PLNT2530 PLANT BIOTECHNOLOGY

FINAL EXAMINATION

Friday, April 12, 2019 09:00 to 11:00 Agriculture 138

Answer any combination of questions totalling to exactly 100 points. If you answer questions totalling more than 100 points, answers will be discarded at random until the points equal 100. This exam is worth 40% of the course grade. The questions available total to 120 points.

Hand in these question sheets along with your exam book. Question sheets will be shredded.

Ways to write a readable and concise answer:
i. Just answer the question. Save time by specifically addressing what is asked. Don't give irrelevant background if it doesn't contribute to the question that was asked.
ii. Avoid stream of consciousness. Plan your answer by organizing your key points, and then write a concise, coherent answer. Make your point once, clearly, rather than repeating the same thing several times with no new information.
iii. Point form, diagrams, tables, bar graphs, figures are welcome. Often they get the point across more clearly than a long paragraph.
iv. Your writing must be legible. If I can't read it, I can't give you any credit.

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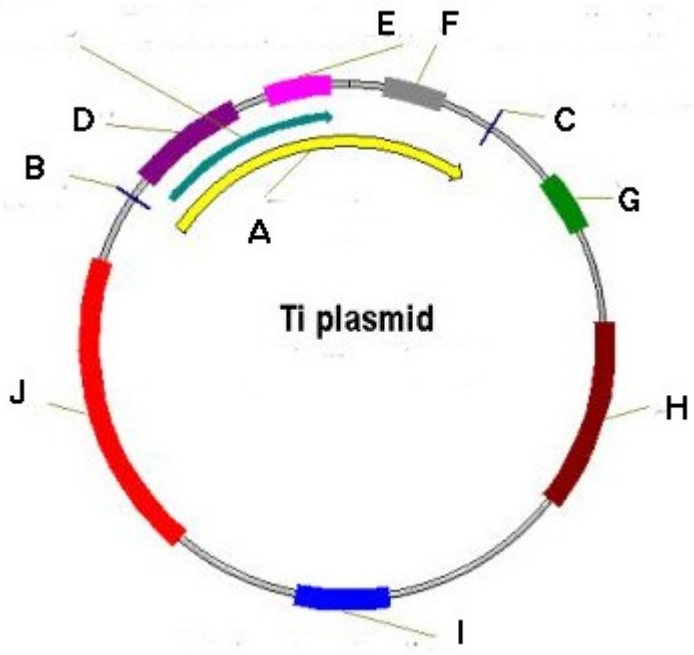
1. (10 points) Explain the distinction between the following terms, with reference to biotech crops: homozygous, heterozygous, hemizygous, and biallelic. Using standard genetic notation (eg. X for dominant, x for recessive and so forth) how would the genotypes for each be represented?

2. (5 points) Describe the distinction between transformation and transient expression.

3. (10 points) For the map of the octopine Ti plasmid shown below, give the name for each feature marked with a letter, A - J. You do not need to describe each feature, just list its name.



4. (10 points) DNA sequencing reads are aligned below, with the final consensus at top. At positions 17 and 72, not all reads agree. One of them is probably a sequencing error. The other is not an error. State which position has the error, and explain your reasoning. Assuming that the other position is not an error, explain the reason for the results shown. (Hint: assume the sequence came from the plant nuclear genome.)

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          1         2         3         4         5         6         7         8         9         10
  12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
  ACTTTTGCTGGAGCTGACCCAATTGTCGCCAAAACTAGAGATATTTCTGTCACTGGTGGTACTGGAGATTTSTTATGCATAGAGGAATTGCTACTAT
6- ACTTTTGCTGGAGCTGACCCAATTGTCGCCAAAACTAGAGATATTTCTGTC
7- ACTTTTGCTGGAGCTGACCCAATTGTCGCCAAAACTAGAGA
5              GCTGACCCAATTGTCGCCAAAACTAGAGATATTTCTGTCACTGGTGGTACTGGAGATTTCT
2  ACTTTTGCTGGAGCTGACCCAAT
4                GGCCCAATTGTCGCCAAAACTAGAGATATTTCTG
12-                          AACTAGAGATATTTCTGTCACTGGTGGTACTGGAGATTTGTTATGCATAGAGG
1-                                                   TGGAGATTTCTTATGCATAGAGGAATTGCTACTAT
3-                                                CACTGGTGGTACTGGAGATTTGTTATGCATAGAGGAATTGCTACTAT
9-                                             TTCTGTCACTGGTGGTACTGGAGATTTGTTATGCATAGAGGAATTGCTACTAT
8                                           ACTAGAGATATTTCTGTCACTGGTGGTACTGGAGATTTCTTATGC
11                                                GATATTTCTGTCACTGGTGGTACTGGAGATTTCTTT
10                                                                          TTGTTATGCATAGAGGAATTGCTACTAT

- after a number indicates that the opposite strand has been used
  S stands for C or G
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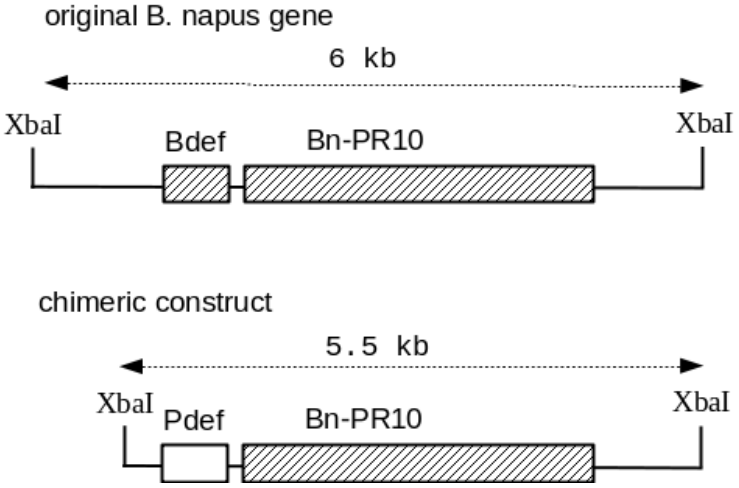
5. (10 points) Draw a simple diagram illustrating how the recombinant Cas9/sgRNA complex works in CRISPR gene-editing. Make sure to label the critical parts, especially the tracRNA and crRNA regions, PAM site, chromosomal DNA, and indicate possible double-stranded cut sites. What is the purpose of the trRNA and crRNA regions in the sgRNA?

6. (5 points) Why is wounding important in *Agrobacterium* infection of plant tissue?

7. (10 points) A construct was made in which the promoter of the Brassica defense gene PR10 was replaced by the a promoter from the PR10 gene of pea (Pdef).

This chimeric construct was transformed into B. napus. In order to identify segregating progeny that had the transgene, it was decided that Southern blots would be done. Draw what you would expect to see in Southern blots from Wild type (WT) or transformants segregating for the gene (TT, T0, 00). Assume that DNA was cut with XbaI. Show the results that would be seen using the following probes:



a) The B. napus coding sequence, Bn-PR10

b) The pea promoter only (Pdef).

8. (10 points) Some insect populations develop resistance to the Bt toxin. Is resistance to Bt dominant or recessive? Explain why.

9. (15 points) The bacterium *Erwinia carotovora* causes tuber soft rot in potatoes. Potato cells from the susceptible line Iwa were transformed with a gene encoding the protein magainin. Magainin inhibits growth of prokaryotic organisms by disrupting their cell membranes. Table 1 lists results from leaves of transformed plants from the T0 generation (MgD1 - MgD50) . Table 2 lists disease scores from tubers of transgenic lines at different times after harvesting, ranging from 6 - 12 weeks. (Recall that potatoes are propagated vegetatively, rather than sexually, by making cuttings from tubers and planting them in the soil. In essence, plants from cuttings are clones of the original parent.) Disease severity is scored on a scale of 0 to 10, where 0 is the least severe, and 10 the most severe.

Table 1. Summary of Molecular Analyses. Southern Analysis, RT-PCR and Western Analysis of the 26 Independently Derived MagaininD-Transgenic Potato Lines. RT-PCR and MagaininD Peptide Expression is Shown as: '+' Indicating Expression, '-' Indicating no Expression, '?' Indicating Possible Expression, but Band was Very Faint

Plant Line	Southern Copy Number	RT-PCR	Peptide Expression
MgD1	1	+	+
MgD2	1	+	?
MgD3	6	+	?
MgD4	1	+	?
MgD5	1	+	+
MgD6	2	+	-
MgD8	6	+	-
MgD9	6	+	+
MgD10	4	+	-
MgD15	2	+	-
MgD16	7	+	-
MgD17	1	+	-
MgD18	5	+	+
MgD19	5	+	-
MgD22	2	+	-
MgD24	4	+	-
MgD25	2	+	-
MgD27	1	-	-
MgD28	1	+	-
MgD29	1	+	-
MgD30	1	+	-
MgD32	1	+	-
MgD33	5	+	-
MgD34	2	+	-
MgD39	5	+	+
MgD50	2	+	-

Table 2. Soft Rot Assays. Four Independent Soft Rot Assays were Performed on Tuber Produced on Field Grown Plants Over Three Consecutive Years: Assay 1 (2000/01 Season, 6 Weeks Tuber Storage); Assay 2 (2001/02 Season, 10 Weeks Tuber Storage); Assay 3 (2002/03 Season, 6 Weeks Tuber Storage) Assay 4 (2002/03 Season, 12 Weeks Tuber Storage)

Line	Assay 1	Assay 2	Assay 3	Assay 4
Iwa	2.62	4.62	4.84	4.67
MgD1	1.46	2.26	2.06	2.26
MgD2	1.96	5.13	4.58	3.99
MgD3	3.77	4.41	5.68	5.87
MgD4	4.11	4.49	4.99	5.45
MgD5	2.15	2.20	3.03	2.92
MgD9	3.73	4.69	4.47	3.94
MgD39	2.05	2.13	1.95	2.03
Vector control	3.21	-	6.20	4.92
Cr4#2	9.81	6.80	9.99	9.49
A206	0.32	1.26	1.05	1.35
LSR 5%	1.737	1.224	1.262	1.328

LSR: Least significant ratio (larger mean/smaller mean) for two means to be significantly different at the 5% level. Degrees of freedom were 99 for assays 1, 3, and 4, 90 for assay 2. The figures are back-transformed values of the mean of the logarithm-transformed data (n=10).

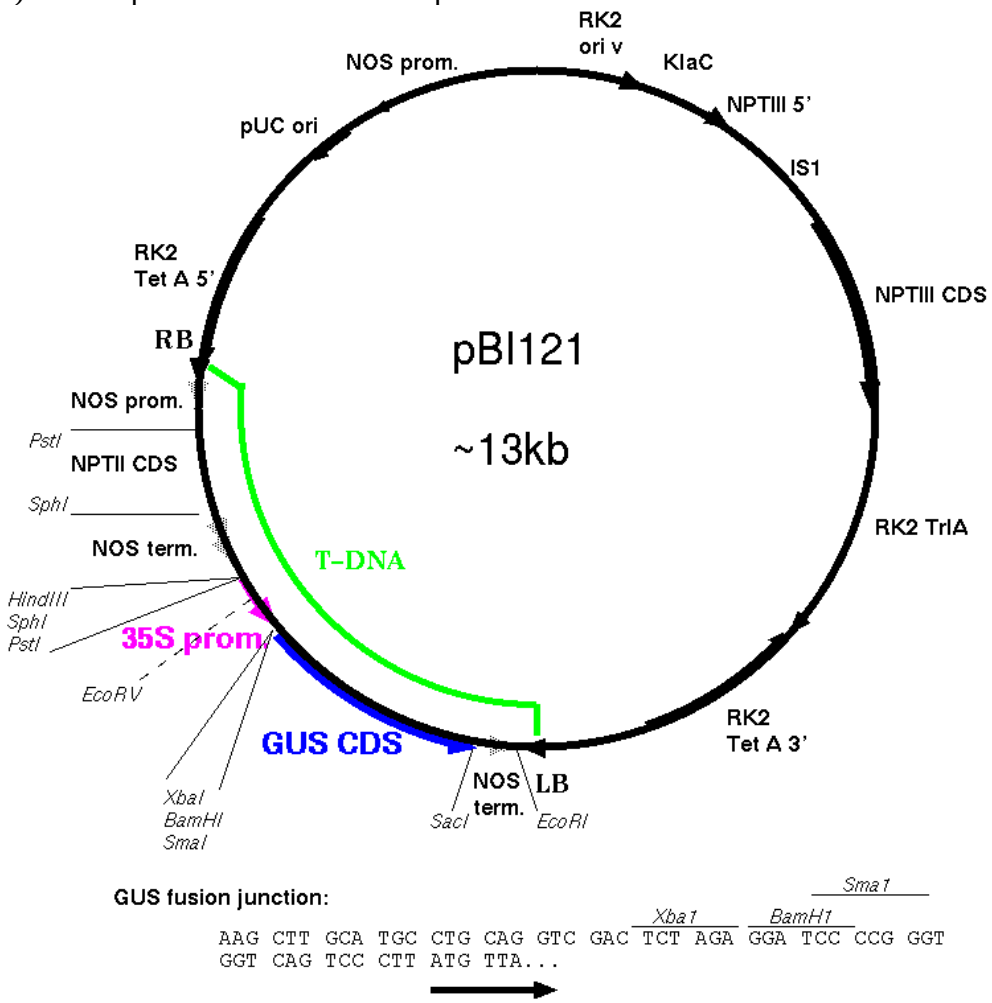
a) Based on these results, what can you conclude about the effects of magainin expression on resistance to *E. carotovora*? Cite results that support your conclusion. (You can ignore Cr4#2 and A206.)

b) Recalling that T0 plants are hemizygous for each transgene, we might expect better results if we could get plants that were homozygous. However, since potatoes are not sexually propagated, we can't do crosses to obtain homozygotes. How might CRISPR technology solve this problem?

c) The authors used "billions" of bacterial cells per inoculum to test for disease resistance. This level of inoculum is unlikely to be encountered in the field. Only 10^6 - 10^7 cells are needed to give disease symptoms on susceptible plants. Based on this information, propose a better test for resistance.

10. (15 points) To which component of peanuts are people allergic? Genetic engineering of peanuts to eliminate allergenicity is far more difficult than most traits eg. herbicide resistance, insect resistance. Why is allergenicity so difficult to eliminate? Describe the approach that was used to decrease allergenicity in peanuts. Was it successful?

11. (10 points) The map of the T-DNA vector pBI121 is shown below.



For each component listed below, use a sentence or phrase to describe its purpose in the vector:

pUC ori
RK2 ori v
NPTII CDS (within T-DNA)
NPTIICDS (outside of T-DNA)
GUS CDS

12. (5 points) *Agrobacterium* is not able to infect monocotyledonous plants such as rice, maize or wheat. List two alternative ways to transform monocots.

13. (5 points) The problem with genetic engineering in apple is that apple trees require 5 years to reach maturity. How did the developers of Arctic apple speed up the process so that they could obtain fruit in a shorter time after transformation?