

PLNT2530 PLANT BIOTECHNOLOGY

MID-TERM EXAMINATION

11:30 am to 12:20 pm

Wednesday, February 11, 2014

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:

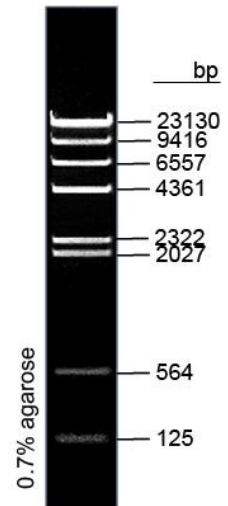
- i. Just answer the question. Save time by specifically addressing what is asked. Don't give irrelevant background if it doesn't contribute to the question that was asked.
- ii. Avoid stream of consciousness. Plan your answer by organizing your key points, and then write a concise, coherent answer. 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 (^). Complete the table by indicating what should appear in boxes labeled **a - e**.

Enzyme	Cutting site	Cohesive ends		Ligates with*
NotI	5' GC [^] GGCCGC 3'	5' -GC 3' -CGCCGG5'	GGCCGC - 3' CG - 5'	c
NspLKI	5' GG [^] CC3'		b	SmaI
SmaI	5' CCC [^] GGG3'	5' -CCC 3' -GGG	GGG - 3' CCC - 5'	NspKI
XmaI	a	5' -C 3' -GGGCC	CCGGG - 3' C - 5'	d
XmaIII	5' C [^] GGCCG3'	5' -C 3' -CCCGG	GGCCC - 3' 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) We have discussed several different types of tissue culture. If tissue explants are cultivated with an equal cytokinin to auxin ratio, cells will _____a_____, and the resultant cells are referred to as _____b_____. In cell suspension culture, cells are maintained and divide as _____c_____. One of the most difficult types of tissue culture is protoplast culture, in which _____d_____ are removed by digestion with enzymes such as cellulase and hemicellulase. This requires strict control of osmolarity to prevent cells from bursting.

It is also possible to cultivate microspores in culture. Plants regenerated from microspores will have a _____e_____ chromosome number.

4. (10 points) The *Arabidopsis thaliana* haploid genome has 5 chromosomes, with a total genome size of 1.2×10^8 bp. The *Glycinie max* haploid genome (soybean) has 10 chromosomes with a total genome size of 1.1×10^9 bp. What is the most important reason for the difference in genome sizes. What role does chromosome number play in determining the genome size, in base pairs?

5. (20 points) Draw a simple diagram illustrating the process of gene expression in eukaryotes. Make sure to include the following: Transcription of the gene (DNA) to pre-mRNA, processing of the pre-mRNA to mRNA, and translation of mRNA into protein. Make sure to label 5' and 3' ends, and amino terminal and carboxy terminal ends.

6. (10 points) List two aspects of DNA structure that cause single-stranded DNA to spontaneously reanneal into double-stranded DNA.

7. (10 points) Briefly describe the differences between cell division and cell elongation. Your answer may be in point form or in a table.

8. (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.

9. (5 points) Why is it necessary to provide a carbon source to plant cells growing in callus culture?

10. (10 points) What is meant by the term "directional cloning"? Use one or more diagrams to illustrate your descriptions.

11. (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?