PLNT2530 PLANT BIOTECHNOLOGY

FINAL EXAMINATION

Friday, April 14, 2020 18:00 to 20:30 UMLearn Quiz

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) 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              GCTGACCCAATTGTCGCCAAAACTAGAGATATTTCTGTCACTGGTGGTACTGGAGATTTC
2  ACTTTTGCTGGAGCTGACCCAAT
4                 GGCCCAATTGTCGCCAAAACTAGAGATATTTCTG
12-                        AACTAGAGATATTTCTGTCACTGGTGGTACTGGAGATTGTTATGCATAGAGG
1-                                                 TGGAGATTCTTATGCATAGAGGAATTGCTACTAT
3-                                              CACTGGTGGTACTGGAGATTGTTATGCATAGAGGAATTGCTACTAT
9-                                           TTCTGTCACTGGTGGTACTGGAGATTGTTATGCATAGAGGAATTGCTACTAT
8                                    ACTAGAGATATTTCTGTCACTGGTGGTACTGGAGATTCTTATGC
11                                         GATATTTCTGTCACTGGTGGTACTGGAGATTCTT
10                                                                    TGTTATGCATAGAGGAATTGCTACTAT
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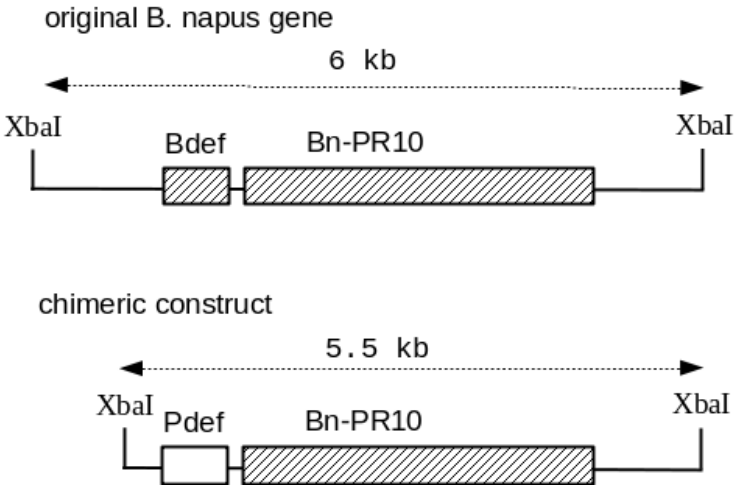
- after a number indicates that the opposite strand has been used
S stands for C or G

2. (5 points) Why is wounding important in *Agrobacterium* infection of plant tissue?

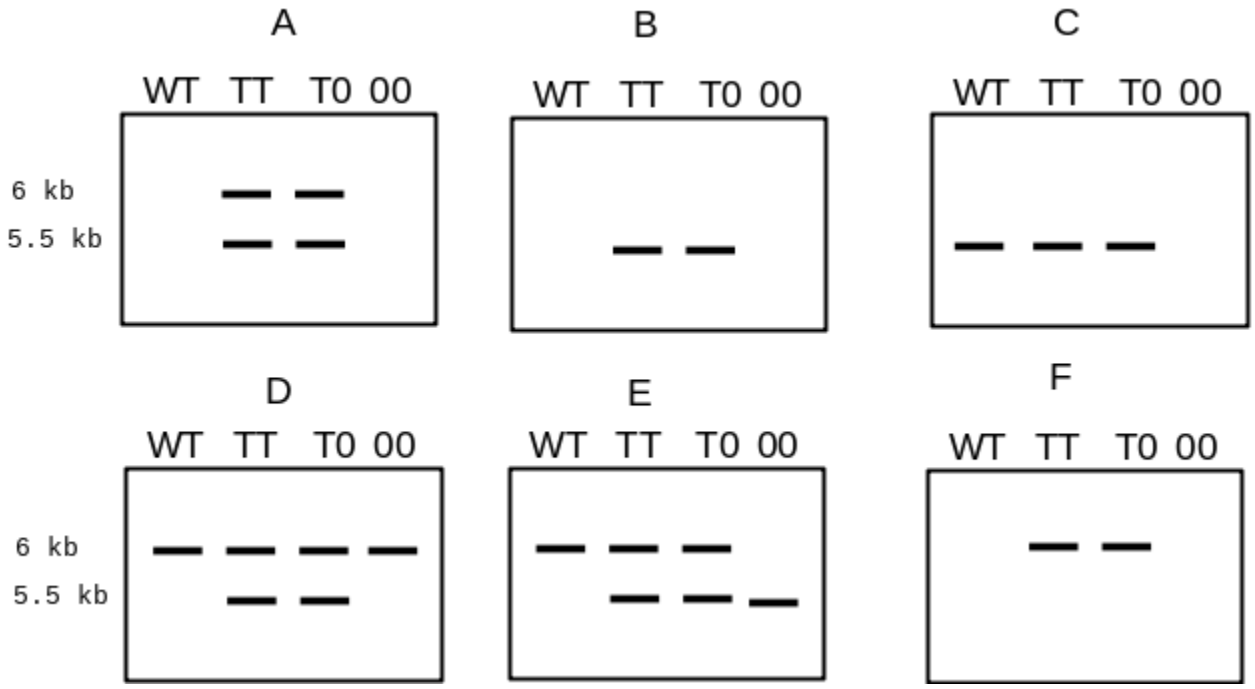
3. (10 points) A construct was made in which the promoter of the Brassica defense gene PR10 was replaced by the a promoter from the PR10 gene of pea (Pdef).

This chimeric construct was transformed into B. napus. To identify segregating progeny that had the transgene, Southern blots were done using DNA from Wild type (WT) or transformants segregating for the gene (TT, T0, 00). Assume that DNA was cut with XbaI. Which of the southern blots (A - F) would be seen if each of the following was used as a probe?



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

ii) The pea promoter only (Pdef).



4. (10 points) Some insect populations develop resistance to the Bt toxin. Is resistance to Bt dominant or recessive? Explain why.

5. (15 points) In class we discussed a paper whose goal was to decrease the allergenicity of peanuts by silencing genes for the allergenic proteins in the Arah2 family.

a) For each of the experiments shown below (Coomassie blue stained SDS gel, Western blot, ELISA assay), what do the results tell us?

b) What are the controls, and what do they tell us?

c) In 1 sentence, state the main conclusion of these experiments, taken together?

ELISA assay for Ara2 protein from peanut seeds.

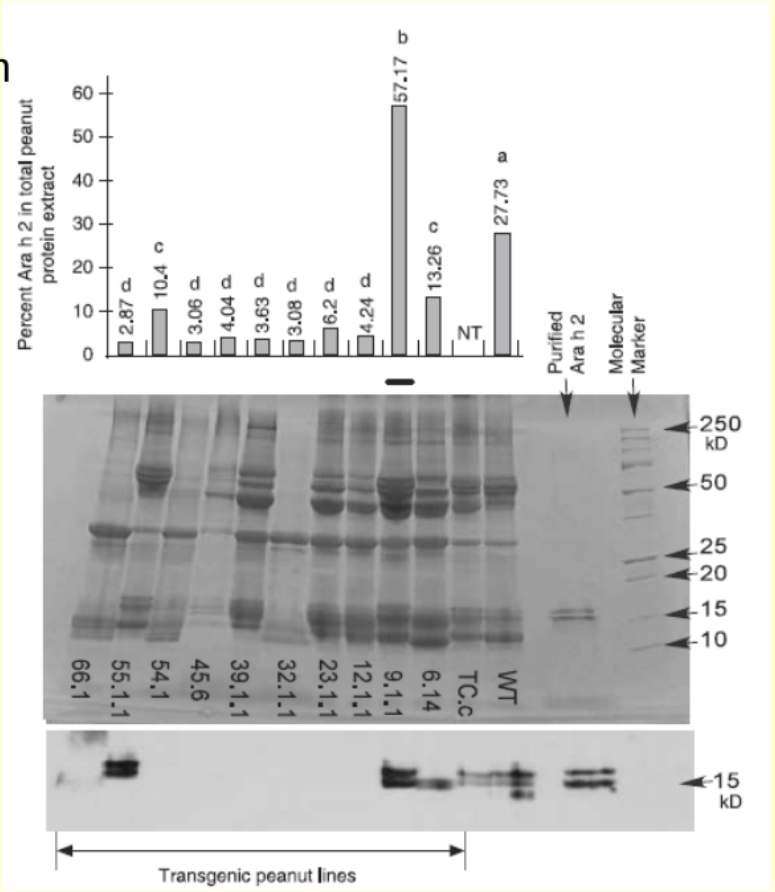
Numbers followed by same letters are not significantly different.

Coomassie blue stained SDS-PAGE gel of proteins from transgenic peanuts

WT - wildtype seeds

TC.c - WT tissue culture cells

Western blot with anti-Arah2 antibodies



6. (5 points) *Agrobacterium* is not able to infect monocotyledonous plants such as rice, maize or wheat. List two physical methods to introduce foreign DNA into moncots.

7. (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?

8. (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 MagininD-Transgenic Potato Lines. RT-PCR and MagininD 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).

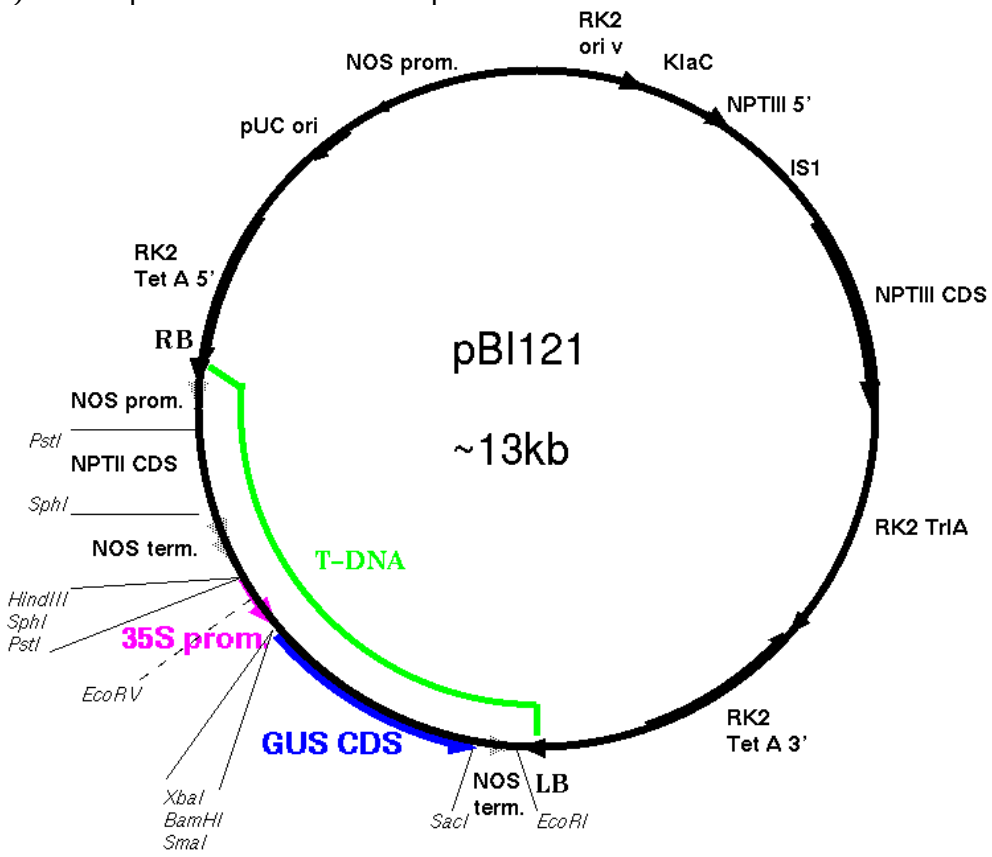
a) Based on these results, what can you conclude about the effects of magainin expression on resistance to *E. carotovora*? Cite results that support your conclusion. (You can ignore Cr4#2 and A206.)

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.

9. (10 points) 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 engineer peanut plants with reduced allergenicity. Was it successful?

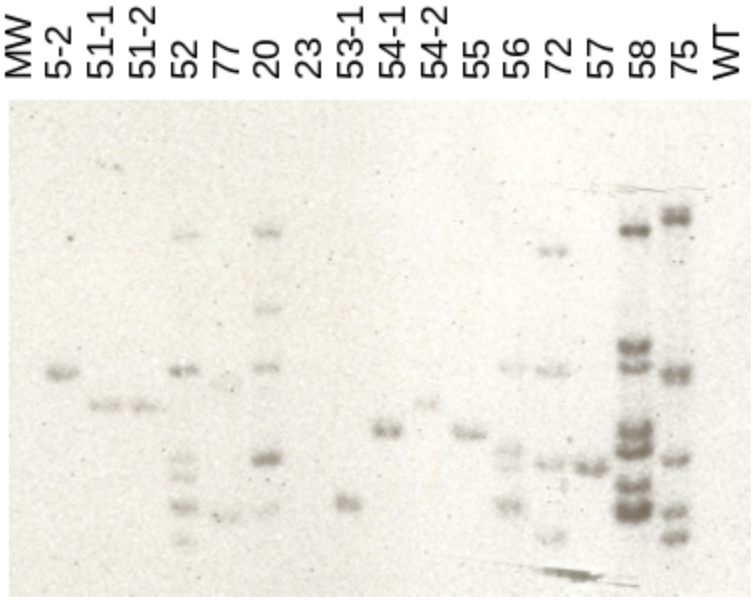
10. (10 points) The map of the T-DNA vector pBI121 is shown below.



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

11. (10 points) Your goal is to create a new canola cultivar expressing a pea gene. *Brassica napus* (canola) plants were transfomed with a pea gene. The DNA from regenerated transformants and non-transformed plants was cut with a restriction enzyme, blotted onto a filter, and probed using the pea gene. The results are shown below.



MW - molecular weight marker; WT - wild type *Brassica napus*

Which plants would you choose as parents for further breeding? State your reasoning.

12. (10 points) In plant biotechnology, the naturally-occurring Ti-plasmid has been modified to create the binary vector system. Why is it not practical to use the unmodified Ti-plasmid as it occurs in nature, for the purposes of plant transformation.

13. (5 points) The following steps describe the process of high-throughput genomic sequencing. Put 1 - 5 into the correct order. Step 6 is given:

- immobilize individual DNA molecules onto a solid surface
- read the sequence by imaging the emission of light in real time
- fragment genomic DNA into small fragments of a few hundred bases
- perform DNA synthesis using nucleotides that emit a characteristic wavelength of light each time a base is added
- amplify each molecule by PCR many thousands of times

6) from the millions of reads, assemble overlapping reads into long contiguous segments known as “contigs”