

## FINAL EXAMINATION

Friday, April 28, 2021

13:30 to 15:30

UMLearn Quiz

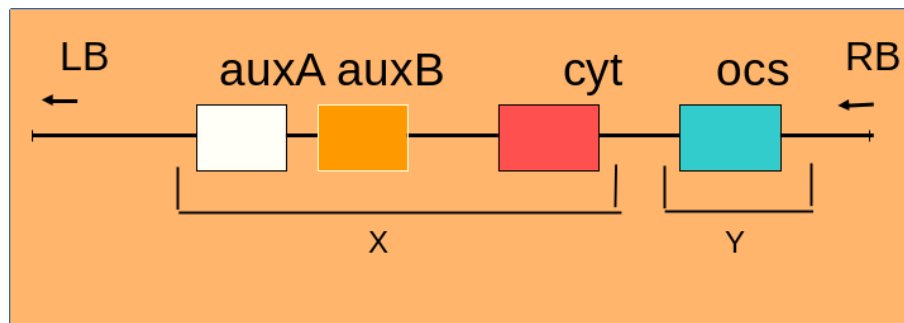
Answer any combination of questions totalling to exactly 100 points. If you answer questions totalling more than 100 points, answers will be discarded at random until the points equal 100. This exam is worth 40% of the course grade. The questions available total to 120 points.

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.

1. (10 points) Two strains of *Agrobacterium* have 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?

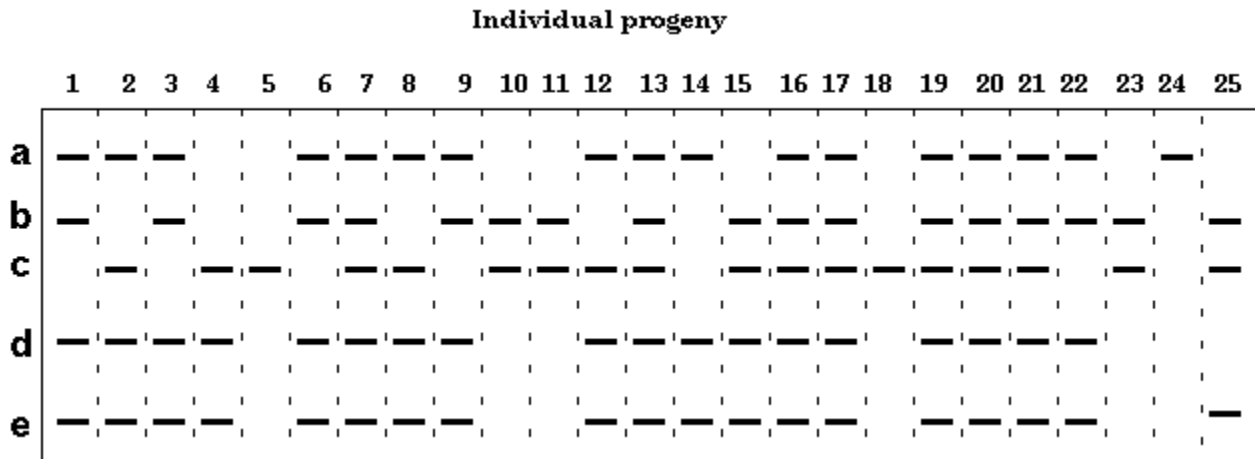


2. (5 points) In nature, *Agrobacterium tumefaciens* lives within its plant host. Is this relationship symbiosis or parasitism? Explain your reasoning.

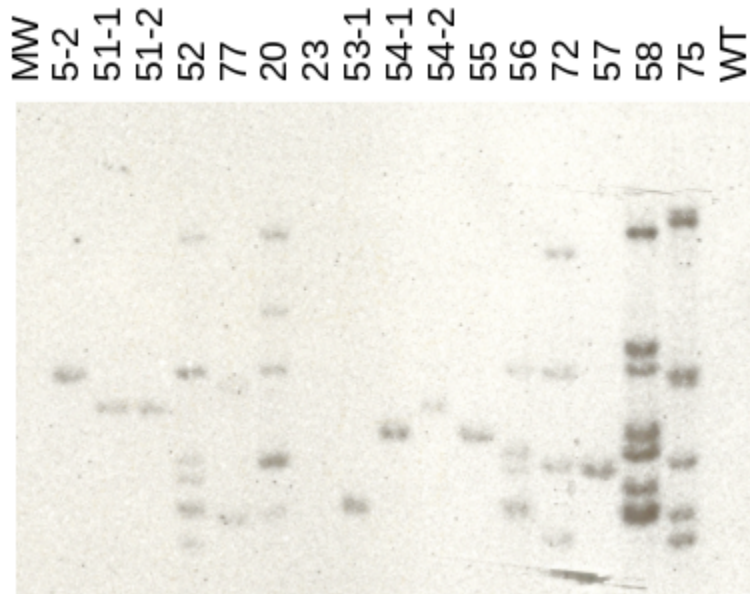
3. (10 points) In comparing genomics with metabolomics, what is the distinction between the two? Which one is more straightforward to do, and which is more complex? Which one is going to be roughly the same regardless of which organism you are working with, and which would differ with species? Explain your reasoning in each case.

4. (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?



5. (10 points) Your goal is to create a new canola (*Brassica napus*) cultivar expressing a pea (*Pisum sativum*) gene. Canola plants were transformed 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*

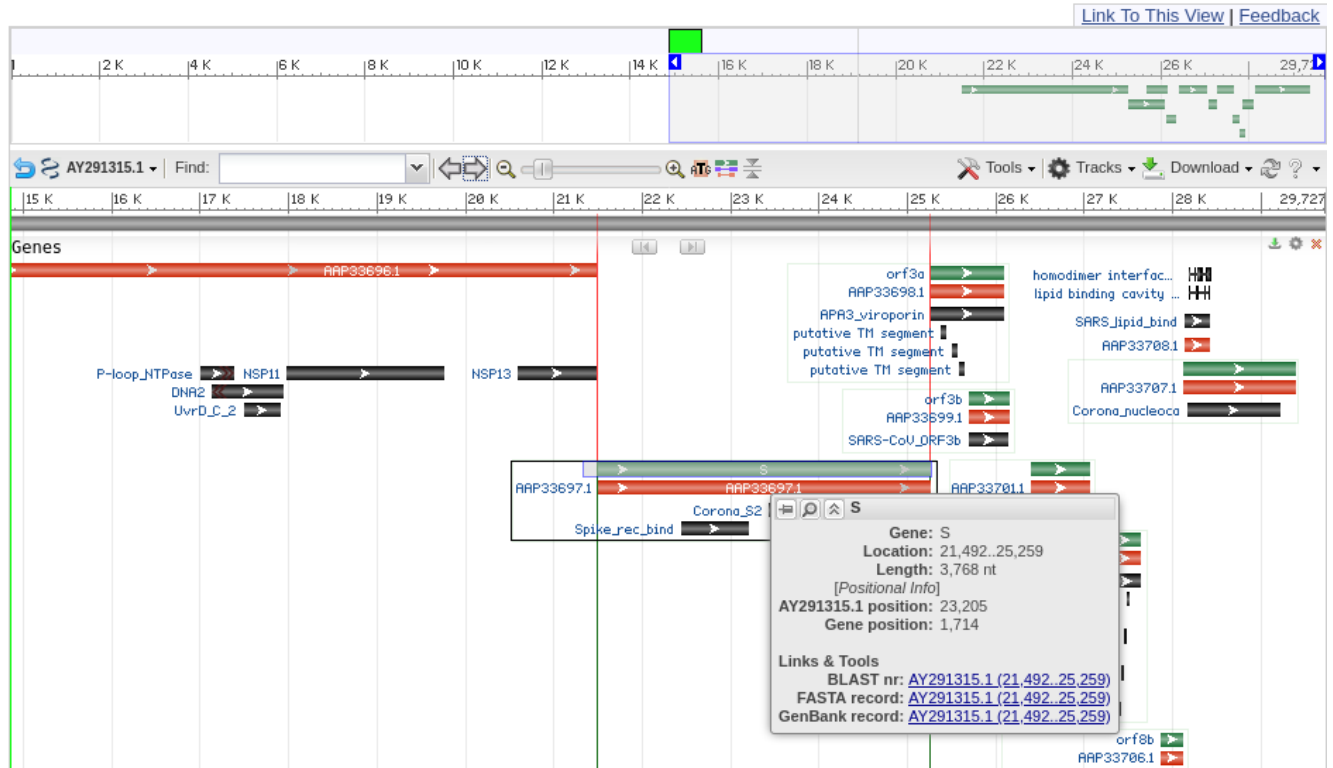
Which plants would you choose as parents for further breeding? State your reasoning.

6. (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.

### SARS coronavirus Frankfurt 1, complete genome

GenBank: AY291315.1

[GenBank](#) [FASTA](#)



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.

7. (5 points) Why is the Bt toxin protein considered non-toxic to humans?

8. (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.

9. (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”

10. (5 points) In nature, there are at least 4 major classes of Bt toxin proteins, encoded by gene referred to as Cry1 - Cry 4. Does this diversity of Cry genes make it easier, or more difficult, to genetically engineer insect resistance? Explain your reasoning.

11. (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.

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 <sup>1</sup>	S	n	PPO Suppression <sup>2</sup>
GD743	294	173	10	91 %
GD	3176	1235	10	
GS784	520	259	10	90 %
GS	5390	2341	10	

<sup>1</sup> SpActivity = Specific Activity of PPO.  
<sup>2</sup> 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 <sup>1</sup>	S	n <sup>2</sup>	PPO Suppression <sup>3</sup>
GD743	207	104	14	82 %
GD	1165	390	6	
GS784	315	95	10	76 %
GS	1297	245	8	

<sup>1</sup> SpActivity = Specific Activity of PPO.  
<sup>2</sup> 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.  
<sup>3</sup> 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 <sup>1</sup>	n <sup>2</sup>	PPO Suppression <sup>3</sup>
GD743	593	2	77 %
GD	2561	6	
GS784	289	2	87 %
GS	2166	4	

<sup>1</sup> SpActivity = Specific Activity of PPO.  
<sup>2</sup> n = number of pooled tissue culture leaf samples per event.  
<sup>3</sup> 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.

13. (10 points) The following Southern blots were done during a project to transform soybean with a gene from pea for fungal resistance (**P**). Explain the results in B and C in light of what we have discussed regarding the basic genetics of transgenes.

**A**  
 A Southern blot was done using DNA from the original, untransformed parent. The southern was probed with gene P, and the blot was washed and reprobated with the probe from a single copy gene from soybean, S. Not surprisingly, the Pea gene does not hybridize with soybean DNA, while a single band is seen with the soybean gene S, indicating that S is a single copy gene.

P	S
[Empty Box]	[Single Band]

**B**  
 Soybean was transformed with gene P, and a Southern blot was done with DNA from a T<sub>0</sub> transformant. The transformant shows many bands for gene P. The blot was reprobated with S as a control.

P	S
[Multiple Bands]	[Single Band]

**C**  
 The T<sub>0</sub> transformant from B is back crossed to the original parent for 3 generations. Each generation, progeny are crossed back to the original untransformed line, referred to as the recurrent parent.

The southern are repeated using DNA from a BC3 plant, with the results shown at right.

P	S
[Empty Box]	[Single Band]
[Single Band]	[Empty Box]
[Single Band]	[Empty Box]

T<sub>0</sub> x untransformed  
 |  
 v  
 BC1 x untransformed  
 |  
 v  
 BC2 x untransformed  
 |  
 v  
 BC3