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B. M. S. College of Engineering, Bengaluru - 560019

Autonomous Institute Affiliated to VTU

September / October 2023 Supplementary Examinations

Programme: B.E. **Semester: V**
Branch: Biotechnology **Duration: 3 hrs.**
Course Code: 19BT5DCGEN **Max Marks: 100**
Course: Genetic Engineering **Date: 20.09.2023**

Instructions: 1. Answer any FIVE full questions, choosing one full question from each unit.
2. Missing data, if any, may be suitably assumed.

UNIT - I

1. a) The sequence of a gene of interest to be cloned in suitable plasmid is given below. Using appropriate components and enzymes insert the gene. Write the process and final design of the recombinant plasmid with GOI. **08**

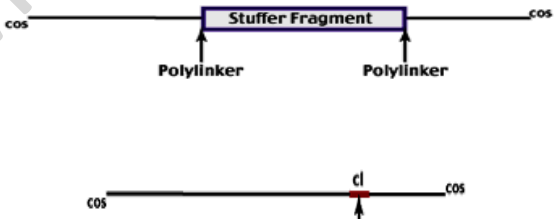
5'CCGGATTTCGACGATGACGATGACGATGCGTAGGCC3'
3'GGCCTAAAGCTGCTACTGCTACTGCTACGCATCCGG5'

b) Identify Type II RE sites in the following sequence and write it/ encircle it. Give the sizes in base pairs of each fragment that will be formed after making cut with the enzyme. **04**

5'- GTGGCCATTCTAGCGCCGAATTCGGCAATTACGATCGTAGGCCATCGATGTAATTC -3'
3'- CACCGGTAAGATCGCGGCTTAAGCCGTTAATGCTAGCATCCGGTAGCTACATTAAG -5'

c) A gene of 10kbp and 20 Kbp needs to be inserted in the phage vectors at the site given below. **04**

a. Justify your answer reasoning the choice of vector.
b. Draw appropriate figures to show how the insertion must be carried out.

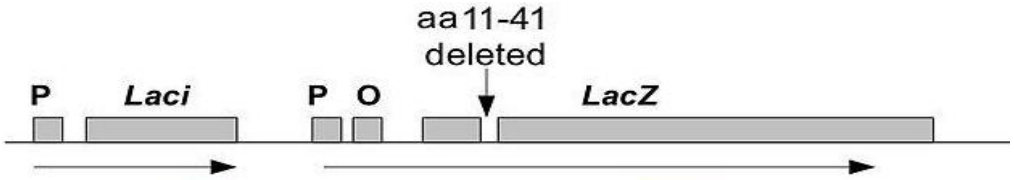


d) RNA polymerase from phages are highly specific and can be used both downstream and upstream to produce RNA with two different orientation. Validate the statement. **04**

UNIT - II

2. a) With a suitable example, compare and contrast expression and cloning vectors. **04**

b) A gene of interest being cloned in the plasmid, the selection of which is based on the following image. Write all the possible outcomes of the research conducted in cloning a GOI. **08**



c) Design and sketch the yeast vector with following features. Name the vector and its size. **06**

i) GOI cloned in SUP4 at SnaB1 site.
ii) Sequences from Centromeric and Telomeric regions of yeast chromosome
iii) URA3 and Trp as selection markers.
iv)

d) Write any two features of strong promoters. **02**

OR

3. a) Draw typical structure of a Cosmid and a Phagemid Vector. **06**

b) Design and draw a figure of vector having following features: **05**

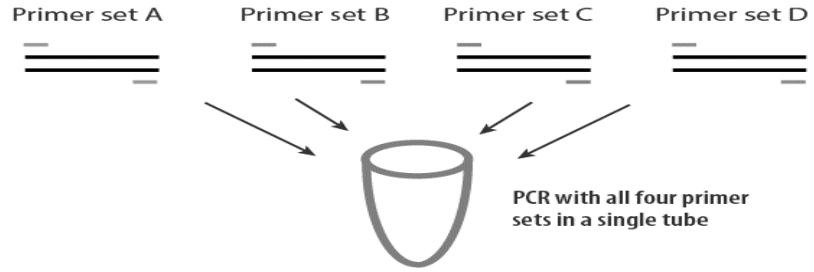
i) MCS flanking *EcoRI* and *HindIII* cloned in lac z gene.
ii) Lac promoter, driving expression of GOI cloned in MCS.
iii) Ori and amp resistance.

c) The *ADE1* and *ADE2* yeast genes encode two enzymes, phosphoribosylamino-imidazolesuccinocarbozamide synthetase and phosphoribosylamino-imidazole-carboxylase respectively in the biosynthetic pathway of adenine. Mutants of these two gene results in phenotypic variation, extensively used in selection of transformants cloned in yeast vectors. Justify the statement with detailed principle and concept behind the selection. **06**

d) Reason why codon optimization is one of the crucial steps in appropriate gene expression. **03**

UNIT - III

4. a) The figure below shows the usage of four sets of primers in a PCR reaction. Name the variant used. Explain the process and product of DNA amplification and add a note on its application. **07**



b) Researcher isolates total RNA from liver cells and would prefer to further create a cDNA library of the genes expressed in liver. Suggest appropriate protocol and the precautions the researcher needs to take while experimenting. **06**

c) The scientist separates a total DNA and transfers on the membrane and it was found that not the entire DNA has been transferred on the gel. Write the steps the scientist follows while transferring the DNA on the membrane. Where did the experiment go wrong? Suggest at least two measures which should be taken during the transfer. **07**

OR

5. a) Given below is the start reaction of a PCR. One of the primers (forward) has single point mutation. Write the variant, the process and the product. Add a note on the significance of this variants in modern Genomics. **07**

b) Genomic DNA Library screening is a cumbersome process and needs skilled technician. With the help of one of the methods justify this statement. **05**

c) Write the purpose of the following in Western blot technique: **08**

i) Transfer of protein on the membrane post SDS PAGE.
ii) Adding secondary antibody
iii) Washing after each step during blot development
iv) Staining the membrane with Ponceau prior to the addition of antibodies

UNIT -IV

6. a) With a suitable diagram, explain how micro particles can be used for delivering gene into the target cells. **06**

b) Differentiate between selectable and reporter marker genes. Categorize the following into selectable and reporter markers: **04**

i. *gfp*
ii. *npt2*
iii. *ß-gus*
iv. *hpt4*
v. *cat*

c) In foreign gene transfer technique reason why **06**

i) Sonopoaration is less efficient means of gene transfer?
ii) Biological vector mediated gene transfer is genotype dependent?
ii) Laser wave induced is preferred over direct laser beam?

d) Draw suitable figure for the following techniques, **04**

a) Microinjection
b) Electroporation

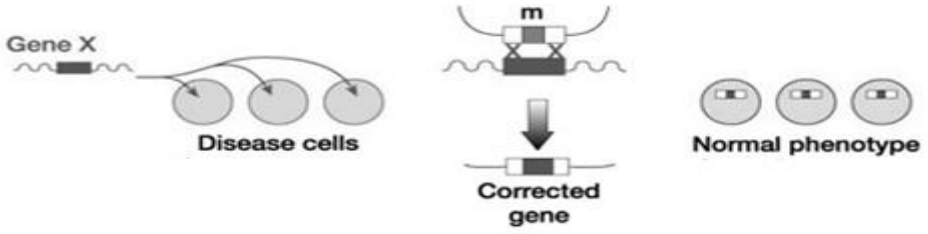
UNIT - V

7. a) CRISPR Cas-9 is an excellent tool for gene editing and can be been applied successfully in developing appropriate therapeutics. Comment on the technique with suitable figures. **04**

b) There are two important ways of gene therapy (a) and (b) in target cells. What are these two techniques? Write briefly on their advantages. **06**

(a)

(b)



c) The creation of second generation golden rice has been made possible through the knowledge of genomes of organisms. How was the rice genome modified to create Golden Rice 2? **04**

d) There are several ways of controlling pests for increase in the production and helping the plant grow healthier. Validate the statement with any two examples. **06**

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