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

Autonomous Institute Affiliated to VTU

August 2024 Supplementary Examinations

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

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) You are working with a piece of DNA of the sequence: **10**

5'-TATTGAGCTCCCGGAT-3'

3'-ATAACTCGAGGGCCTA-5'

You cut the above piece of DNA with a restriction enzyme that recognizes the sequence 5'-GAGCTC-3' and cuts on the 3' side of the A within this sequence.

(i) Draw all products that result from this digestion. Make sure to draw the nucleotide sequence of both strands, and label all 5' and 3' ends.

Now you ligate the DNA you produced in part (i) to the sequence below, which you have also cut with the same restriction enzyme.

5'-TAGAGCTCCGCAATG-3'

3'-ATCTCGAGGCGTTAC-5

(ii) Draw all products that result from this digestion. Make sure to draw the nucleotide sequence of both strands, and label all 5' and 3' ends.

Draw the shortest DNA product that could form from ligating a piece of DNA from part (i) to a piece of DNA from part (ii). Make sure to draw the nucleotide sequence of both strands, and label all 5' and 3' ends.

b) What is the significant difference between adaptors and linkers? Why adapters are better than linkers? **04**

c) It is very important to inactivate the alkaline phosphatase before you mix the vector and the fragment. Why is this? **03**

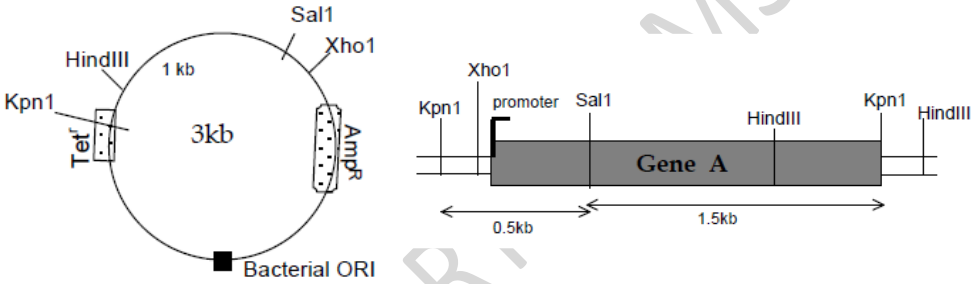
d) What is the role of polynucleotide kinase in gene cloning? **03**

UNIT - II

2 a) You purify a protein from a plant cell that can act as a potential appetite suppressant. Owing to its possible commercial application you decide to clone the gene, Gene A, which encodes this protein. You isolate this gene from the plant cell, clone it into a plasmid vector and amplify it in the bacterial cells. **10**

(i) List the important features that a plasmid must have to allow the cloning and expression of Gene A in bacterial cells.

You decide to use the following plasmid to clone Gene A. To achieve this you digest both the genomic DNA and plasmid DNA using a restriction enzyme. You then ligate the Gene A DNA into the digested plasmids. Finally, you transform the *E. coli* bacterial cells with the ligation mix (the recombinant plasmids).



Note: The recognition sites for *Kpn*I and *Sal*I on plasmid are 1 kb apart.

(ii) Which restriction enzyme (*Kpn*I, *Hind*III, *Sal*I or *Xho*I) did you use to digest Gene A for insertion into the plasmid? Briefly explain why.

(iii) Which restriction enzyme (*Kpn*I, *Hind*III, *Sal*I or *Xho*I) did you use to digest the plasmid before insertion of Gene A? Briefly explain why.

You then plate these transformed bacterial cells onto media that will allow you to distinguish between bacterial cells that obtained the plasmid and those that did not.

(iv) Onto what type of growth medium will you plate your transformation mix? Explain your answer.

b) pYAC3 is the yeast artificial chromosome that can be used to clone long pieces of DNA in yeast. **06**

(i) What can you say about the cloning strategy with pYAC3?

(ii) How do you check the presence of the insert DNA in the vector?

c) When cloning a foreign DNA fragment into a plasmid, it is often useful to insert the fragment at a site that interupts a selectable marker, which is used to identify the clones containing recombinant plasmids with foreign DNA. With **04**

a bacteriphage λ vector, it is not necessary to do this, yet one can easily distinguish vectors that incorporate large foreign DNA fragments from those that do not. How are these recombinant vectors identified?

OR

3 a) The shorthand way of describing the antibiotic resistance phenotype of bacteria that have been transformed with a plasmid carrying an antibiotic resistance gene is to write amp^R (ampicillin resistant) or ter^R (tetracyclin resistant), or if they are sensitive to both the antibiotics amp^R, tet^R. If bacteria are expressing β-galactosidase, they are said to be lacZ positive, which can be written as $lacZ^+$; if not, then lacZ negative, which can be written as $lacZ^-$. Write down the shorthand for the phenotype of: **08**

(i) *E. coli* transformed with pBR322.

(ii) *E. coli* transformed with a recombinant plasmid derived from pBR322 but with an insert cloned into the tetracycline resistant gene.

(iii) A strain of *E. coli*, with a deletion of the portion of the lac Z gene which encodes the peptide of β-galactosidase, transformed with pUC18.

(iv) What color would the colonies be if a fragment of DNA had been cloned into the multiple cloning site of pUC18 and the transformants are plated on ampicillin and X-gal? Justify your answer.

b) NASA astronauts found a mysterious gene from the H zone of outer space. They needed your help to know more about the gene product, so they cloned it into a bacterial expression vector and they sent you the plasmid, that they named pSET (plasmid "Sequence ExtraTerrestrial"). **08**

(i) Which one of the following elements should be in the vector? Circle all that apply.

Centromere	Multiple cloning sites	Nuclear localization signal
Telomere	Inducible terminator	Bacterial origin of replication
Selectable marker	Nucleoid insertion sites	Cytosolic targeting sequences
Inducible promoter	Topoisomerase gene	Gene encoding restriction enzyme

You rename the gene present in the pSET vector as GOSH for "gene from the outer space H" and the protein coded by this gene as POSH for "protein from the outer space H". In the hope of expressing gene, you transformed bacterial cells (*E. coli)* with recombinant pSET vector and tried to express the gene.

Surprisingly you found that gene product was toxic to the host cell even when synthesized in small amounts. To overcome this problem you had drawn the strategy for regulating the expression of gene. As a part your strategy you cut the GOSH gene out of this vector and cloned into pET vector.

(ii) What motive is there in selecting pET vector?

(iii) Explain the general strategy involved in regulating the expression of gene using pET vector.

c) The retroviral vectors make possible the efficient intergation of foreign DNA into a mammalian genome. Explain how these vectors, which lack genes for replication and viral packaging (*gag*, *pol*, *env*), are assembled into infectious viral paticles. Suggest why it is important that these vectors lack the replication and packaging genes. **04**

UNIT - III

4 a) In a hypothetical scenario you wake up one morning in the hostel to your roommate exclaiming about her sudden hair growth. She has been supplementing her diet with a strange new fungus purchased at the local market. You take samples of the fungus to your lab and you find that this fungus does indeed make a protein (the harE protein) that stimulates hair growth. You construct a fungal genomic DNA library in E. Coli with the hope of cloning the harE gene. If you succeed you will be a billionaire! You obtain DNA from the fungus, digest it with a restriction enzyme, and clone DNA fragments into a vector. **12**

You could screen your library by hybridization with a probe.

(i) What information would you need to do this screen?

(ii) What would you use as a probe?

(iii) Would the entire harE gene need to be present in a recombinant vector for your screen to work? Explain.

You screen your library by hybridization with a probe and identify a recombinant vector that contains the complete harE gene. In the meantime, you have developed an antibody to the harE protein. You use cells carrying the recombinant vector that contains the complete harE gene to test how well this antibody reacts with the harE protein. You do the experiment and find that the antibody does not react with the cells containing the recombinant vector.

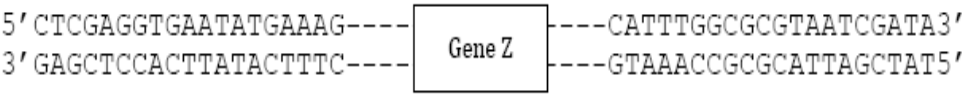
(iv) Does this result indicate that your antibody does not react to the harE protein? Explain.

You make a second library, a cDNA library. You plan to transform bacterial cells with this new library and then screen for a colony whose cells are making the harE protein using your antibody.

(v) Describe what is cDNA and how it differs from genomic DNA.

(vi) What features must be present on your cloning vector that will allow you to use this to make fungal cDNA library and successfully identify a colony whose cells are making the harE protein using your antibody?

b) The following is the DNA sequence of the wild type allele of Gene Z that you want to amplify using the polymerase chain reaction (PCR). **08**



(i) If you amplify a DNA sequence through PCR what are the reaction components that you would absolutely need? Briefly state the function of each of these components.

(ii) Which set of following primers would you use for PCR reaction in part (i)?

Set 1: 5’TACACTTATACTTTC3’ and 3’GTAAACCGCGCATTAG5’

Set 2: 5’CTCGAGGTGAATAT3’ and 3’CCGCGCATTAGCTAT5’

Set 3: 5’GAGTTACACTTATAC3’ and 3’TGGCGAGTAATCGATA5’

(iii) In the PCR reaction, you need a three-step reaction cycle, which results in a chain reaction that produces an exponentially growing population of identical DNA molecules. Each step of a reaction cycle is performed at a specific temperature i.e. 95℃ for Step 1, 55 ˚C for step 2 and 70 ˚C for Step 3. Briefly explain why the three steps are performed under different temperatures.

OR

5 a) Your Major project focuses on two genes, Par1 and Mst5, which you suspect are involved in the embryonic development of the mouse. To study these genes, you isolate total DNA, total RNA, and total protein samples from mice at the different developmental stages. The expression of Par1 and Mst5 during development may be controlled at the RNA level (by regulating either transcription or mRNA stability) or at the protein level (by regulating translation or protein stability). To begin to understand Par1's regulation, you decide to perform Northern and Western blots on your samples. **10**

Compare and contrast the Northern and Western blot procedures by

completing the following table:

	Northern blot	Western blot
What method(s) will you use to denature your samples?		
What type of gel will you run?		
In what direction will your samples migrate in the gel? What ensures this?		
What specific type of "probe" will you use to detect Par1 mRNA and protein?		

b) How do you separate supercoiled DNA from linear and open-circular DNA molecules based on their buoyant density? Justify your answer. **06**

c) A researcher is doing a PCR-based experiment using the following temperature profile: **04**

- Melt for 1 minute at 94°C
- Anneal for 30 seconds at 37°C
- Extend for 30 seconds at 70°C

Unfortunately, her gels show way too many bands. Her primers have perfect complementarity to the target sequence. Give a simple way to reduce the number of bands.

UNIT - IV

6 a) In order to make a transgenic plant one can use direct gene transfer, which is very inefficient, or Agrobacterium-mediated transfer. Answer the following questions about Agrobacterium-mediated gene transfer. **10**

(i) What is Agrobacterium tumefaciens and what are the important features of tumor inducing (Ti) plasmid?

(ii) How are plant derived signals recognized and how is T-DNA transferred from agrobacterium to plant cells?

(iii) Binary vector systems for plant transformation employ two plasmids. Why must a binary vector (co-transformation) scheme be used to make a transgenic plant?

b) One objection that has been raised to the use of GM crops is the possibility that the cloned gene might escape from the engineered plant and become established in a weed species. However, one way of making such transfer totally impossible would be to place the cloned gene not in the nucleus but in **06**

the plant's chloroplasts. How do you achieve the synthesis of a protein (δ-endotoxin) in transgenic chloroplast?

c) Electroporation is a method of direct gene transfer. It is only effective on animal cells or plant protoplasts (i.e. cells without walls). How does this process work and what are its drawbacks? **04**

UNIT - V

7 a) Recently, CRISPR-Cpf1 has emerged as a new tool for efficient genome editing, including DNA-free editing in plants, with higher efficiency, specificity, and potentially wider applications than CRISPR-Cas9. How does CRISPR-Cpf1 differ from CRISPR-Cas9 in four important ways? **08**

b) Examine the use of antisense RNA technology involved in the engineering of plants for extented shelf-life of fruits. **08**

c) "Gene therapy is boon to mankind". Apprise the role of suicide gene therapy in the treatment of cancer. **04**

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