PLNT2530 PLANT BIOTECHNOLOGY

FINAL EXAMINATION

Monday, May 1, 2022   9:00 to 11:00   UMLearn Quiz

Answer any combination of questions totalling to <u>exactly</u> 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.

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) Two strains of Agrobacterium haved been constructed with deletions in the T-DNA of either region X or Y. Compare the expected results in plants inoculated with strains X, Y or wild type Agrobacterium. How would these deletions affect the plant? How would they affect the bacterium?
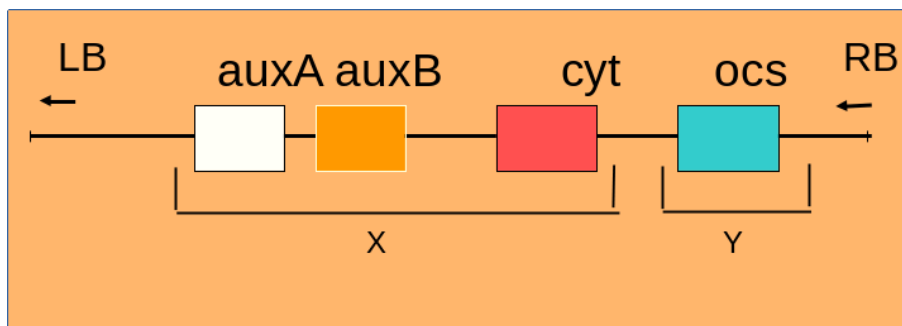

2. (15 points) In the event that a gene from a transgenic crop gets transferred to a wild relative, what factors might govern the "fixation" of that gene in the wild population? (Fixation means that eventually, all plants in the population would have the gene.)
Discuss the likelihood for fixation of the following traits, introgressed from a transgenic plant into a wild plant species:

      a) a gene for antibiotic resistance
      b) a gene for herbicide resistance
      c) a gene for improved nutritional content

3. (10 points) In a genome mapping experiment, 5 PCR reactions (a - e) were done on genomic DNA from 25 segregating progeny. Each reaction used a primer pair for a different locus. For comparison, reaction products from all 5 primer pairs were combined, and combined reactions for each of the 25 plants were loaded on a gel.

Which loci are closely linked on the chromosome? For those linked loci, what is the most likely order on the chromosome?

Individual progeny
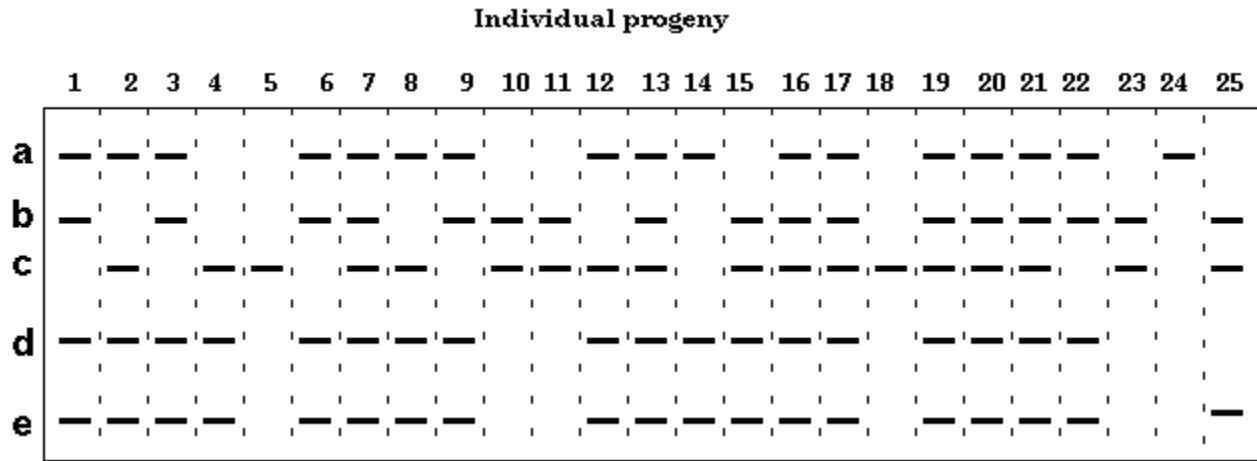
4. (10 points) Your goal is to create a new canola (*Brassica napus*) cultivar expressing a pea (*Pisum sativum*) gene. Canola plants were transfomed with a pea gene. The DNA from regenerated transformants and non-transformed plants was cut with a restriction enzyme, blotted onto a filter, and probed using the pea gene. The results are shown below.
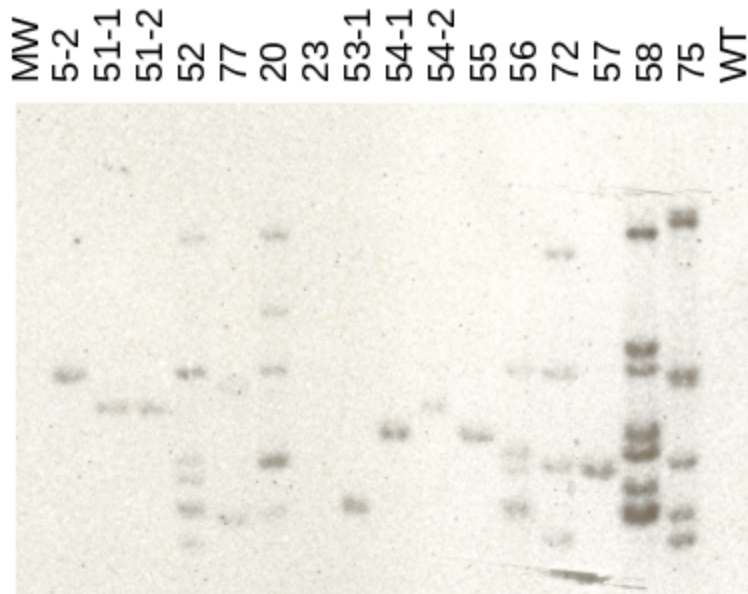
MW - molecular weight marker; WT - wild type *Brassica napus*

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

5. (20 points) Suppose that your goal was to create a plant-based vaccine by expressing the Covid19 S (spike) protein in a transgenic plant. A map of part of the Covid (SARS-2) genome is shown below, indicating the location of the S gene shown in green.
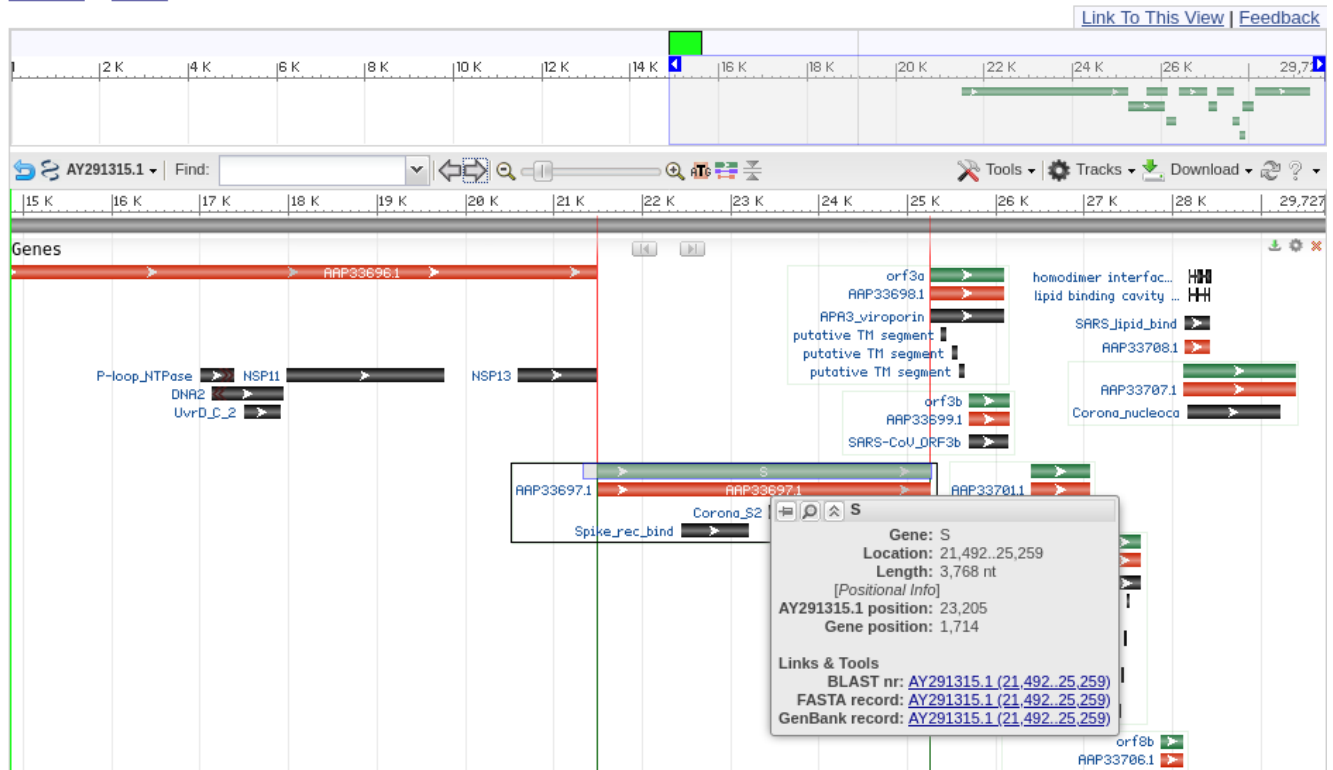
a) (5 points) One potential complication is that the viral genome is RNA, not DNA. Why does this make your strategy more complex? How would you overcome that problem?

b) (5 points) Even if this had been a DNA molecule, it turns out that there are no good restriction sites that would give you the S gene as a single fragment. For your cloning strategy, what is an alternative way of getting a single fragment containing just the S gene?

c) (10 points) Based on these considerations, outline a cloning strategy for cloning this gene into pBI121 to express the S protein in transgenic plants.

6. (5 points) Why is the Bt toxin protein considered non-toxic to humans?

7. (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?

8. (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"

9. (5 points) One potential problem with Bt crops is the concern that if you transform a crop with a Bt gene, eventually the insect population will develop resistance to that gene. What characteristic of the Cry gene family could be leveraged to prevent the insect population from evolving resistance? (We are not talking about refugia.)

10. (10 points) Mutations created using CRISPRs can be identified in the initial M0 generation. In contrast, mutations created by chemicals or radiation can only be identified in the M1 or M2 generations. Explain why this is true.

11. (10 points) Peanuts are known to be highly allergenic to some people, so that even a trace amount of peanut in food products can induce a severe allergic reaction. Most of the allergenicity of peanut is due to 3 classes of allergenic proteins:

Ara h 1	major allergen, glycoprotein	63.5kd	2 isoforms
Ara h 2	major allergen, glycoprotein	17.5kd	3 isoforms
Ara h 3	minor allergen, globulin	60 kd	2 isoforms

That means that at least 7 loci would have to be be silenced to completely eliminate allergenicity.

One possible way to create non-allergenic peanuts would be to knock out all 7 loci using CRISPR constructs targeting each of the genes.

a) Which do you think is dominant, allergenicity or non-allergenicity? Explain your reasoning.

b) In the field, it has been demonstrated that peanuts can outcross at a rate of anywhere from 0 - 4%. Therefore, it is of some concern that non-allergenic peanuts in one field might cross with conventional allergenic fields in an adjacent field. Discuss the likelihood of the following two possibilities

i) some plants in the conventional field would become non-allergenic
ii) some plants in the field of gene-edited plants would become allergenic

Explain your reasoning.

12. (10 points) Three sets of data are given for Arctic non-browning apples, in which polyphenol oxidase genes have been knocked out, at different stages in product development. If you were deciding whether to invest in this company, which of the three would make a more compelling case to invest? Explain your reasoning by comparing what the 3 experiments tell you, and what they don't tell you.

A

Table 18: PPO Activity in GD743 and GS784 - Mature Fruit

Event	Mean SpActivity[1]	S	n	PPO Suppression[2]
GD743	294	173	10	91 %
GD	3176	1235	10	
GS784	520	259	10	90 %
GS	5390	2341	10	

[1] SpActivity = Specific Activity of PPO.
[2] PPO Suppression = ((Mean SpActivity of Control – Mean SpActivity of Event) / Mean SpActivity of Control)*100

B

Table 16: PPO Activity in GD743 and GS784 - Field Leaves

Event	Mean SpActivity[1]	S	n[2]	PPO Suppression[3]
GD743	207	104	14	82 %
GD	1165	390	6	
GS784	315	95	10	76 %
GS	1297	245	8	

[1] SpActivity = Specific Activity of PPO.
[2] n = number of pooled field leaf samples per event. In the field, generally, two pooled leaf samples were taken from each tree. Therefore, the number of samples approximately equals twice the number of trees sampled.
[3] PPO Suppression = ((Mean SpActivity of Control – Mean SpActivity of Event) / Mean SpActivity of Control)*100

C

Table 14: PPO Activity in GD743 and GS784 - Tissue Culture Leaves

Event	Mean SpActivity[1]	n[2]	PPO Suppression[3]
GD743	593	2	77 %
GD	2561	6	
GS784	289	2	87 %
GS	2166	4	

[1] SpActivity = Specific Activity of PPO.
[2] n = number of pooled tissue culture leaf samples per event.
[3] PPO Suppression = ((Mean SpActivity of Control – Mean SpActivity of Event) / Mean SpActivity of Control)*100

GD, GS - non-transgenic parents
GD743, GS784 - transgenic varieties in which polyphenol oxidase has been knocked out using siRNA.