

PLNT2530 PLANT BIOTECHNOLOGY

MID-TERM EXAMINATION

11:30 am to 12:20 pm      Monday, March 8, 2021

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 total points equal 100. The questions total to 120 points. This exam is worth 20% of the course grade.

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

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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) Describe the purpose of a laminar flow hood. Explain how the function of the hood accomplishes that purpose.

2. (10 points) Why is it that plants cells in tissue culture can regenerate into complete organ systems such as shoots or roots? In particular, in what way do plants lend themselves to regeneration, where animals do not?

3. (5 points) Briefly explain the distinction between transposons and retrotransposons.

4. (10 points) For the following macroelements found used in tissue culture media, match each element with the phrase that best describes its role in the cell. To answer the question, simply write a list a - e, with the corresponding phrase. Only use each phrase once.

- a) Nitrogen (N)
- b) Potassium (K)
- c) Calcium (Ca)
- d) Magnesium (Mg)
- e) Phosphorus (P)

- i) nucleic acids, energy transfer, req'd in respiration and photosynthesis
- ii) enzyme cofactor, component in chlorophyll
- iii) proteins, nucleic acids
- iv) regulates osmotic potential, main inorganic cation
- v) cell wall structure, membrane function, signaling

5. (10 points) Consider the double-stranded duplexes shown below. Which duplex will be more stable, A or B? Which duplex will be more stable, C or D? In each case, explain your reasoning.

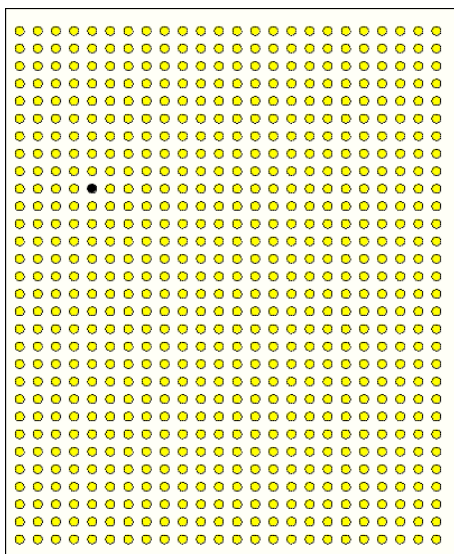
<p><b>A</b></p> <p>5' AACAAATCGTAAG 3'</p> <p>3' TTGTTAGCATTTC 5'</p>	<p><b>B</b></p> <p>5' AACTATCGTAAG 3'</p> <p>3' TTGTTAGCATTG 5'</p>
<p><b>C</b></p> <p>5' TCTGCTAACTGA 3'</p> <p>3' AGACGATTGACT 5'</p>	<p><b>D</b></p> <p>5' CCACTGACCTGG 3'</p> <p>3' GGTGACTGGACC 5'</p>

6. (5 points) Based on the data in the table below, which species, do you expect to have longer chromosomes, *Glycine max* or *Zea mays*? Explain your reasoning.

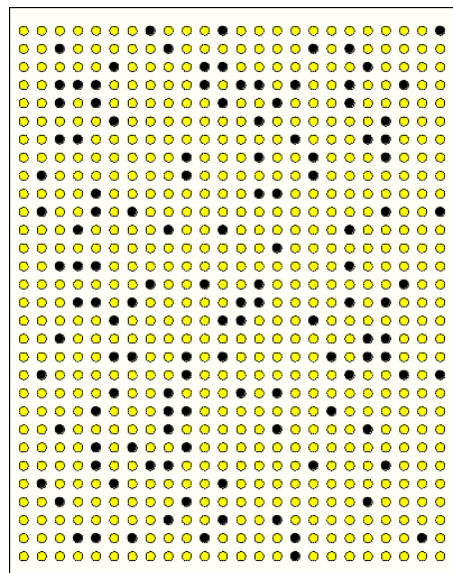
Organism	Chromosome number (n)	Genome size (bp)	Gene No.
<i>Arabidopsis thaliana</i>	5	$1.19 \times 10^8$	25,400
<i>Fragaria vesca</i>	7	$2.80 \times 10^8$	25,050
<i>Brassica rapa</i>	10	$2.84 \times 10^8$	41,174
<i>Oryza sativa</i>	12	$4.66 \times 10^8$	58,000
<i>Glycine max</i>	20	$1.10 \times 10^9$	46,430
<i>Zea mays</i>	10	$2.80 \times 10^9$	63,000
<i>Triticum aestivum</i>	21	$1.70 \times 10^{10}$	41,910

7. (10 points) A BAC library from pea (*Pisum sativum*) was screened with two probes. The first probe was a cDNA clone for pea defense gene DRR206. An identical filter was also probed using as a probe a 6 kb genomic fragment containing the DRR206 gene. Hybridization results for the two experiments are shown below. What is the most likely explanation for the different results? You can ignore trivial explanations such as probe contamination.

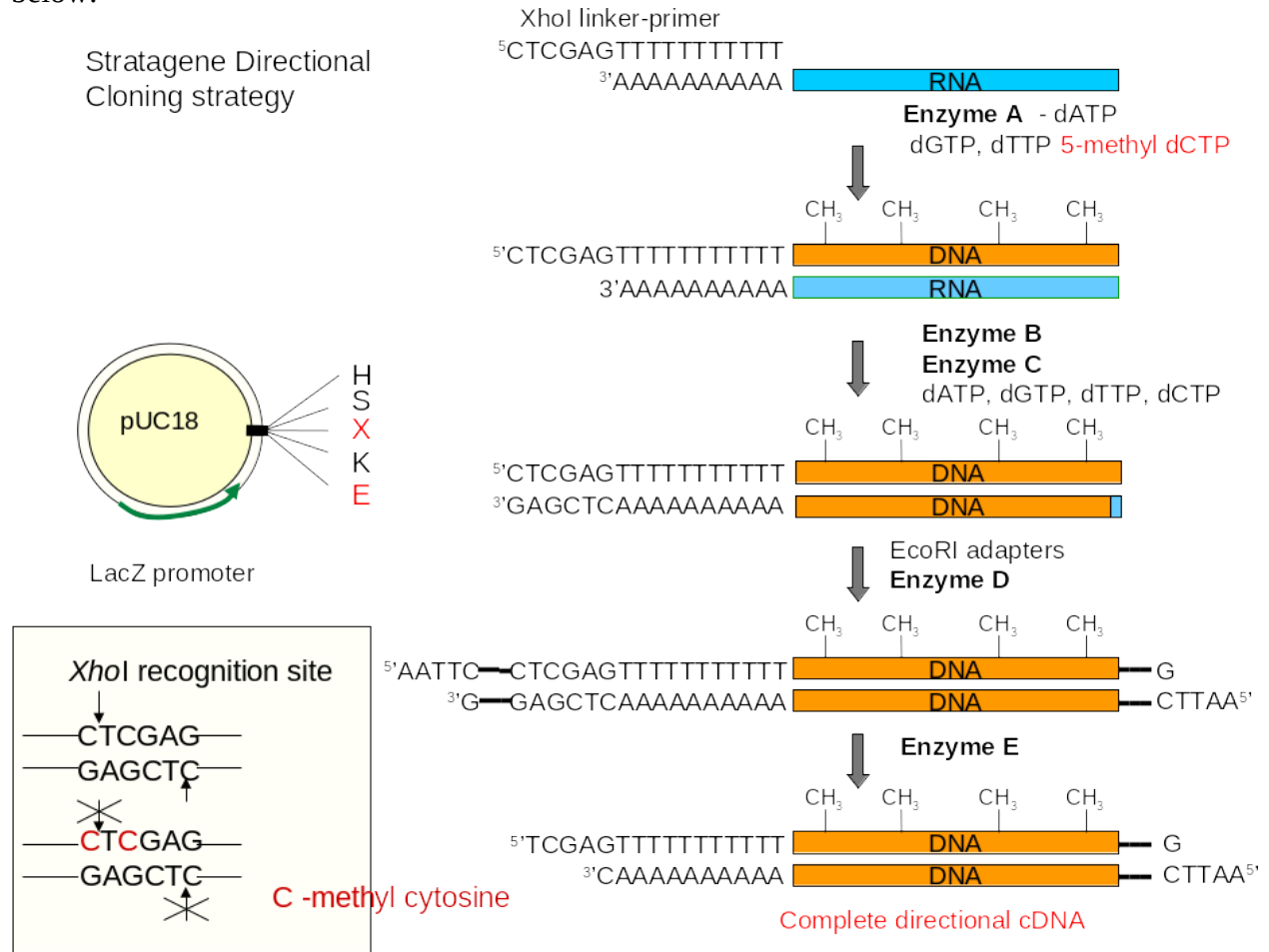
cDNA probe



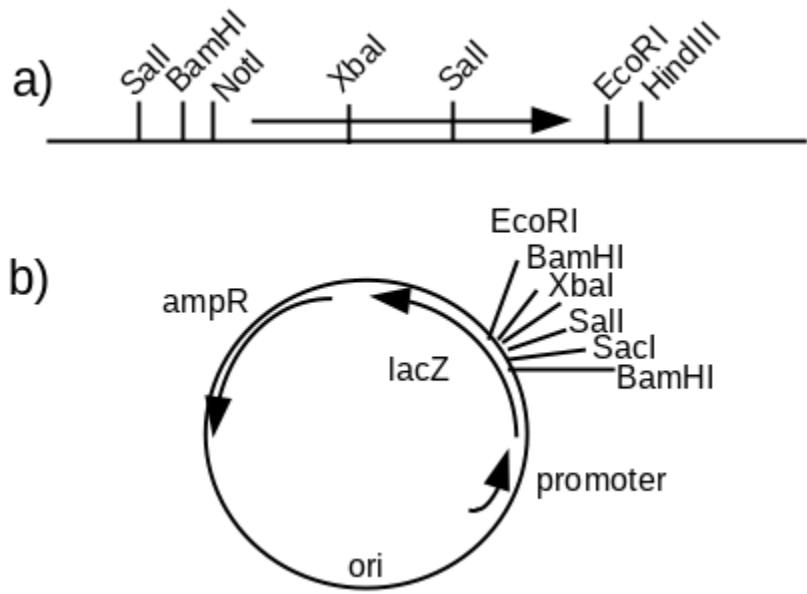
6 kb genomic probe



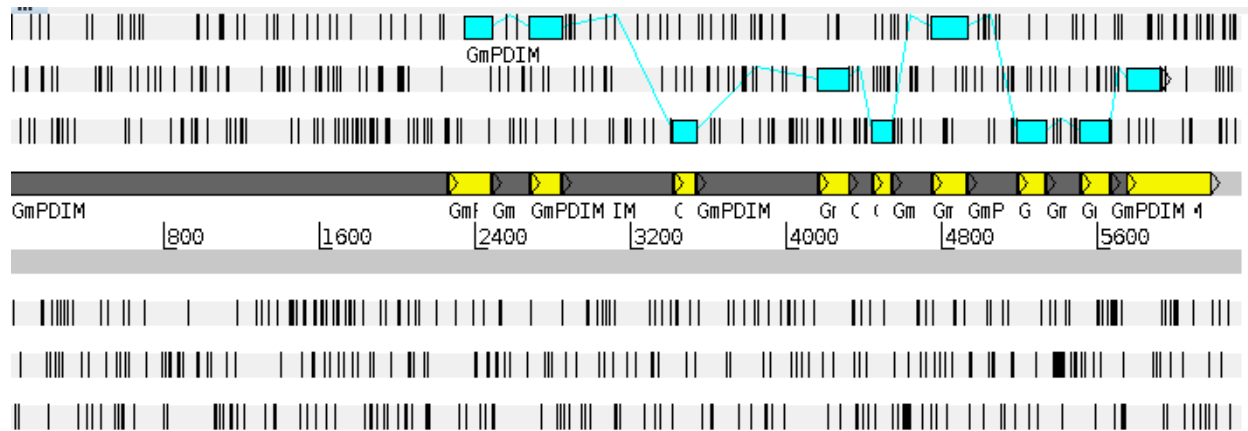
8. (10 points) List the names of Enzymes A - E, used in the cDNA cloning protocol shown below.



9. (10 points) Based on the restriction maps for a gene (a) and a vector (b), choose a pair of restriction enzymes that could be used to cut the entire gene from a, to be cloned between any two compatible sites in the vector.



10. (15 points) The image below depicts a clone for a gene for disulfide isomerase. In each of the 3 reading frames on the forward strand (top) and 3 reading frames on the reverse strand (bottom), stop codons are shown as vertical tick marks. A map of the gene is shown between the two strands.



a) Which boxes are exons, and which are introns? What lets you distinguish between exons and introns in this diagram?

b) Is this from a cDNA library, or a genomic library?

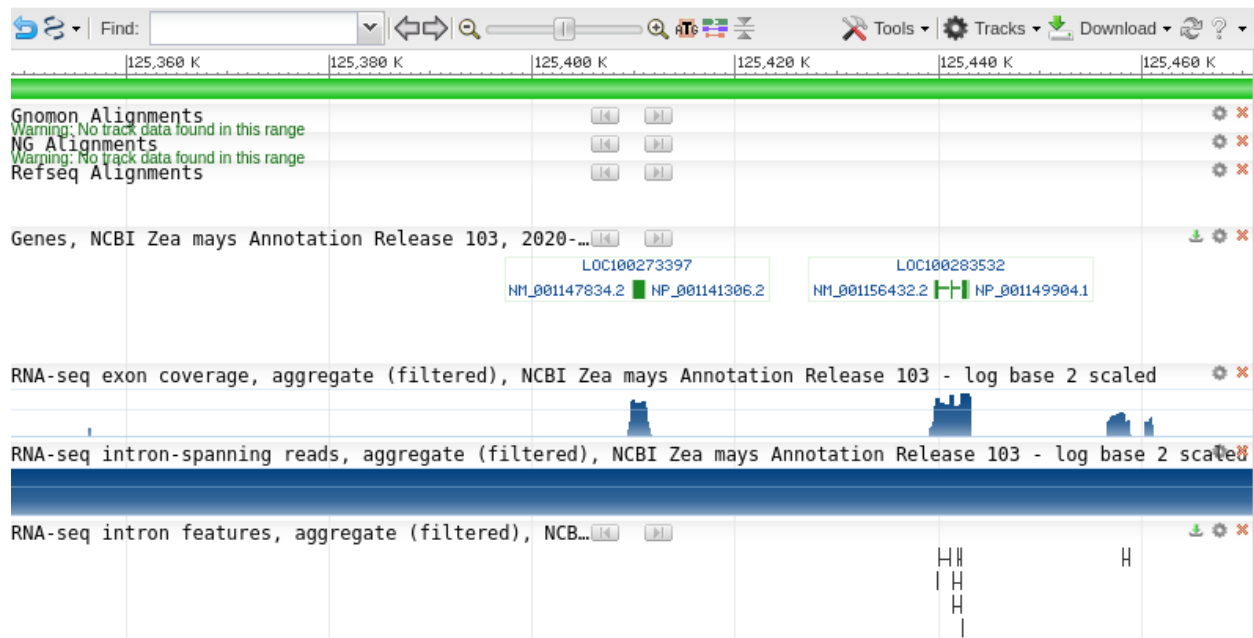
c) The gene shown was cloned into the multiple cloning region of the pUC18 cloning vector. Many white colonies were screened, but the disulfide isomerase protein could not be detected in any of the clones. What is the most likely reason that the protein was not produced in any of the clones?

11. (5 points) Media for co-cultivation of *Agrobacterium* with plant tissue needs Acetosyringone in order to induce *Agrobacterium* genes required for transformation. All other components are heat stable, but Acetosyringone would be destroyed by autoclaving. How could you sterilize the acetosyringone so that it could be added to the media after autoclaving?

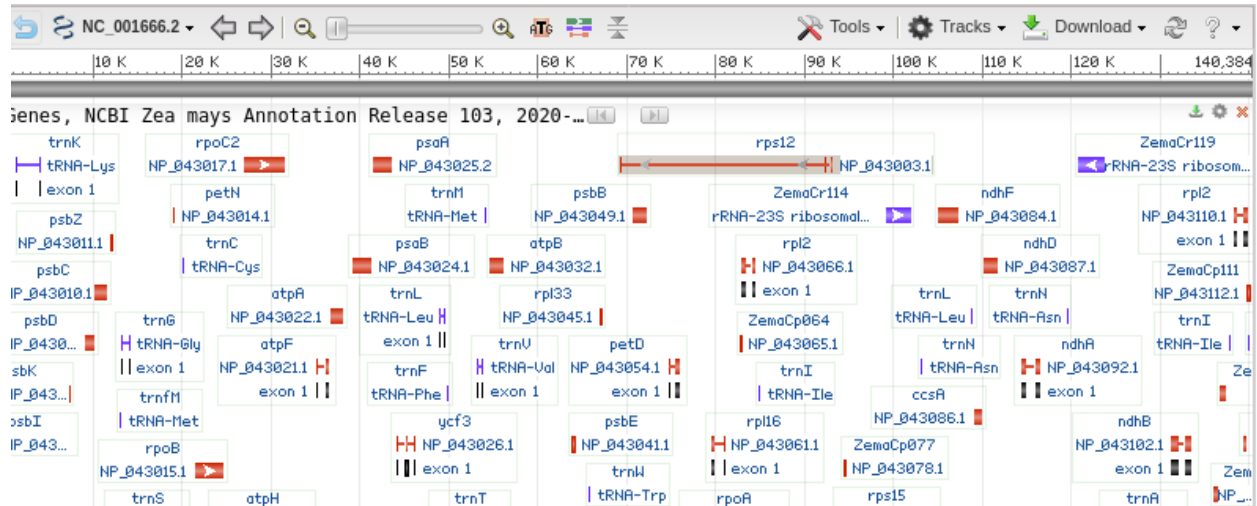
12. (10 points) The  $T_d$  for a 100 bp DNA fragment is 72 °C at standard hybridization conditions. What would be the  $T_d$  for a 500 bp fragment, given similar conditions? Show your work.

13. (10 points) One of the figures below is from a region of a maize nuclear chromosome. The other is from the maize chloroplast genome. Both diagrams span around 140 Kb of DNA. State which is genomic, and which is from chloroplast. What observations support your choices?

A



B



# Genetic code

		Second base							
		U		C		A		G	
First base	U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
		UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
		UUA	Leu	UCA	Ser	UAA	STOP	UGA	STOP
		UUG	Leu	UCG	Ser	UAG	STOP	UGG	Trp
	C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
		CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
		CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
		CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
	A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
		AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
		AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
		AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly	
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly	
	GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly	

Met  
tRNA  
UAC

5' AUG codon  
mRNA

## Frequencies of Restriction Sites (or other oligonucleotides)

length n	frequency: occurs every 4 <sup>n</sup>	example	sequence
1	4	Single nucleotide	G
2	16	Di-nucleotide	GT
3	64	Codon	ATG
4	256	Taq I	TCGA
5	1024	MbolI	GAAGA
6	4096	Hind III	AAGCTT
7	16384	Abe I	CCTCAGC
8	65536	Not I	GCGGCCGC