 x 3 = 9M]**

(a) 70% ethanol (instead of 100% DNA) is used for final wash of DNA during DNA isolation?

Ans.

(b) A dry spin of two minutes (step before elution) while isolating plasmid using kit.

Ans.

(c) Chloroform/isoamyl alcohol treatment after phenol/chloroform/isoamyl alcohol extraction during purification of DNA.

Ans.

Q4. (a) *NotI* and *Bsp120I* enzymes have the following recognition sequences. How these two enzymes can be correlated? Justification is required. **[3M]**

Not I	*Bsp120 I*
5' - GC*GGCC GC – 3'	5' - G*GGCC C – 3'
CG CCGG*CG	C CCGG*G

Ans.

(b) Following is the sequence of a single strand of double stranded DNA. How many restriction sites of SacI (5' GAGCTC 3') and EarI (5' CTCTTC 3') are present in this dsDNA? Write the sequence in your answer and underline the mentioned sequences. [4M]

5' - TCGGCTCGAGATGAAGAGGTACTTTCTCTTCTGCAAGAGCTCCGCTCAGGTGGATTCTGCAAAT - 3'

Ans.

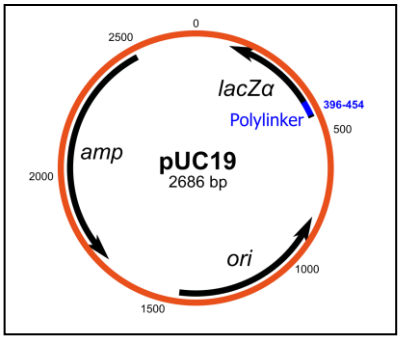
Q5. (i) Bacteriophage λ can be used as a vector, but non-essential region should be removed for efficient cloning. Which is this region and why is that region not considered essential for a cloning vector? How long DNA can generally be cloned in a λ phage based vector? [4M]

Ans.

(ii) Dam methylase catalyzes methylation at the N^6 position of the adenine in the sequence GATC. Dcm methyltransferases catalyzes methylation at the C^5 position of the second cytosine in the sequences CCAGG and CCTGG. What is the importance of these properties in bacteria with respect to restriction enzymes? **[3M]**

Ans.

Q6. (i) How does blue-white selection work for pUC19 plasmid? Write a proper mechanism. **[4M]**



Ans.

(ii) How will you select your recombinant clones? Show through flow chart. Describe wherever needed. **[3M]**

Ans.

(iii) Theoretically, how this pUC19 vector can be converted to a shuttle vector? Justify. **[3M]**

Ans.

Q7. You are working in a molecular biology lab and your mentor asked you to check the stocks of minus 80℃ deep freezer and clean the freezer. While checking the old stocks, you found a micro-centrifuge tube containing unknown plasmid DNA without label. Your mentor asked you to map this DNA by using routinely used restriction enzymes. You observed the following DNA fragments by digestion with the mentioned restriction enzymes. **[10M]**

Digesting enzyme	Size of Fragments
HindIII	8.5 Kb
BamHI	5.5, 3 Kb
EcoRI	3.5, 2.9, 2.1 Kb
HindIII and *SmaI*	8.5 Kb
BamHI and *EcoRI*	3.5, 2.4, 1.5, 0.6, 0.5 Kb
EcoRI and *HindIII*	3.5, 2.1, 1.5, 1.4 Kb

Draw a circular restriction map of the plasmid and mark each restriction site with the distance between each site.

Ans.

All the Best

Rough work