PLNT2530 PLANT BIOTECHNOLOGY

MID-TERM EXAMINATION

11:30 am to 12:20 pm Friday, February 12, 2016

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.

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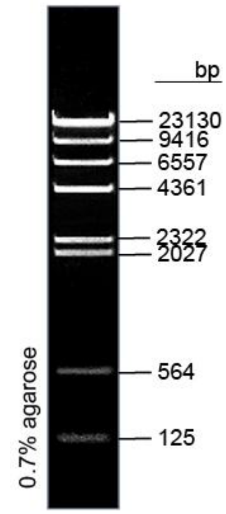
1. (15 points) The following table lists restriction enzymes and their cutting sites, indicated by the caret (^). Complete the table by indicating what should appear in boxes labeled **a - e**.

Enzyme	Cutting site	Cohesive ends	Ligates with*
NotI	5'GC^GGCCGC3'	5'-GC GGCCGC-3' 3'-CGCCGG5' CG-5'	**c**
NspLKI	5'GG^CC3'	**b**	SmaI
SmaI	5'CCC^GGG3'	5'-CCC GGG-3' 3'-GGG CCC-5'	NspKI
XmaI	**a**	5'-C CCGGG-3' 3'-GGGCC C-5'	**d**
XmaIII	5'C^GGCCG3'	5'-C GGCCC-3' 3'-CCCGG G-5'	**e**
*With which other sites would the cohesive ends generated by this enzyme ligate? If none of the other sites are compatible, answer "NA"			

2. (5 points) The figure at right shows Lambda phage DNA digested with HindIII, separated by electrophoresis and stained with Ethidium bromide. The length of each restriction fragment is indicated at right. Explain why some bands are brighter than others on the gel.



3. (10 points) List two aspects of DNA structure that cause single-stranded DNA to spontaneously reanneal into double-stranded DNA.

4. (10 points) Naturally-occurring plasmids in *E. coli* typically have copy control sequences which, if present, limit the plasmid to one copy per cell. Plasmid cloning vectors typically have these sequences removed. In contrast, BAC vectors typically do include copy number control sequences. Explain why copy control is useful to have in BACs, but needs to be deleted in plasmid vectors.

5. (5 points) Why is it necessary to provide a carbon source to plant cells growing in callus culture?

6. (15 points) Wheat and rye are two closely-related monocot species. It is therefore a good assumption that a probe from one species will detect related sequences in the other genome. Suppose you had BAC libraries from both wheat and rye. Based on DNA sequencing of one of the rye BAC clones, you have identified a rye clone containing the PR1 gene, a gene that is activated when plants are infected by fungi or bacteria. You would like to find the homologous PR1 gene in the wheat library.

a) What would be two problems associated with labeling the rye BAC DNA for use as a probe that to identify a BAC clone from wheat containing the wheat PR1 gene?

b) How could you use PCR to get around these problems?

7. (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.

8. (10 points) Beginning with transcription, diagram the main steps which occur in the eukaryotic nucleus for expression of protein coding genes.

9. (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

10. (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?

11. (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.

12. (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.

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