

PLNT2530 PLANT BIOTECHNOLOGY

MID-TERM EXAMINATION

11:30 am to 12:20 pm      Wednesday, March 1, 2023

Answer any combination of questions totaling to exactly 100 points. If you answer questions totaling 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) When making a cDNA library with a Lambda phage vector, the protocol typically includes cutting with a restriction enzyme, followed by dephosphorylation of the vector using alkaline phosphatase. Alternatively, one might consider dephosphorylating the cDNA instead of the Lambda arms. What would be the problem with this alternative approach?

2. (5 points) The simplest way to construct an expression library would be to synthesize a double-stranded cDNA, make the ends blunt using DNA polymerase, and then ligate identical adaptors for a restriction enzyme to both ends. For example, if the adaptors had overhanging ends for a BamHI site, you could clone the cDNA into the BamHI site of a vector.

Suppose you screened the library using a hybridization probe, and found 24 positive clones. How many of them do you expect would express the protein?

Hint: This is NOT directional cloning.

3. (5 points) If a diploid genome undergoes genome doubling, resulting in a tetraploid, the number of genes could be said to double. Why is that an oversimplification?

4. (10 points)

a) Imagine that you are testing a self-driving car on a street with 20 houses. In each trial, the computer chooses an address at random, and drives to that address. After 20 trials, would you expect to have visited all 20 houses? Explain your answer.

b) Now, let's apply this reasoning to genomic libraries. The maize haploid genome is  $2 \times 10^9$  bp long. You have made a BAC library with an insert size of  $2 \times 10^5$  bp. The library contains 10,000 clones. Would you expect that, somewhere in your library, every gene in the maize genome is represented? Explain your answer.

5. (10 points) Which do you think would yield more DNA: 100 mg of embryos, or 100 mg of tissue from fully expanded leaves?

Hint: Think about cell division and cell elongation as part of development.

6. (10 points) Choose the correct answer for each of the following:

a) For any eukaryotic gene, which clone in a library would have a longer insert (cDNA or genomic)

b) Which of the following would NOT be found in a cDNA clone (polyadenylation signal, start codon, 5' untranslated region, TATA box)

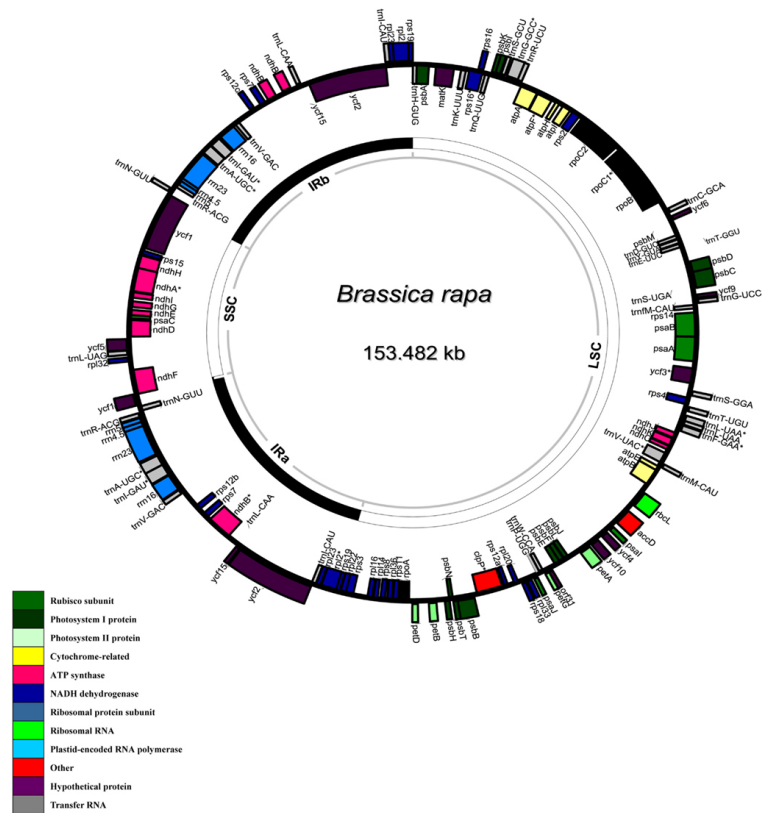
c) Which of the following would NOT be seen in a genomic clone (enhancer, CAAT box, polyA-tail, stop codon)

d) Which would give you more moles: (1 ng of genomic DNA, 1 ng of cDNA)?

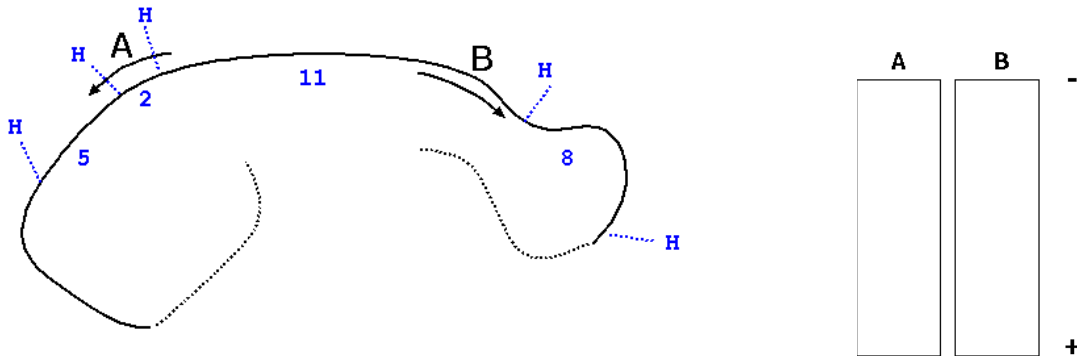
e) If you had a clone containing 500 bp of genomic DNA, what is the thing you would be most likely to find (transposon, rRNA, exon, promoter, telomere, centromere)

7. (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?

8. (5 points) The map of the plastid genome for *Brassica napus* indicates that part of the original genome was duplicated in some ancient plant species. Without knowing the exact coordinates of the two inverted repeats, we can get a crude estimate of their size by noting that IRb spans about 10 o'clock to 12 o'clock on the circle. Give an estimate of the size of the plastid genome before the ancient duplication event occurred. Show your work.



9. (10 points) A map of a small part of a chromosome is shown below. The locations of mRNAs for two genes, A and B are indicated by arrows. HindIII restriction sites are marked as "H", and the distances between sites are given in kilobases.



Two identical Southern blots of HindIII-digested genomic DNA were prepared. Draw the predicted bands if the blot was probed with a cDNA clone for gene A, and show the bands predicted if the other blot was probed with a cDNA for gene B. Next to the Southern, indicate the sizes of the bands in kb.

10. (10 points) Suppose you had cDNA libraries from several cDNA sources as listed below. For each gene a - e, specify which library would give you the best chance of finding a clone for that gene. Write your answer in tabular form, as shown below.

gene	source for cDNA library
a) light-harvesting chlorophyll protein (Cab)	
b) histone	
c) a protein required for DNA recombination	
d) a cotyledon-specific storage protein	
e) a seed storage protein	

Hint: Each source is used only once. Some sources might not be used in the table.

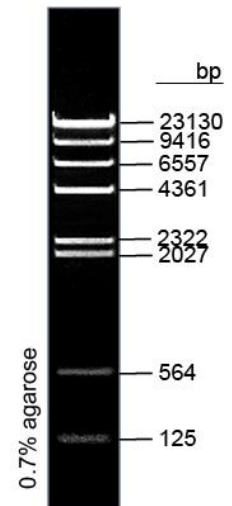
torpedo stage embryo  
 root  
 immature (rapidly growing) leaf  
 mature (fully expanded) leaf  
 anther (male reproductive organ)  
 none of these libraries

11. (15 points) In your exam booklet, re-write the table, using the terms below. Some terms may be used more than once. Some may not be used at all.

	plastid	mitochondrion	nucleus
genome size (bp)			
copies of genome per cell			
chromosome topology			
number of genes			
inheritance			

maternal      paternal      both parents  
 1      2      <100      > 100      50 - 200       $10^5$   
 >  $10^4$        $10^5 - 10^6$        $10^7 - 10^{12}$   
 linear      circular      branched

12. (5 points) The figure at right shows Lambda phage DNA digested with HindIII, separated by electrophoresis and stained with RedSafe. The length of each restriction fragment is indicated at right. Explain why some bands are brighter than others on the gel.



13. (10 points) Match statements a - e with one of the choices.

Enzyme	Cutting site
BamHI	G <sup>^</sup> GATCC
ApaI	GGGCC <sup>^</sup> C
NotI	GC <sup>^</sup> GGCCGC
AoxI	<sup>^</sup> GGCC
HaeII	RGCGC <sup>^</sup> Y

- a) Digestion with this enzyme gives the largest fragments
- b) Digestion with this enzyme gives the smallest fragments
- c) Enzymes whose overhangs are compatible for ligation
- d) Generates a 3' protruding end
- e) Has an asymmetric recognition sequence

Choices:

- 1. none
- 2. HaeII
- 3. NotI
- 4. AoxI
- 5. ApaI
- 6. BamHI
- 7. ApaI, AoxI
- 8. AoxI, NotI
- 9. ApaI, NotI

14. (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

