Ahmedabad University

School of Arts and Sciences

BIO502: Plant Biotechnology and Tissue Culture

End Term Examination 2024 (Winter Semester)

Total Marks = 50 **Time – 2.5 hours**

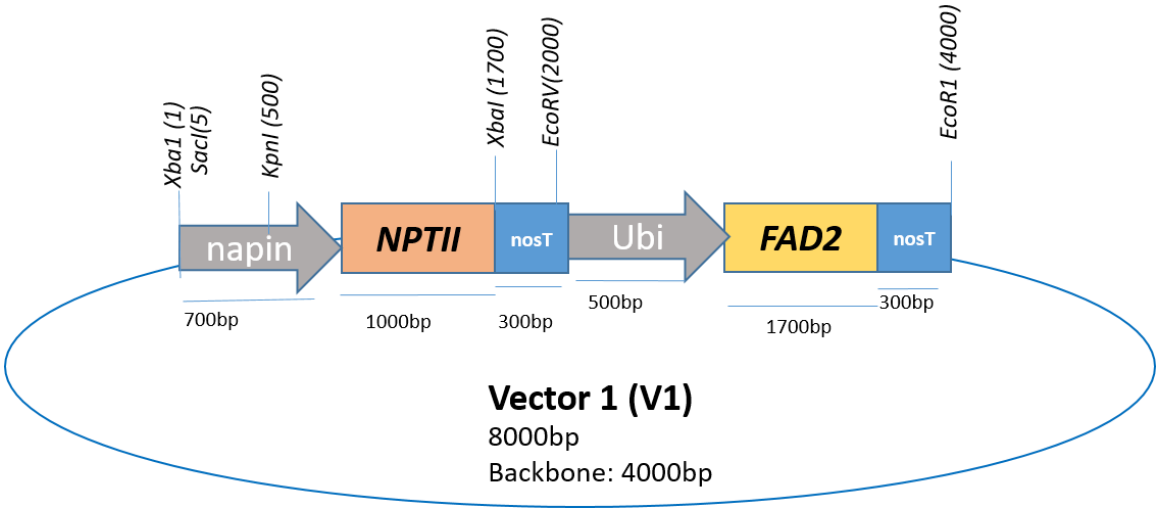
Date: 20-04-2024
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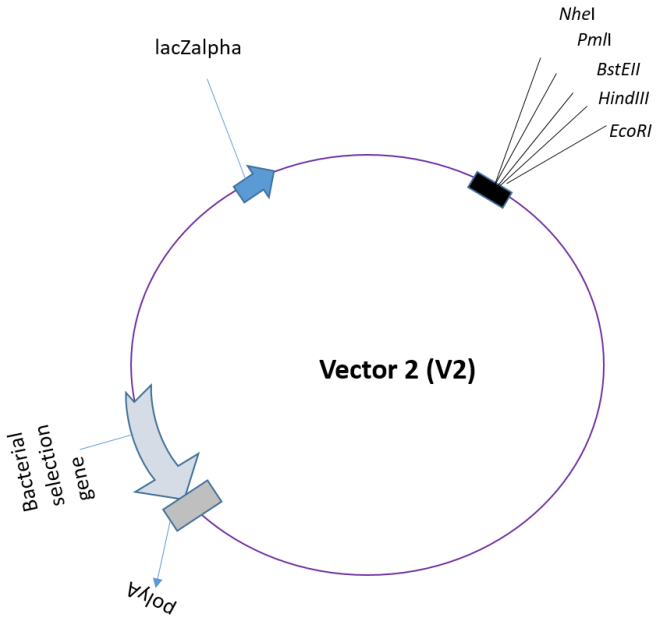
Instructions:

- This is a 50 marks and 2.5 hour question paper.
- There are three sections in the question paper
- The bold numbers written in the brackets indicate marks of each question.
- For the crossword puzzle, answer in the boxes, and not in the answer sheet. The number of alphabet characters for each crossword is written in the bracket.
- Answer using pens only; answers written with pencil(s) will not be graded
- Be precise and concise in your answer. Lengthy answers could result in the grade loss.

Section 1 (30 points)

You are given two vectors – vector 1 (V1) and vector 2 (V2). In V1, the *NPTII* is expressed under seed specific napin promoter, while oil producing *FAD2* enzyme is expressed under constitutive 35S promoter. With the help of the figures and information provided, answer the following:





1. You want to clone *NPTII* and *FAD2* expression cassettes from V1 into V2. Briefly describe the cloning strategy in terms of restriction enzymes selection, and its impact on cloning into V2 (**5 points**)

2. Using gene gun, you performed a transformation of vector V1 (as it is) without any alteration in maize embryogenic calli. Describe the impact of transformation the generation of transgenic events. (**5 points**)

3. If you are given a chance to design vector to enhance oil production; which expression cassettes, restriction enzyme combinations and vector backbone features you will choose. Support your answer with figures and its description?(**10 points**)

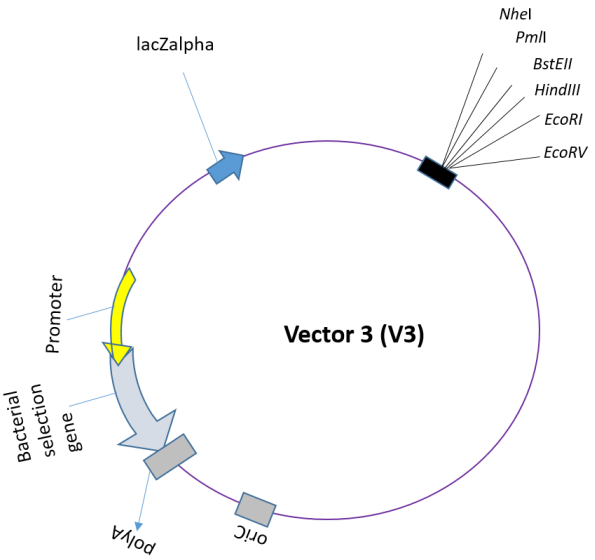
4. Refer the sequences 1 (DNA) and 2 (coding) of the same gene from *Arabidopsis* and vector 3 (V3) and answer the following questions (the small letters indicate introns)

Sequence 1

AGATATTTTGATTTCTGCTAAAGCTGTCTGATAAAAAGAAGAGGAAAACTCGAAAAAGCTACACACAAG
AAGAAGAAGAAAAGATACGAGCAAGAAGACTAAACACGAAAGCGATTTATCAACTCGAAGGAAGAGAC
TTTGATTTTCAAATTTCGTCCCCTATAGgtacgttgatttctgatttcttacaaattttcaattaatttctctgattaggtctcgtcaattggtgattctagggtt
tatcttcgtctttaggttttcgattatttccttttaggtatagctgtgaaattgggaaattttaggtgttcctaatttgataacaaataagtaaatcgatctgattttgaaccaagtttaagttc
tcttgccttatgtttcagcttgtgttctgatattgaagtgttgttgtattgtagATTGTGTTGTTTCTGGGAAGGAG**ATG**GCAGTTTATGATCA
GAGTGGAGATAGAAACAGAACACAAATTGATACATCGAGGAAAAGGAAATCTAGAAGTAGAGGTGACG
GTACTACTGTGGCTGAGAGATTAAAGAGATGGAAAGAGTATAACGAGACCGTAGAAGAAGTTTCTACCA
AGAAGAGGAAAGTACCTGCGAAAGGGTCGAAGAAGGGTTGTATGAAAGGTAAAGGAGGACCAGAGAAT
AGCCGATGTAGTTTCAGAGGAGTTAGGCAAAGGATTTGGGGTAAATGGGTTGCTGAGATCAGAGAGCCT
AATCGAGGTAGCAGGCTTTGGCTTGGTACTTTCCCTACTGCTCAAGAAGCTGCTTCTGCTTATGATGAGGC
TGCTAAAGCTATGTATGGTCCTTTGGCTCGTCTTAATTTCCCTCGGTCTGATGCGTCTGAGGTTACGAGTA
CCTCAAGTCAGTCTGAGGTGTGTACTGTTGAGACTCCTGGTTGTGTTCATGTGAAAACAGAGGATCCAGA
TTGTGAATCTAAACCCTTCTCCGGTGGAGTGGAGCCGATGTATTGTCTGGAGAATGGTGCGGAAGAGATG
AAGAGAGGTGTTAAAGCGGATAAGCATTGGCTGAGCGAGTTTGAACATAACTATTGGAGTGATATTCTG
AAAGAGAAAGAGAAACAGAAGGAGCAAGGGATTGTAGAAACCTGTCAGCAACAACAGCAGGATTCGCT
ATCTGTTGCAGACTATGgttggcccaatgatgtggatcagagtcacttggattcttcagacatgtttgatgtcgatgagcttctacgtgacctaaatggcgacgat
gtgtttgcagGCTTAAATCAGGACCGGTACCCGGGGAACAGTGTTGCCAACGGTTCATACAGGCCCGAGAGTC
AACAAAGTGGTTTTGATCCGCTACAAAGCCTCAACTACGGAATACCTCCGTTTCAGCTCGAGGGAAAGGA
TGGTAATGGATTCTTCGACGACTTGAGTTACTTGGATCTGGAGAAC**TAA**ACAAAACAATATGAAGCTTTT
TGGATTTGATATTTGCCTTAATCCCACAACGACTGTTGATTCTCTATCCGAGTTTTAGTGATATAGAGAAC
TACAGAACACGTTTTTTCTTGTTATAAAGGTGAACTGTATATATCGAAACAGTGATATGACAATAGAGAA
GACAACTATAGTTTGTTAGTCTGCTTCTCTTAAGTTGTTCTTTAGATATGTTTTATGTTTTGTAACAACAGG
AATGAATAATACACACTTGTGAAGCTTTATGGGCC

Sequence 2:

ATGGCAGTTTATGATCAGAGTGGAGATAGAAACAGAACACAAATTGATACATCGAGGAAAAGGAAATCT
AGAAGTAGAGGTGACGGTACTACTGTGGCTGAGAGATTAAAGAGATGGAAAGAGTATAACGAGACCGTA
GAAGAAGTTTCTACCAAGAAGAGGAAAGTACCTGCGAAAGGGTCGAAGAAGGGTTGTATGAAAGGTAA
AGGAGGACCAGAGAATAGCCGATGTAGTTTCAGAGGAGTTAGGCAAAGGATTTGGGGTAAATGGGTTGC
TGAGATCAGAGAGCCTAATCGAGGTAGCAGGCTTTGGCTTGGTACTTTCCCTACTGCTCAAGAAGCTGCT
TCTGCTTATGATGAGGCTGCTAAAGCTATGTATGGTCCTTTGGCTCGTCTTAATTTCCCTCGGTCTGATGC
GTCTGAGGTTACGAGTACCTCAAGTCAGTCTGAGGTGTGTACTGTTGAGACTCCTGGTTGTGTTCATGTGA
AAACAGAGGATCCAGATTGTGAATCTAAACCCTTCTCCGGTGGAGTGGAGCCGATGTATTGTCTGGAGAA
TGGTGCGGAAGAGATGAAGAGAGGTGTTAAAGCGGATAAGCATTGGCTGAGCGAGTTTGAACATAACTA
TTGGAGTGATATTCTGAAAGAGAAAGAGAAACAGAAGGAGCAAGGGATTGTAGAAACCTGTCAGCAAC
AACAGCAGGATTCGCTATCTGTTGCAGACTATGGTTGGCCCAATGATGTGGATCAGAGTCACTTGGATTC
TTCAGACATGTTTGATGTCGATGAGCTTCTACGTGACCTAAATGGCGACGATGTGTTTGCAGGCTTAAATC
AGGACCGGTACCCGGGGAACAGTGTTGCCAACGGTTCATACAGGCCCGAGAGTCAACAAAGTGGTTTTG
ATCCGCTACAAAGCCTCAACTACGGAATACCTCCGTTTCAGCTCGAGGGAAAGGATGGTAATGGATTCTT
CGACGACTTGAGTTACTTGGATCTGGAGAAC**TAA**



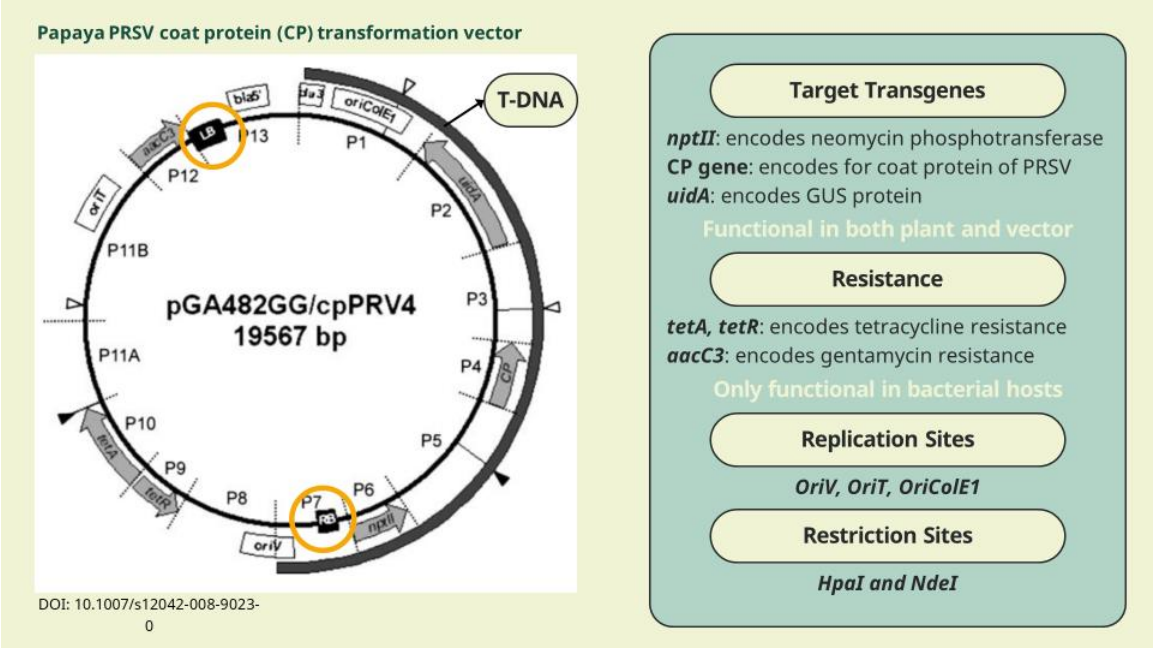
4a: A researcher wanted to clone the two DNA and coding sequences in the vector V3 separately. Describe a strategy of cloning the sequence from Arabidopsis. (**5 points**).

4b: After successful cloning and transformation of the both the sequences in rice calli, researcher observed that the cloned DNA sequence in the regenerated transgenic seedling did not work well, while the coding sequence showed the positive integration and phenotype. What factors could have led to the results variability. Justify your answers with the reasoning (**5 points**).

Section 2 (13 points):

5. The *VirD1/D2* genes got mutated in the Agrobacterium plasmid, while other *vir operon* remained intact. Describe the sequential effect of the mutations on the host-pathogen interaction (**5 points**)

6: Refer the following figure from transgenic papaya group presentation and answer:



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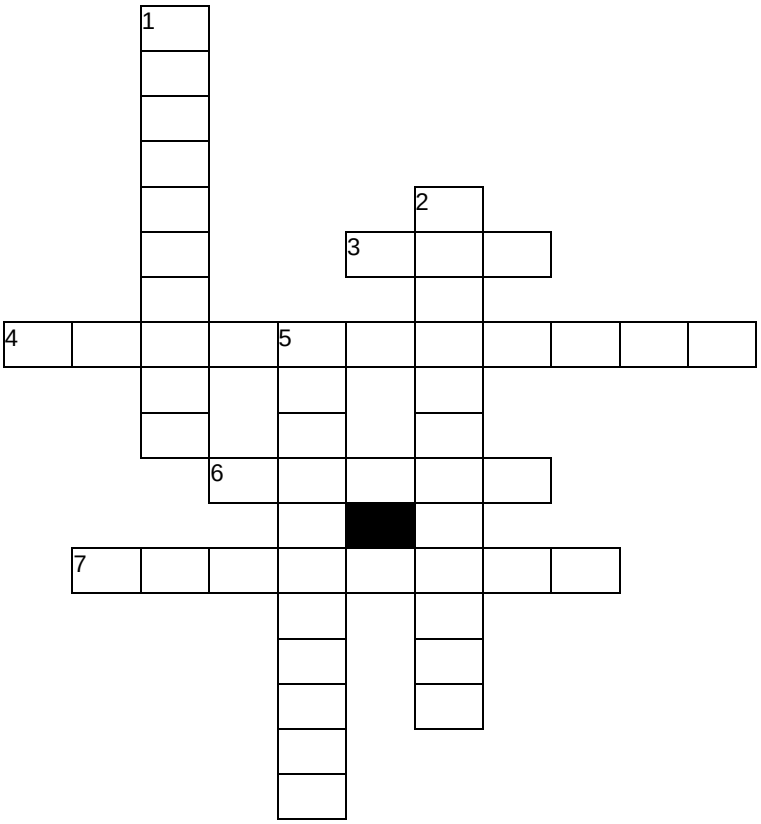
6a: Which mode of transformation was used for creating transgenic papaya and why? (**3 points**)

6b: Outline functions of each target transgenes and resistance genes. Why there were two or more replication sites in the plasmid? (**5 points**)

Section 3 (7 points)

Name: _____ Date: _____

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Across

3. Agrobacterium plasmid is a ..... copy plasmid

4. DNA is complexed with histones to form.....

6. Disruption of lacZ gene in the TOPO cloning results in .....colony appearance; potentially a positive clone

7. Red Fluroscent Protein and luciferase are .....genes

Down

1. HPTII hydrolyses. ..... is the tissue culture media

2. CaMV35S is a .....promoter

5. Single stranded T-DNA integration occurs in the..... region of the host cell chromosome