PLNT2530 PLANT BIOTECHNOLOGY

MID-TERM EXAMINATION

11:30 am to 12:20 pm Monday, February 26, 2018

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.

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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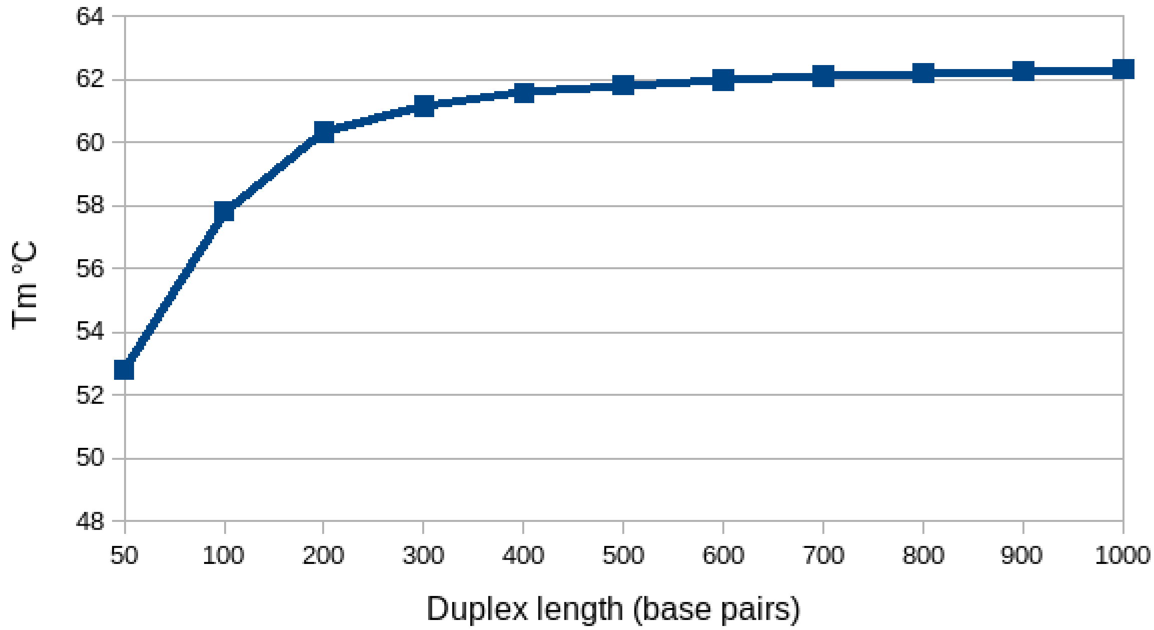
1. (15 points) The following table lists restriction enzymes and their cutting sites, indicated by the caret (^). For boxes labeled **a - e**, indicate what should be in the box.

Enzyme	Cutting site	Cohesive ends	Ligates with*
BamHI	**a**	5'-G GATCC-3' 3'-CCTAG G-5'	MboI
EcoRV	5'GAT^ATC3'	5'-GAT ATC-3' 3'-CTA TAG-5'	HpaI
KpnI	5'GGTAC^C3'	5'-GGTAC C-3' 3'-C CATGG-5'	**b**
Asp718	5'G^GTACC3'	5'-G GTACC-3' 3'-CCATG G-5'	**c**
HpaI	5'GTT^AAC3'	5'-GTT AAC-3' 3'-CAA TTG-5'	EcoRV
MboI	5'^GATC3'	**d**	**e**

N - wild card ie. any of A,G,C or T

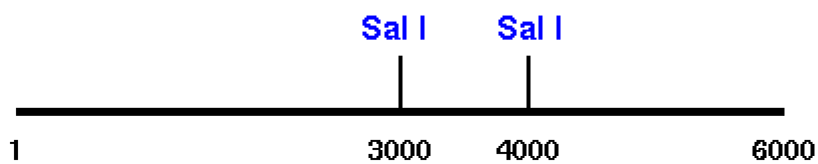
*With which other sites would the cohesive ends generated by this enzyme ligate? If none of the other sites are compatible, answer "NA"

2. (10 points) A plot of melting temperature Tm as a function of duplex length is shown for double-stranded DNAs of various sizes. What generalizations can you draw from the results?

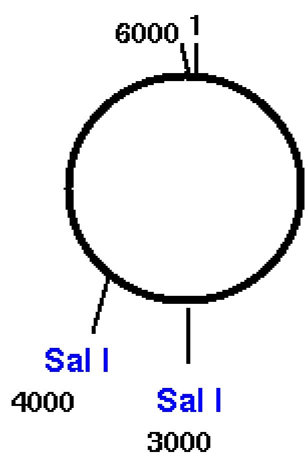


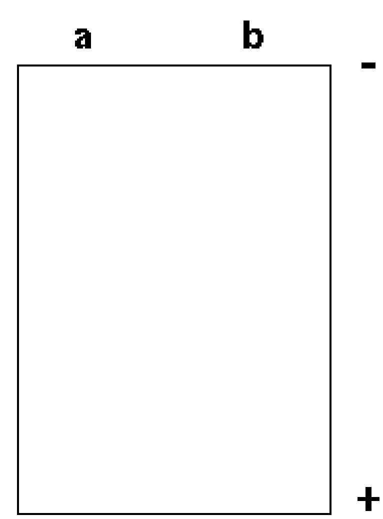
3. (10 points) Restriction sites for SalI are shown for two different DNA molecules, as indicated in a) and b). Reference coordinates are also indicated on the maps. Draw a diagram, similar to the one shown at right, of the expected bands seen for SalI digests of each of these molecules, if loaded side by side on a gel.

a)

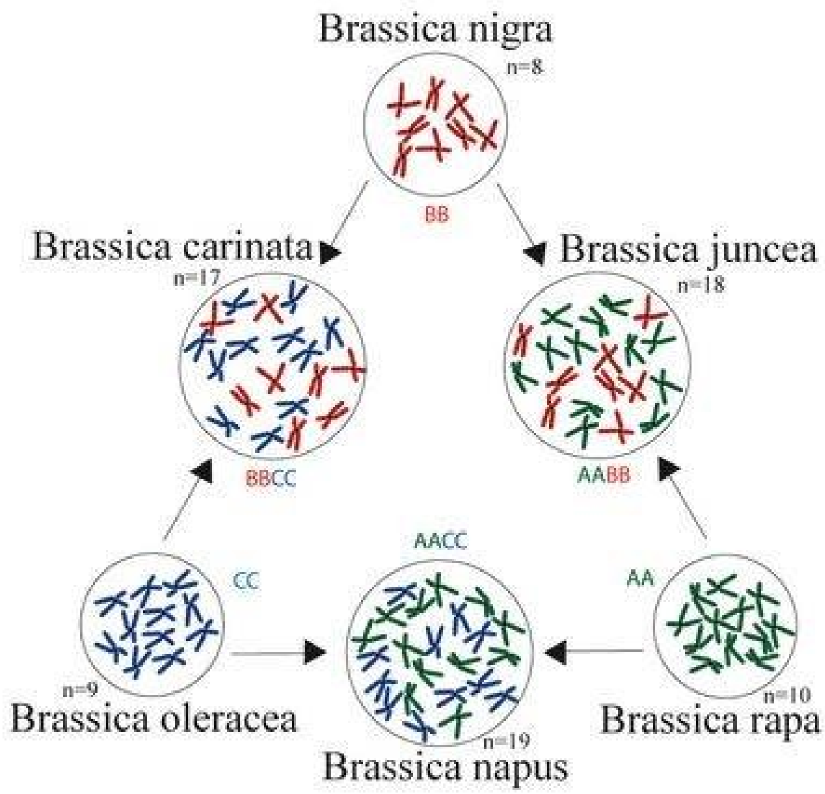


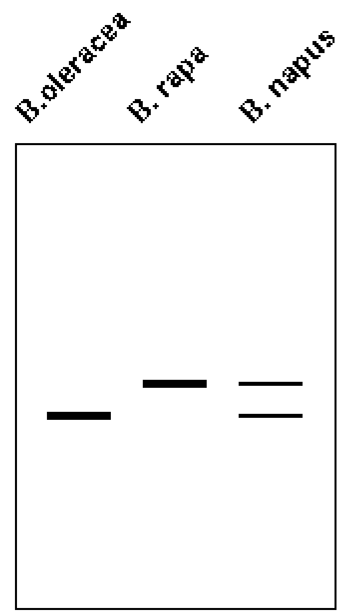
b)





4. (10 points) As illustrated in the figure at left, *B. napus* is a hybrid derived from *B. rapa* and *B. oleracea*. The figure at right shows a Southern blot of DNA that has been cut with a restriction enzyme, from three *Brassica* species and probed with cloned sequence taken from a gene. You can assume that 1 µg of genomic DNA was added in each lane.





a) How do you account for the fact that *B. rapa* and *B. oleracea* each shows one band, while *B. napus* shows two bands?

b) In replicate experiments, Southerns hybridization consistently shows more intense bands with *B. rapa* and *B. oleracea* DNA than with *B. napus*. Based on what we discussed in class, is there something about these genomes that would account for the different signal intensities? Explain your reasoning.

5. (10 points) Design a set of DNA hybridization probes for a protein that contains the following amino acid sequence:

Ile Met His Ser Trp Tyr

For reference, the standard Genetic Code is included on the last page of this exam.
In total, how many unique oligonucleotides would you have to make to account for all possible 18-mers that could encode this polypeptide?

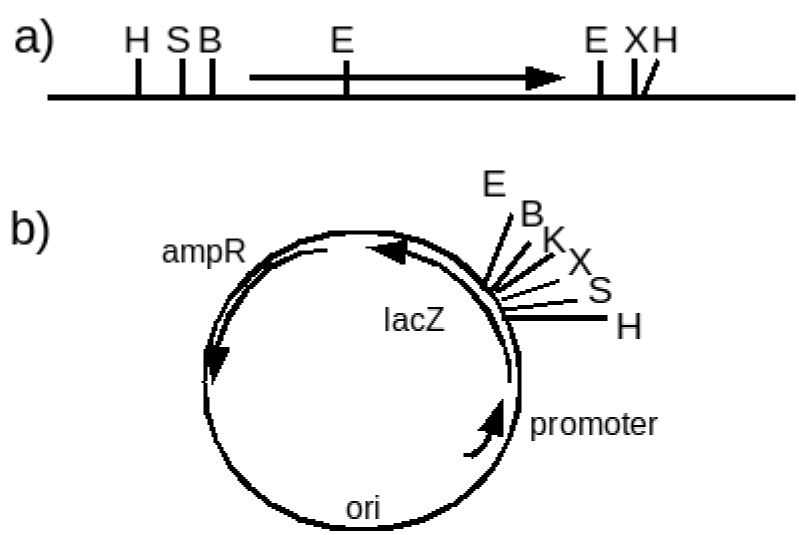
6. (10 points) In expression libraries, each clone expresses the protein encoded by a single cDNA insert. Why is it not possible to make expression libraries in BAC vectors carrying large fragments (eg. 100 kb) of plant genomic DNA as inserts? Give at least 2 reasons.

7. (15 points) A restriction map of a gene that you want to clone is shown in a). The direction of transcription of the gene is indicated by an arrow. Your goal is to use directional cloning to clone the transcribed sequence of the gene into the vector (b) in the sense orientation. In other words, your construct should have the insert in the correct orientation to produce the protein coded for by the gene, under the control of the lacZ promoter.

Describe a directional cloning strategy with the following infomation

- Which enzyme(s) would you use to cut the gene?
- Which enzyme(s) would you use to cut the vector?
- Draw a map of the final construct

There are several possible cloning strategies. Only describe one of those.



H = HindIII; S=SalI; B=BamHI; E=EcoRI; K=KpnI; X=XbaI

8. (15 points) When growing cells in callus culture, there are three categories of components that must be included in the media: essential elements, organic supplements, and a carbon source. Briefly define what each of these does and give an example of each.

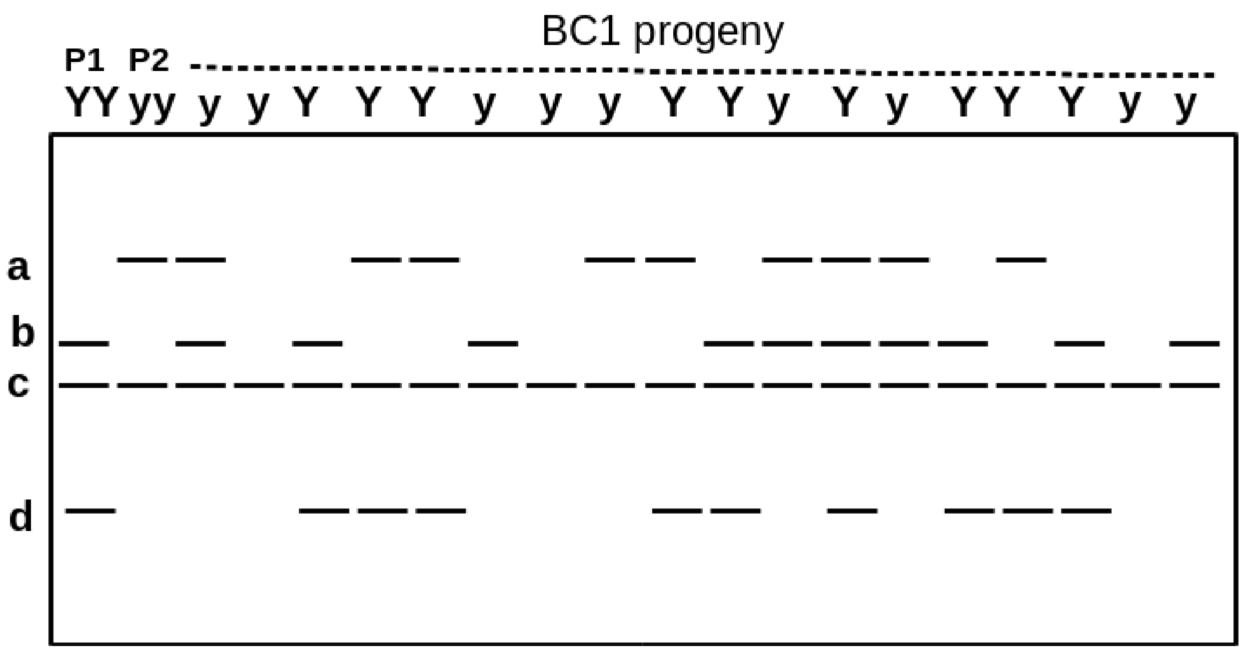
Explain why we don't need to provide these things to mature plants growing in soil.

9. (5 points) Draw a simple diagram illustrating the posttranscriptional processing of pre-mRNA transcripts into mature mRNA. Also show the translation step, indicating the N-terminal and C-terminal ends of the polypeptide product. Show steps in the order in which they occur.

10. (5 points) In cell elongation, what is the mechanism by which plant cells can increase their total volume without having a substantial increase in cytoplasmic volume?

11. (5 points) Briefly state the difference between a transposon and a retrotransposon?

12. (10 points) *Brassica rapa* plants with brown (YY) seed were crossed with yellow plants (yy) to give an F1 generation. F1 plants were crossed back to the yy homozygote to give a back cross generation, BC1. Eighteen BC1 progeny were scored for seed color, and for four PCR markers. Which of the four loci, a, b , c or d is linked to the Y locus? Briefly explain your reasoning.



Genetic code

	Second base			
First base	U	C	A	G
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