

PLNT2530 PLANT BIOTECHNOLOGY

MID-TERM EXAMINATION

11:30 am to 12:20 pm

Wednesday, February 12, 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.
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1. (10 points) Describe the concepts of phenotypic plasticity and totipotency. Why are both important for plant transformation?

2. (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.

3. (10 points) Describe the technique of PCR. What is the purpose of PCR, and how does it work?

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. (15 points) Describe the main characteristics of BAC vectors, in terms of sequences for maintenance in *E. coli*, preventing clones with no inserts, and working with large inserts. You may use point form or a table to organize your answer.

7. (10 points) Raw RNA extracted from plant cells will contain mRNA, tRNA and rRNA, with rRNA making up the vast majority of the RNA population. Describe the technique, used in cDNA cloning, for removing tRNA and rRNA from an RNA sample.

8. (10 points) What are the differences between cDNA libraries and genomic libraries? (You may use a table or point form to organize your answer.)

9. (10 points) Suppose two cDNA libraries were made from the same mRNA population. In both cases, an expression vector is used, containing a promoter to drive expression, and a lacZ gene with a multiple cloning site. Library A was made using a single restriction enzyme for cloning the cDNA. Library B was made by directional cloning, using two restriction enzymes.

For any given clone, what are the chances that it will correctly express the protein coded for by the cDNA, in Library A, and in Library B? Explain your reasoning.

10. (5 points) Why is transformation of plastid genomes considered a useful mechanism for preventing the spread of transgenes from transformed crops to conventional crops?

11. (10 points) What is meant by the term "directional cloning"? Use one or more diagrams to illustrate your descriptions.