PLNT2530 PLANT BIOTECHNOLOGY

FINAL EXAMINATION

6:00 pm to 8:00 pm Wednesday, April 16, 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 points equal 100. This exam is worth 40% of the course grade. The questions available total to 120 points.

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. (10 points) You are planning a research project in canola (*Brassica napus*), a tetraploid species very closely-related to *B. rapa*. There is available at NCBI a high quality genomic sequence for *B. rapa*, although only 60% of the genome has so far been sequenced. Also available is the complete genome for *Arabidopsis thaliana*, for which 100% of the genomic sequence is available.

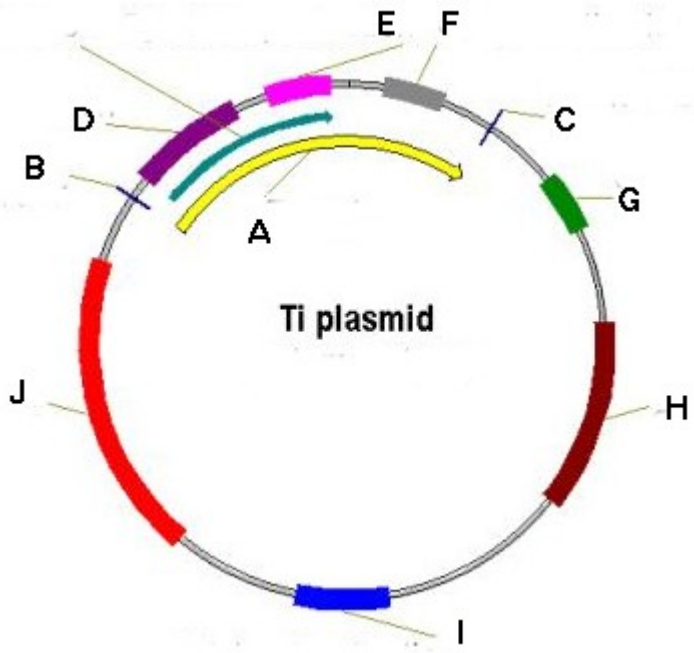
Describe the tradeoffs associated with constructing a microarray consisting of 60-mer oligonucleotides, designed from either *B. rapa* or *A. thaliana*, to measure gene expression levels in *B. napus*

2. (10 points) Glyphosate (Roundup) is a herbicide that targets the _____a_____ pathway, leading to the production of many different classes of secondary metabolites. One example of a product from this pathway is _____b_____. In plant cells, the shikimic acid pathway occurs in the _____c_____ . The target of glyphosate is the enzyme EPSP synthase.

Two different genes have been used for engineering glyphosate resistance in crops. One is an EPSP synthase gene from *Agrobacterium* for which differs from the plant enzyme because _____d_____. The other enzyme used is a bacterial enzyme called glyphosate oxidoreductase that confers glyphosate resistance by _____e_____.

3. (10 points) *Agrobacterium* has evolved an elaborate mechanism for transforming plant cells. Describe what the function of the T-DNA in transformed plants, and explain how this benefits *Agrobacterium*.

4. (10 points) For the map of the octopine Ti plasmid shown below, give the name for each feature marked with a letter, A - J. You do not need to describe each feature, just list its name.



5. (15 points) In the event that a gene from a transgenic crop gets transferred to a wild relative, what factors might govern the "fixation" of that gene in the wild population? (Fixation means that eventually, all plants in the population would have the gene.)
Discuss the likelihood for fixation of the following traits, introgressed from a transgenic plant into a wild plant species:

a) a gene for antibiotic resistance
b) a gene for herbicide resistance
c) a gene for improved nutritional content

6. (15 points) What is the basis of resistance to Bt. toxin that arises in populations of insect pests? Explain the principle behind the use of refugia with crops expressing the Bt protein. What does the planting of refugia hope to accomplish, and how does this work?

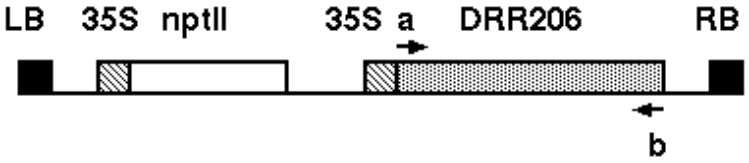
7. (10 points) What are the difficulties associated with genetic engineering to eliminate allergenicity from peanut? What experimental strategy was used to overcome these difficulties, and how well did they work?

8. (5 points) What is the best type of cloning vector for creating a library if you plan to do chromosome walking? Explain your reason.

9. (15 points) There are many ways of verifying transformation of a plant, each with its advantages and disadvantages. The construct shown below contains two genes, under the control of the 35S promoter, between the T-DNA left and right borders. The DRR206 gene has been shown to provide some resistance to the blackleg fungus in transgenic canola. The nptII gene encodes neomycin phosphotransferase.

The following table lists a number of possible experiments, and the types of information that might be obtained. A "yes" means that the experiment is likely to provide a particular type of information. A "no" means that the experiment will not tell us that information. Some of the table has already been filled in.

Your job is to rewrite the table in your answer book, and complete it using yes/no answers. To save time, feel free to shorten or abbreviate the column and row headings, as long as you keep them in the same order.



	What the experiment tells us:			
	Presence of DRR206 gene	Copy number of DRR206 gene	Expression of DRR206	Presence of nptII gene
Experiment:				
PCR using primers a and b	**yes**	**no**	**no**	**no**
Southern blot using DRR206 gene as a probe				
Northern blot using DRR206 as a probe				
assay for root growth in kanamycin				
test for resistance to blackleg		**no**		

10. (5 points) Binary vector systems for plant transformation employ two plasmids. Briefly explain the function of the two types of plasmids in *Agrobacterium*-mediated transformation.

11. (5 points) List at least 2 advantages of transformation using biolistics (the “Gene Gun”) over *Agrobacterium* transformation.

12. (10 points) Which of the AFLPs in the accompanying gel would be considered informative? Which would you use in a genetic mapping experiment, and why?