PLNT2530 PLANT BIOTECHNOLOGY

FINAL EXAMINATION

Friday, April 21, 2023 9:00 to 11:00

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.

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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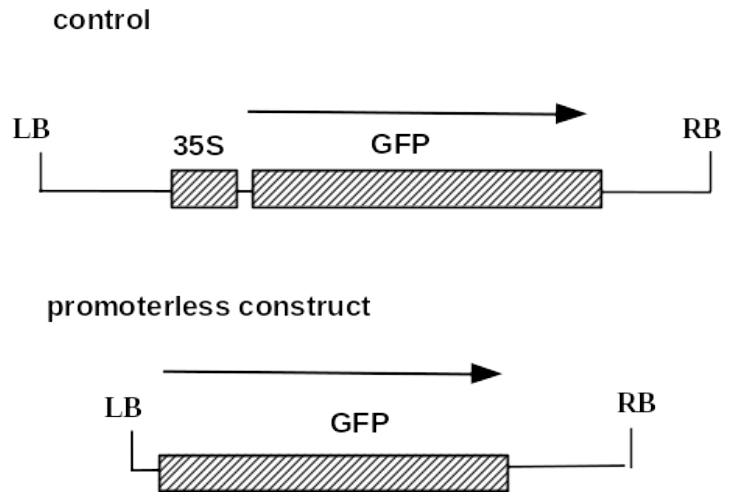
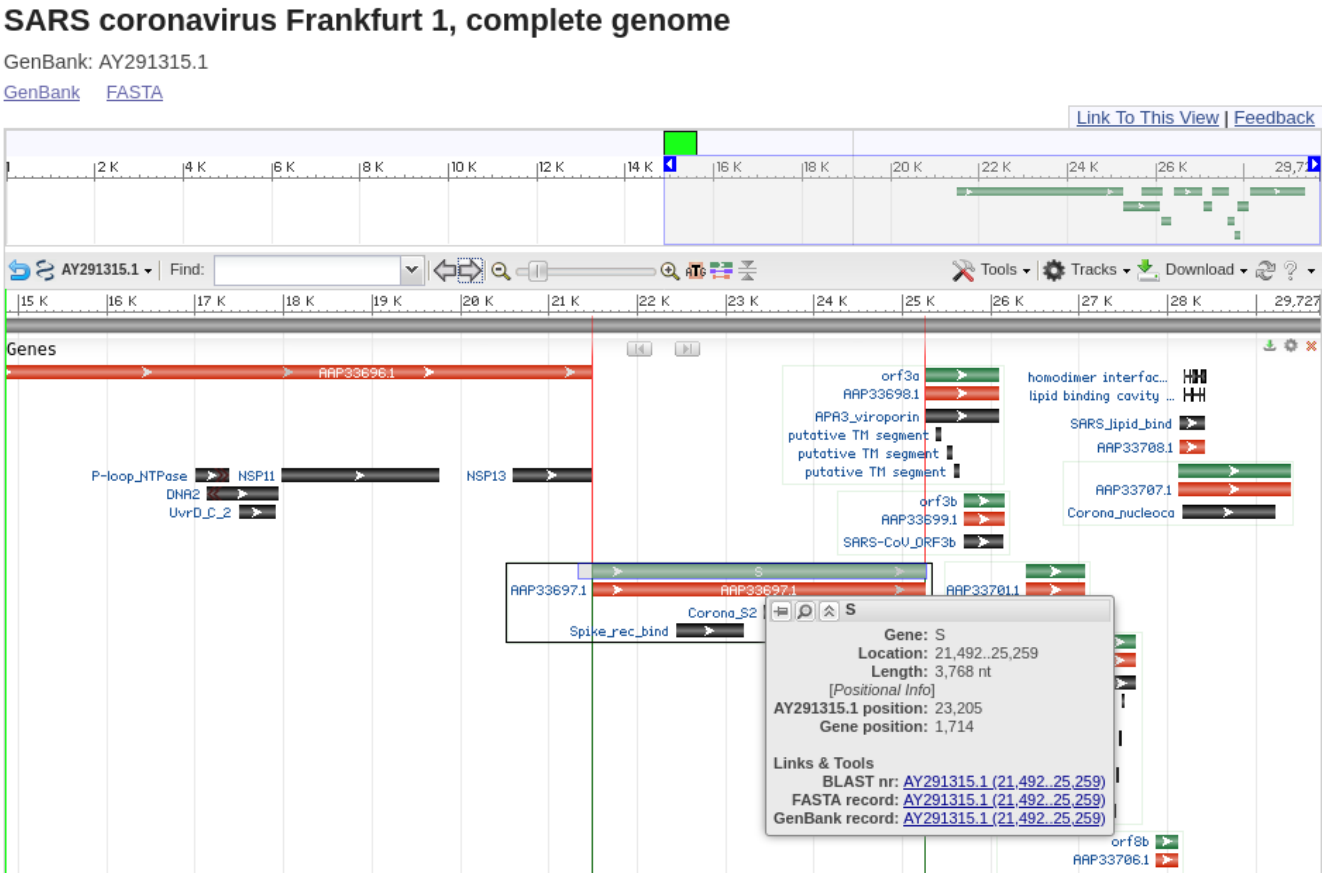
1. (10 points) Two constructs were transformed into Arabidopsis using biolistics. The plants with the control construct showed fluorescence in all tissues. The promoterless construct is identical to the control, except that there is no promoter in front of the GFP coding sequence. Almost all progeny had no GFP expression, but in a very small number of progeny GFP expression was seen. The expression varied from event to event. For example, in one event, expression was seen only in leaves, while in another, expression was seen in anthers. What would be the explanation for these observations?



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 quality in foods

3. (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) 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) 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) Based on these considerations, briefly describe how you would clone this gene into pBI121 to express the S protein in transgenic plants.

4. (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.

5. (10 points) To optimize expression of an Agrobacterium EPSPS gene in chloroplasts, Monsanto had to modify the codon strategy for the EPSPS gene. An alternative genetic code used in plant plastids is given below. In the modified gene, list the best codons to use to code for Leucine. Which codons should not be used for Leucine?

11. The Bacterial, Archaeal and Plant Plastid Code (transl_table=11)

TTT F Phe	TCT S Ser	TAT Y Tyr	TGT C Cys
TTC F Phe	TCC S Ser	TAC Y Tyr	TGC C Cys
TTA L Leu	TCA S Ser	TAA * Ter	TGA * Ter
TTG L Leu i	TCG S Ser	TAG * Ter	TGG W Trp
CTT L Leu	CCT P Pro	CAT H His	CGT R Arg
CTC L Leu	CCC P Pro	CAC H His	CGC R Arg
CTA L Leu	CCA P Pro	CAA Q Gln	CGA R Arg
CTG L Leu i	CCG P Pro	CAG Q Gln	CGG R Arg
ATT I Ile i	ACT T Thr	AAT N Asn	AGT S Ser
ATC I Ile i	ACC T Thr	AAC N Asn	AGC S Ser
ATA I Ile i	ACA T Thr	AAA K Lys	AGA R Arg
ATG M Met i	ACG T Thr	AAG K Lys	AGG R Arg
GTT V Val	GCT A Ala	GAT D Asp	GGT G Gly
GTC V Val	GCC A Ala	GAC D Asp	GGC G Gly
GTA V Val	GCA A Ala	GAA E Glu	GGA G Gly
GTG V Val i	GCG A Ala	GAG E Glu	GGG G Gly

i - codons that are sometimes used as initiation codons.

6. (5 points) The genome of Chinese white poplar (Populus tomentosa) consists of n=38 chromosomes. However, the current sequence assembly has 4022 contigs. The sequencing project estimates about 75 fold coverage (redundancy). What is the most likely reason for the discrepancy? In other words, why are there 4022 contigs, rather than 38?

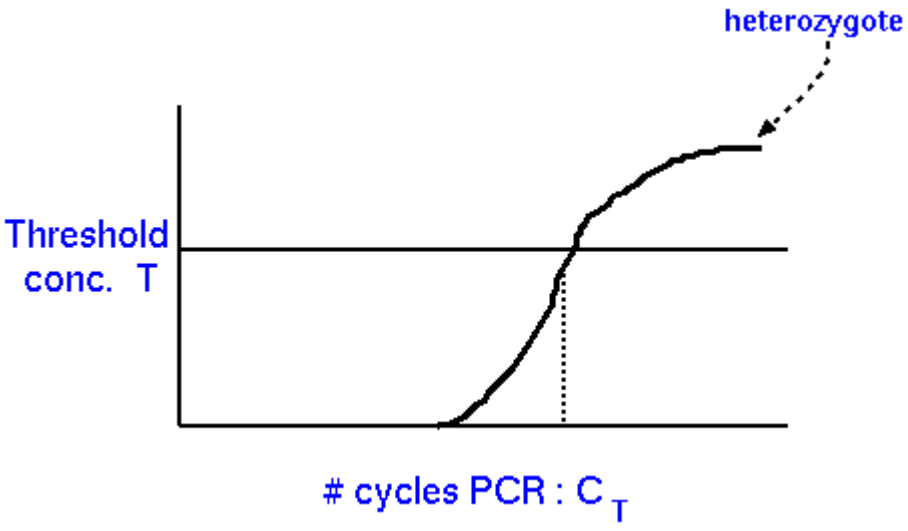
7. (5 points) Describe the distinction between transformation and transient expression.

8. (10 points) Explain why mutations generated by the CRISPR-Cas9 system will be essentially indistinguishable from naturally-occuring mutations. Why is this important in light of regulation and labeling of genetically-modified crops?

9. (10 points) Since the T-DNA inserts at random in the genome, at some frequency we would expect it to insert within a gene, thereby inactivating that gene. Discuss the potential for using T-DNA insertion as a method for generating mutations. What factors would make this method practical, or impractical?

10. (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.)

11. (10 points) The amplification curve for a typical qPCR reaction is shown for a gene in a plant that is heterozygous for a transformant.



Draw a similar diagram comparing the curve for the heterozygous plant with the curve for a homozygous plant.

12. (10 points) In class we showed a simple root assay for distinguishing transformed plants from non-transformed controls. Briefly, since the construct included a kanamycin resistance gene, seeds from selfed T0 plants were germinated on media containing kanamycin. Seedlings (ie. T1) were allowed to grow for several days. Seedlings which developed long roots were deemed to be kanamycin resistant, and seedlings which showed very little root growth were scored as kanamycin sensitive.

For A - D, specify how many copies of the transgene are present: 0 (ie. untransformed), 1, 2 or 3. Explain your reasoning.