PLNT2530 PLANT BIOTECHNOLOGY

MID-TERM EXAMINATION

11:30 am to 12:20 pm Friday, March 3, 2017

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. 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.

---

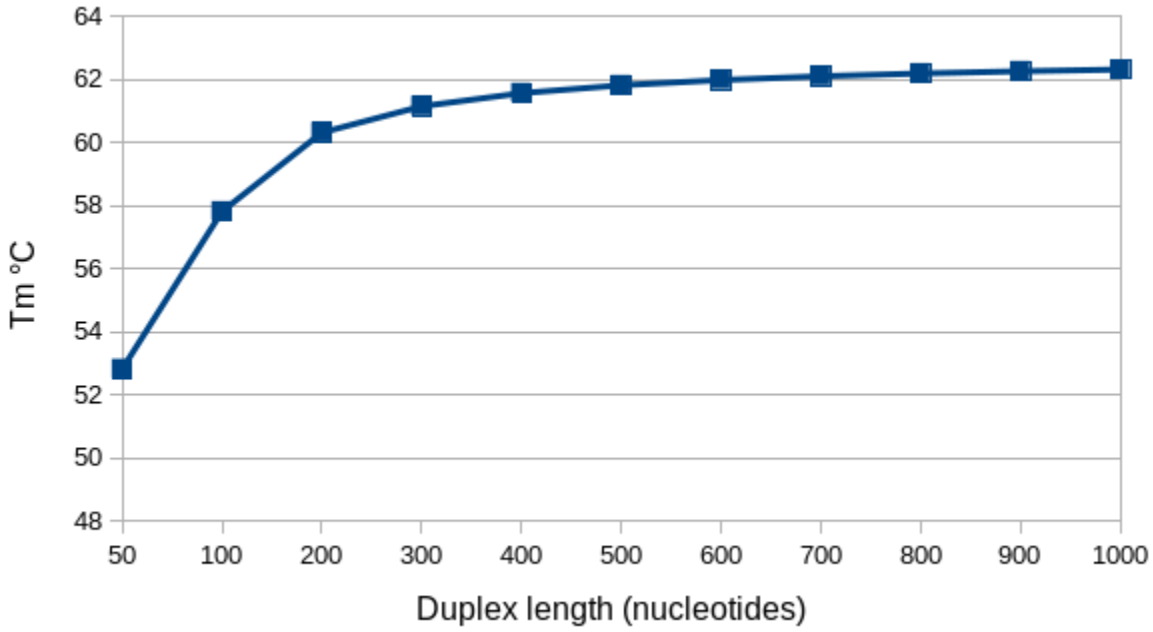
1. (15 points) The following table lists restriction enzymes and their cutting sites, indicated by the caret (^). For boxes labeled **a - e**, indicate what should be in the box.

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

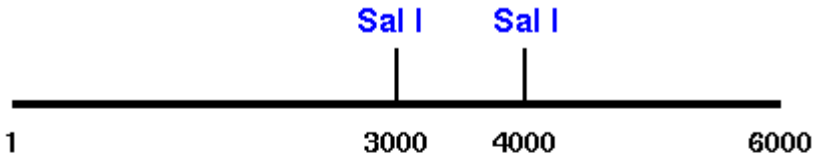
*With which other sites would the cohesive ends generated by this enzyme ligate? If none of the other sites are compatible, answer "NA"

2. (10 points) A plot of melting temperature Tm as a function of temperature is shown for DNA duplexes of various lengths. What generalizations can you draw from the results?

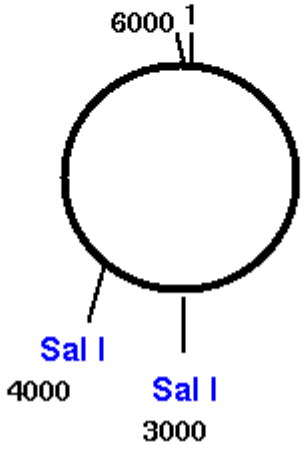


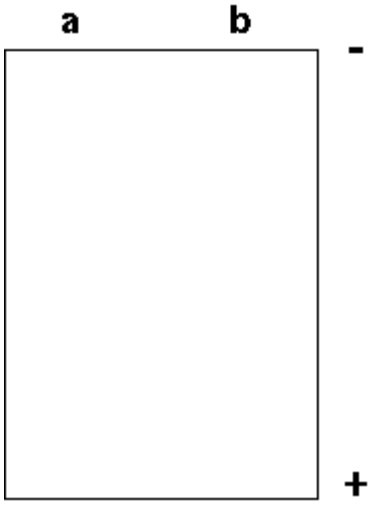
3. (10 points) Restriction sites for SalI are shown for two different DNA molecules, as indicated in a) and b). Reference coordinates are also indicated on the maps. Draw a diagram, similar to the one shown at right, of the expected bands seen for SalI digests of each of these molecules, if loaded side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

a)

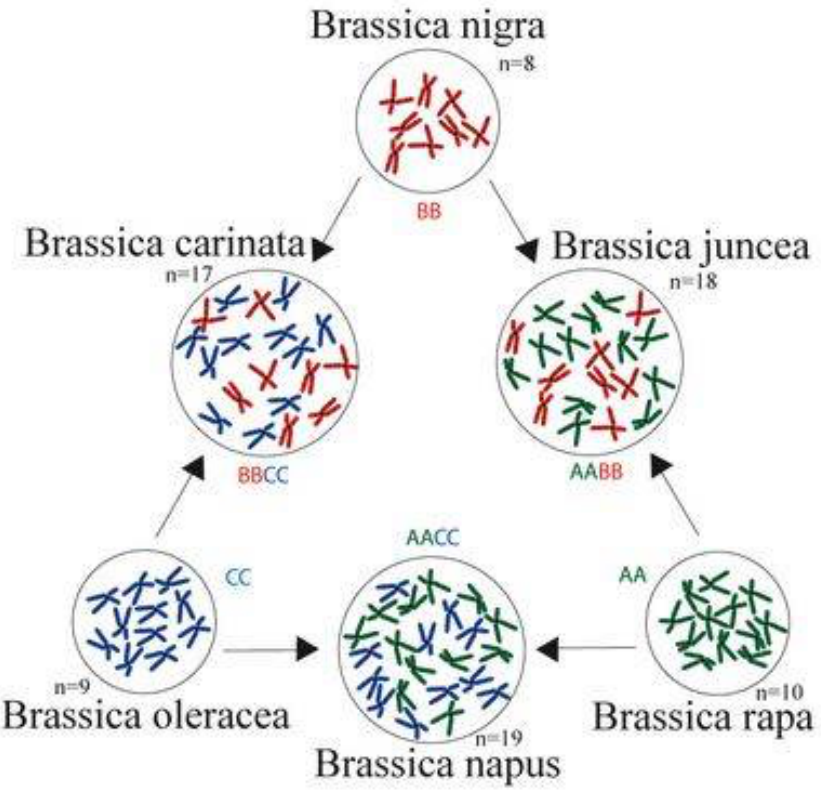


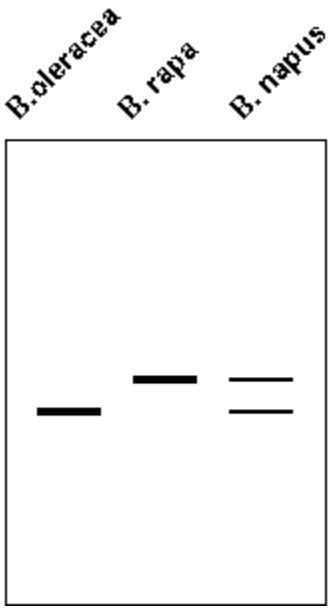
b)





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) Fill in the blanks - choose one of two possible answers for a - e.

Cell elongation is distinct from cell division in a number of ways. During cell elongation, mitosis a) occurs/does not occur. Cell elongation is more characteristic of b) young immature/ older, more mature tissue. In cell elongation, cell volume increases through turgor pressure, primarily due to an increase in volume in the c) vacuole/cytoplasm.

When cells are grown in culture as callus tissue, cells are d) highly-differentiated/de-differentiated. They will grow primarily by e) cell division/cell elongation.

6. (10 points) Beginning with transcription, diagram the main steps which occur in the eukaryotic nucleus for expression of protein coding genes.

7. (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.
Arabidopsis thaliana	5	1.19×10^8	25,400
Frageria vesca	7	2.80×10^8	25,050
Brassica rapa	10	2.84×10^8	41,174
Oryza sativa	12	4.66×10^8	58,000
Glycine max	20	1.10×10^9	46,430
Zea mays	10	2.80×10^9	63,000
Triticum aestivum	21	1.70×10^{10}	41,910

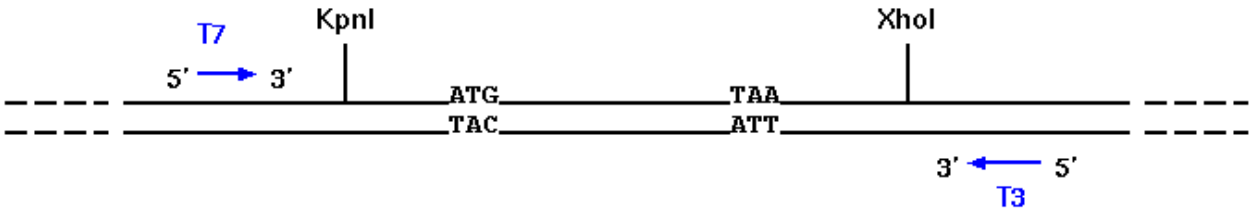
8. (5 points) If a diploid genome undergoes genome doubling, resulting in an autotetraploid, the number of genes could be said to double. Why is that an oversimplification?

9. (10 points) The majority of the DNA in plant genomes consists of repetitive sequences. Some of these sequences are genes, and some are not. Describe the distinction between genic and non-genic repetitve DNA.

10. (10 points) Suppose that you are screening a cDNA library from rice leaves using a probe for a gene in the starch biosynthesis pathway. After screening 60,000 clones from a library containing millions of clones, you don't find any which hybridize with your probe. You have already probed a genomic Southern blot of rice DNA using this probe, and found several bands that hybridize with your probe. Give two possible reasons why you didn't find a cDNA clone that hybridized with your probe, and tell how you would solve the problem in each case.

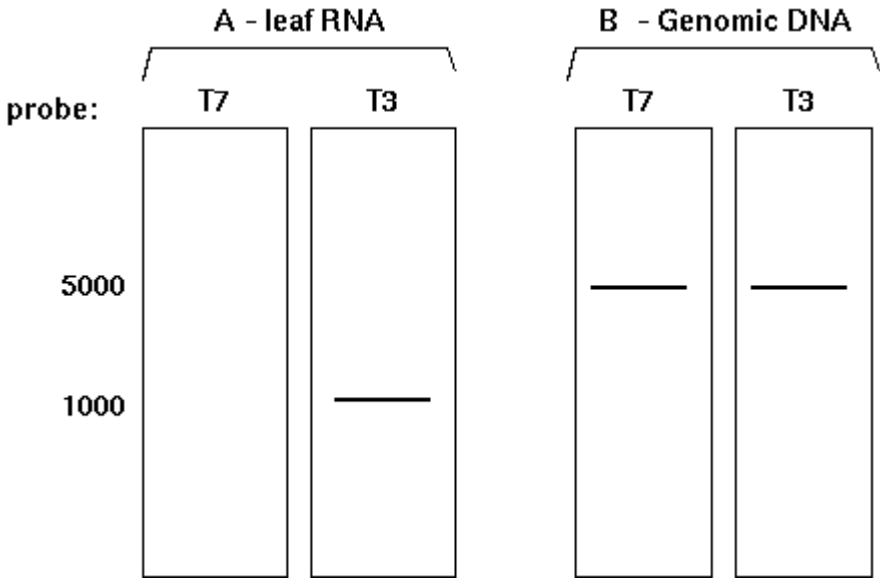
11. (5 points) What is the difference between a transposon and a retrotransposon?

12. (10 points) For the purpose of creating a hybridization probe, almost any DNA or RNA polymerase can be used to synthesize a DNA or RNA strand. In all cases, a nucleotide carrying either a radioactive, chemiluminescent or flourescent tag can be included in the polymerase reaction, to be detected by autoradiography. To make it easy to synthesize single stranded RNA probes, some cloning vectors include promoters for either T3 or T7 RNA polymerase. The following diagram illustrates part of a plasmid in which an Arabidopsis gene has been inserted between the KpnI and XhoI sites in a cloning vector. This vector also has promoters for both T7 and T3 RNA polymerases, as shown. The orientation of the gene is given by noting the start and stop codons for the protein coding region.



To create hybridization probes, two reactions were done:

- T7 - The plasmid was digested with XhoI, followed by incubation with T7 RNA polymerase and labeled nucleotides.
- T3 - The plasmid was digested with KpnI, followed by incubation with T3 RNA polymerase and labeled nucleotides.



Two sets of blots were made:

- Experiment A - RNA was extracted from leaves and run on two identical gels, to produce two identical Northern blots ie. RNA on the filter.
- Experiment B - total genomic DNA was extracted from leaves, digested with HindIII (5'AAGCTT3') and run on two identical gels, to produce two identical Southern blots ie. DNA on the filter. (There are no HindIII sites within the gene.)

Blots from experiments A and B were hybridized using either the T7 or T3 probes, as indicated on the diagram.

a) Explain why bands are seen with either probe on the genomic southern, while in the Northern blot, a band is only seen using the T3 probe.
b) In a similar experiment, the T7 and T3 probes were used to hybridize a Northern blot containing RNA extracted from root tissue. In that experiment no bands were seen on the Northern with either probe. What does the difference between the two Northern blot experiments tell us?

13. (10 points) For each of the diagrams A - E, match the name of the enzyme from the list below that BEST fits the reaction. Note that some enzymes in the list do not have a corresponding diagram.

alkaline phosphatase
DNA ligase
E. coli DNA polymerase I
polynucleotide kinase
Restriction endonuclease XmaI
Ribonuclease H (RNAse H)
DNA polymerase Klenow Fragment