

PLNT3140 INTRODUCTORY CYTOGENETICS

FINAL EXAMINATION

December 19, 2011

Time: 9:00 - 11:00 pm

Location: University Centre, Rm 210 - 224, seats 222-239

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. There are 10 questions to choose from, totaling 120 points. This exam is worth 35% of the final grade.

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)

a) In experiments with artificial chromosomes, it is not typically possible to do traditional recombinant DNA manipulations. For example, one might want to clone a BAC insert from a BAC vector into an artificial chromosome. In other words, the large size of BAC inserts makes it impractical to excise the insert with a restriction enzyme, isolate the fragment, and ligate it to an artificial chromosome vector. What is the alternative approach for doing such cloning steps?

b) Suppose that you had transformed maize with a new artificial chromosome. How would you find maize lines in which the chromosome was stably-maintained as an independent chromosome?

2. (5 points) In your own words, what does the equation at right tell us? No points are given for simply restating the equation by substituting words for each term in the equation.

$$\frac{C}{C_0} = \frac{1}{1 + kC_0t}$$

3. (10 points) You have isolated a genomic clone containing an important gene. Using PCR with fluorescent nucleotides, you create a labeled hybridization probe for the coding region of the gene (going roughly from the start codon to the stop codon) and probe a genomic Southern blot with this probe. Surprisingly, you see that hundreds of bands light up with this probe. If you probe an identical Southern using the cDNA clone for this same gene, only one band is seen. Propose a hypothesis that would explain this result, and an experiment to test that hypothesis.

4. (10 points) The accompanying table shows data from an experiment in which various relatives of wheat were crossed, followed by selfing, to create amphiploid hybrids. The amount of DNA in nuclei was measured by flow cytometry of chromosomes. DNA amounts are given in picograms (pg) per nucleus.

a) From these data, what can you conclude about genome size in resynthesized amphiploid hybrids?

b) Propose a mechanism that could account for these observations.

Parents and amphiploids	Generation	2n	Genome	Observed DNA value	Expected DNA value
<i>T. turgidum</i> ssp. <i>carthlicum</i>		28	BBAA	23.64 ± 0.23	
<i>Ae. tauschii</i>		14	DD	10.16 ± 0.02	
<i>T. turgidum</i> ssp. <i>carthlicum</i> – <i>Ae. tauschii</i>	S ₁	42	BBAADD	32.13 ± 0.13	33.80
	S ₂	42	BBAADD	31.23 ± 0.06	33.80
	S ₃	42	BBAADD	31.44 ± 0.06	33.8
<i>T. turgidum</i> ssp. <i>dicoccoides</i>		28	BBAA	23.97 ± 0.04	
<i>Ae. tauschii</i>		14	DD	10.16 ± 0.06	
<i>T. turgidum</i> ssp. <i>dicoccoides</i> – <i>Ae. tauschii</i>	S ₂	42	BBAADD	31.80 ± 0.11	34.13
<i>Ae. longissima</i>		14	S ^l S ^l	14.35 ± 0.09	
<i>Ae. umbellulata</i>		14	UU	10.87 ± 0.11	
<i>Ae. longissima</i> – <i>Ae. umbellulata</i>	S ₂	28	S ^l S ^l UU	23.21 ± 0.04	25.22
<i>Ae. sharonensis</i>		14	S ^h S ^h	14.65 ± 0.07	
<i>Ae. umbellulata</i>		14	UU	10.80 ± 0.09	
<i>Ae. sharonensis</i> – <i>Ae. umbellulata</i>	S ₁	28	S ^h S ^h UU	23.15 ± 0.06	25.45
	S ₃	28	S ^h S ^h UU	23.17 ± 0.10	25.45
<i>T. turgidum</i> ssp. <i>durum</i>		28	BBAA	23.91 ± 0.12	
<i>Ae. sharonensis</i>		14	S ^h S ^h	14.66 ± 0.07	
<i>T. turgidum</i> ssp. <i>durum</i> – <i>Ae. sharonensis</i>	S ₃	42	BBAASS	36.52 ± 0.10	38.57
<i>T. urartu</i>		14	AA	11.76 ± 0.07	
<i>Ae. tauschii</i>		14	DD	10.16 ± 0.13	
<i>T. urartu</i> – <i>Ae. tauschii</i>	S ₁	28	AADD	19.67 ± 0.26	21.92
	S ₂	28	AADD	19.80 ± 0.07	21.92

S - selfing generations

5. (10 points) The genome size of the garden pea, *Pisum sativum*, is 4.8 x 10⁹ bp. A pea BAC library was made with an average insert size of 100 kilobases. 50,000 clones were obtained from the library. The library was screened using a pea cDNA clone for the gene DRR206. However, no clones showed hybridization with the probe. What is a simple explanation for this negative result?

6. (10 points) The first step in creation of a chromosome painting kit (spectral karyotyping) involves separation of fluorescently-labeled chromosomes using a FACS (fluorescence-activated cell sorter.) The result is samples of chromosomal DNA highly-enriched for DNA from a single chromosome. DNA from each sample is amplified using PCR, to create a pool of chromosome-specific DNA. However, at this step, DNA cannot be used for chromosome painting.

- a) What would be the problem with using this DNA for chromosome painting?
- b) What is the next step that is necessary to eliminate the problem in part a)?

7. (15 points) Define the following terms for chromosomes: metacentric, acrocentric, telocentric. With each term provide a simple drawing of each type of chromosome.

8. (20 points) For each of the following chromosomal rearrangements, draw a simple diagram of how chromosomes pair at meiosis in heterozygotes.

- a) Terminal deletion
- b) Intercalary deletion
- c) Paracentric inversion
- d) Reciprocal translocation

9. (20 points) For each of the following discuss how it might influence the process of speciation, or whether it would most likely not contribute to speciation.

- a) Translocations
- b) Amplification or deletion of middle-repetitive sequences

10. (10 points) The pairwise distances in cM between five loci are shown in Table 1.

<i>Table 1</i>				
	T175	C35	T93	C66
C35	4.2			
T93	18.7	15.6		
C66	26.1	25.5	12.1	
T50B	30.4	30.5	21.1	12.2

- a) Draw a map, showing the order of markers and the distances between adjacent markers.
- b) The distances between markers do not appear to be additive. That is, if the map order was BCA, the BA distance is not equal to BC + CA. What is the most likely reason for this observation? Can you suggest a potential solution to this problem?