PLNT3140 INTRODUCTORY CYTOGENETICS

MID-TERM EXAMINATION

1 p.m. to 2:15 p.m. Tuesday, October 17, 2023

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. There are 14 questions to choose from, totaling 120 points. This exam is worth 25% of the final grade.

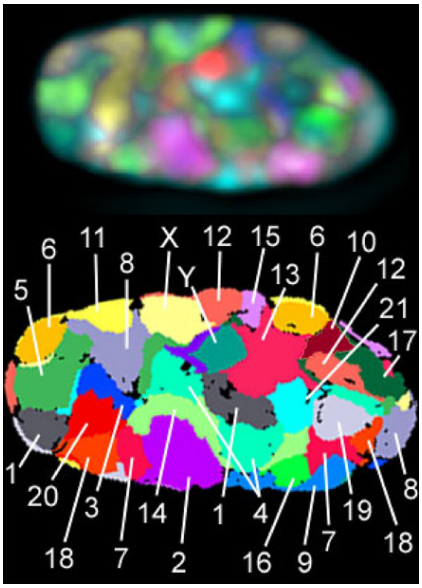
Hand in these question sheets along with your exam book.

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. (5 points) In the Mitosis lab, one of the effects of treatment with 1N HCl would be to break down cellulose in plant cell walls. How would that help in getting good cells to view under the microscope?

2. (10 points) In the figure at right, we see an interphase diploid nucleus in which each chromosome has been painted with a specific combination of fluorescent tags. A series of images was acquired, each at a different focal plane within the nucleus. We are seeing only one of those focal planes. At bottom is an interpretation of the image, showing the locations of different chromosomes.



a)For some chromosomes, we see both copies, while for others, we see only one copy of a chromosome. What is the reason we can't see both copies?

b) Is the area occupied by a chromosome in this 2-D image a good indicator of the actual volume of the chromosome, or of its length?

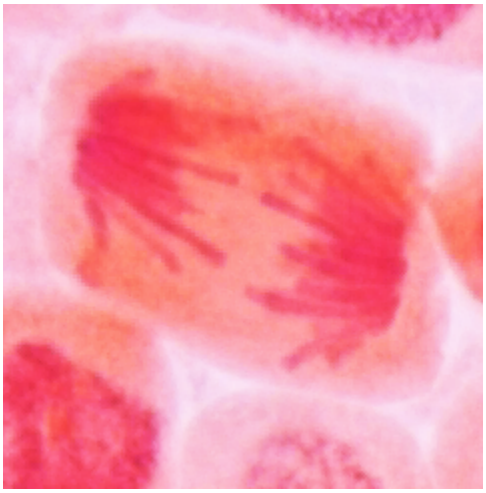
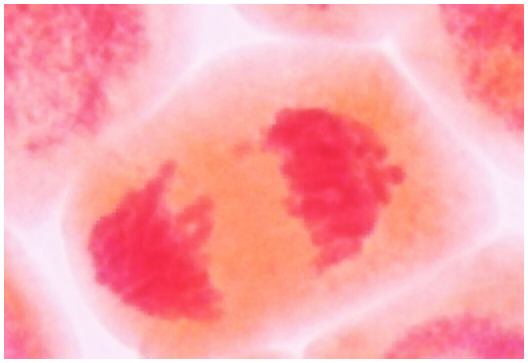
3. (10 points)The Drosophila melanogaster and human genomes are compared in the table below.

	Drosophila melanogaster	Homo sapiens
haploid complement	3 autosomes + 1 XY pair = 4 chromosomes	22 autosomes + 1 XY pair = 23 chromosomes
Total length of haploid genome	1.38×10^8 bp	3×10^9 bp

a) Ignoring crossing over, does meiosis in Drosophila generate more genetic diversity, or less, compared to meiosis in humans? Give calculations to support your answer.

b) Next, consider the contribution of crossing over to genetic diversity. Would you expect crossing over to be a more effective mechanism for generating genetic diversity in Drosophila compared to human? Give a calculation to support your answer.

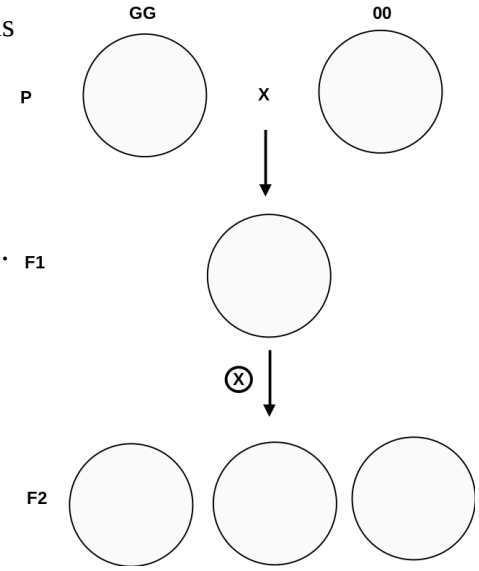
4. (10 points) The appearance of chromosomes during anaphase is the result of the kinetochores "climbing" along the spindle fibers toward the centrosomes, while the arms lag behind.

Early anaphase	Late anaphase
	

While we haven't gone into a lot of detail about telophase, one possibility is that chromosomes largely stay in place, after reaching the opposite poles, and that telophase proceeds by uncoiling of chromosmes where they are, and reforming the nuclear envelope around them. In other words, this model predicts that by the end of telophase or the beginning of G1, chromosomes will be in essentially the same orientation and relative locations that they were in late in anaphase.

Given fluorescent hybridization probes for centromeric and telomeric sequences, how could you use those probes to test that hypothesis? Draw a diagram showing the expected FISH results look like if that hypothesis were true.

5. (10 points) Suppose you have a transformed plant which is homozygous for insertion of a foreign gene into one of the chromosomes. You cross the transformant line with a plant lacking the transformant gene. That cross could be represented as GG x 00, where G represents a transformant chromosome, and 0 represents a chromosome lacking the gene. In the diagram below, nuclei are represented as circles.
a) Re-draw that diagram showing expected FISH results, using a probe for gene G.
b) What would be the ratio of progeny seen in the F2 generation?



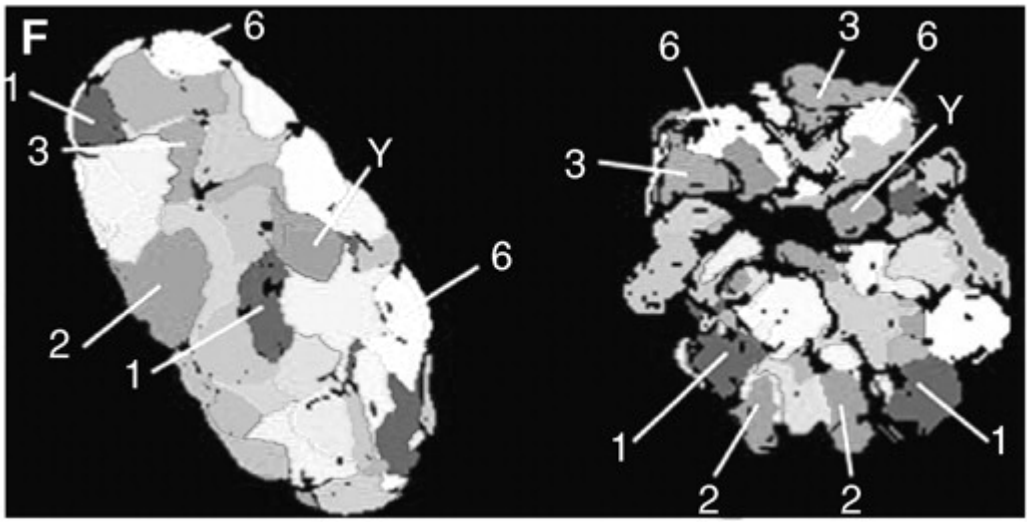
6. (5 points) Eukaryotes generally have linear chromosomes. Is it impossible to have circular chromosomes in eukaryotes? Give evidence to support your reasoning.

7. (10 points) We have demonstrated that if you do a restriction digest of genomic DNA from any eukatyotic genome, you get smear of DNA on the gel, because there are so many bands that they cannot be resolved as separate bands. Would the same be true of a prokaryotic genome?

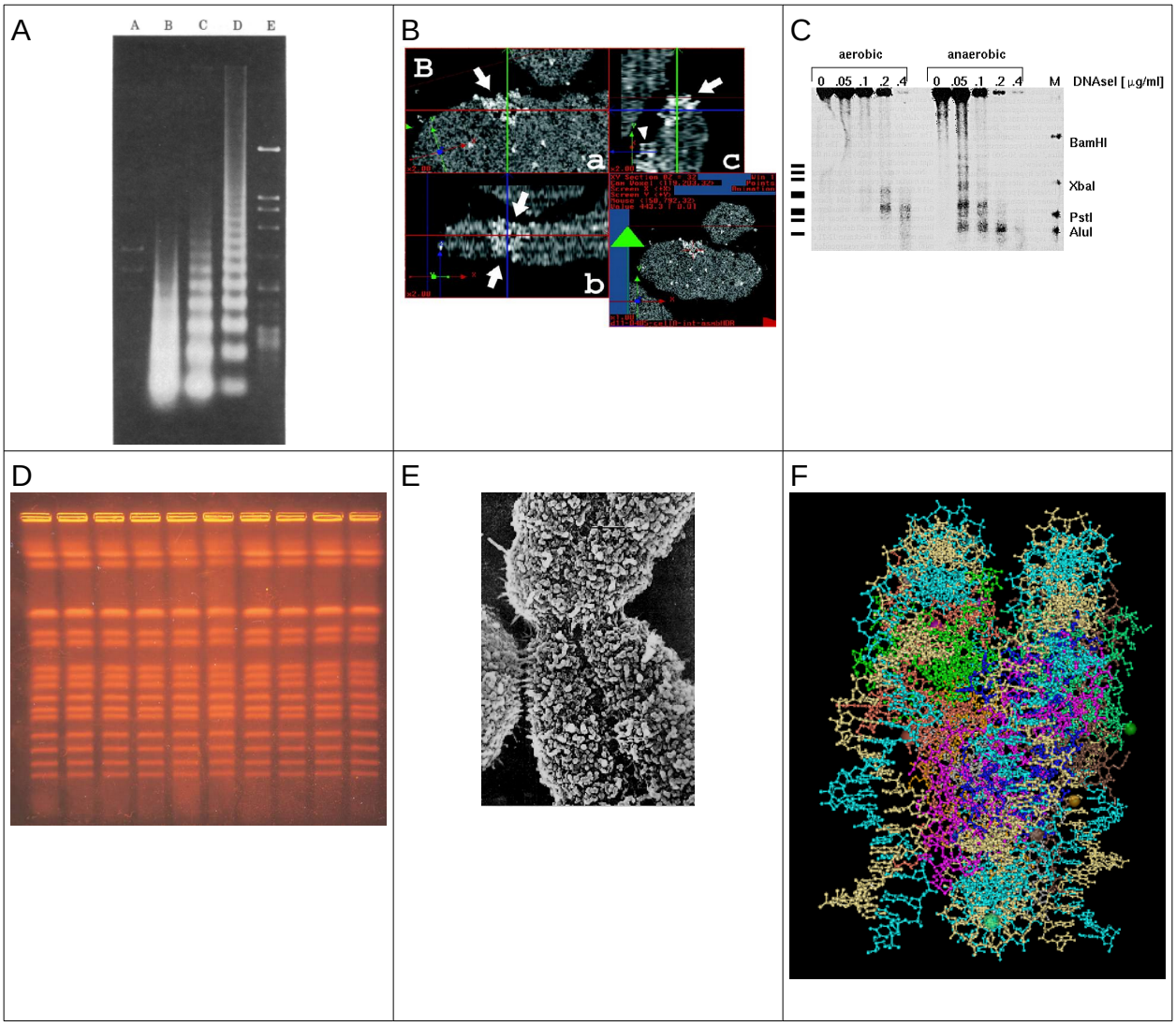
a) For example, consider a restriction digest of E. coli (genome size = 5.5×10^6 bp) digested with any enzyme that recognizes a 6 base sequence (eg. BamHI, HindIII, EcoRI). Do a calculation to answer this question.

b) What if you used an restriction enzyme that recognizes an 8 base sequence (eg. NotI). Do a similar calculation to demonstrate whether or not an 8-cutter would improve the result.

8. (10 points) A 3D reconstruction of a human nucleus is shown in the left panel of Figure F. The identities of chromosome territories, as visualized by FISH, are marked with numbers. A similar image from another cell is shown in the right panel. Which stage of mitotis is represented in the right panel? Explain your choice.



9. (10 points) Below are five statements and six figures. Match each statement with the figure that best describes it. One of the figures is not relevant to any statement, and has no match.

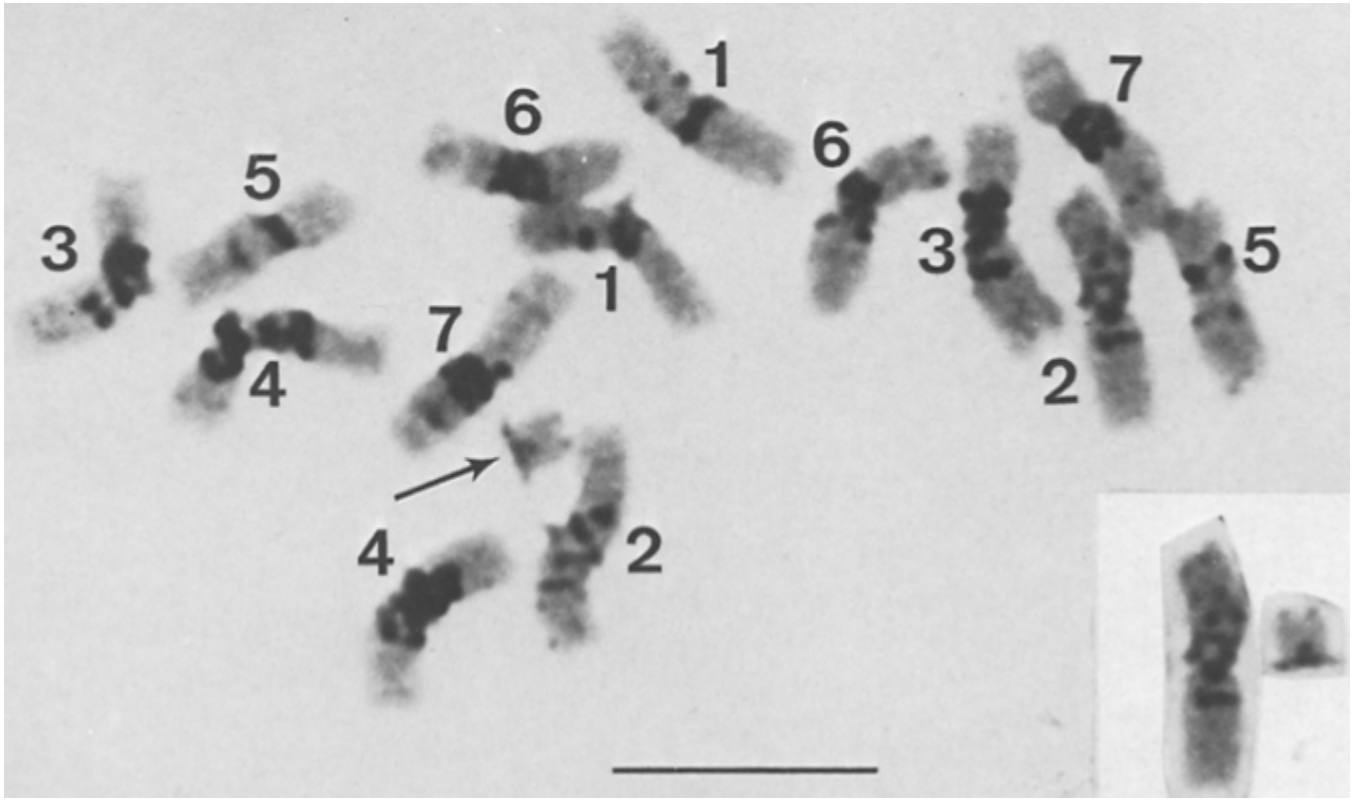


I. The eukaryotic chromosome is a single DNA molecule.
II. At least in some chromosomes, there are at least two higher-order levels of chromatin folding/coiling, between the domain level of organization, and the final mitotic chromosome.
III. DNA is associated with chromatin proteins in units with a periodicity of about 200 bp.
IV. The DNA double helix wraps twice around the nucleosome core particle.
V. Transcriptionally active genes have a more open chromatin conformation than inactive genes.

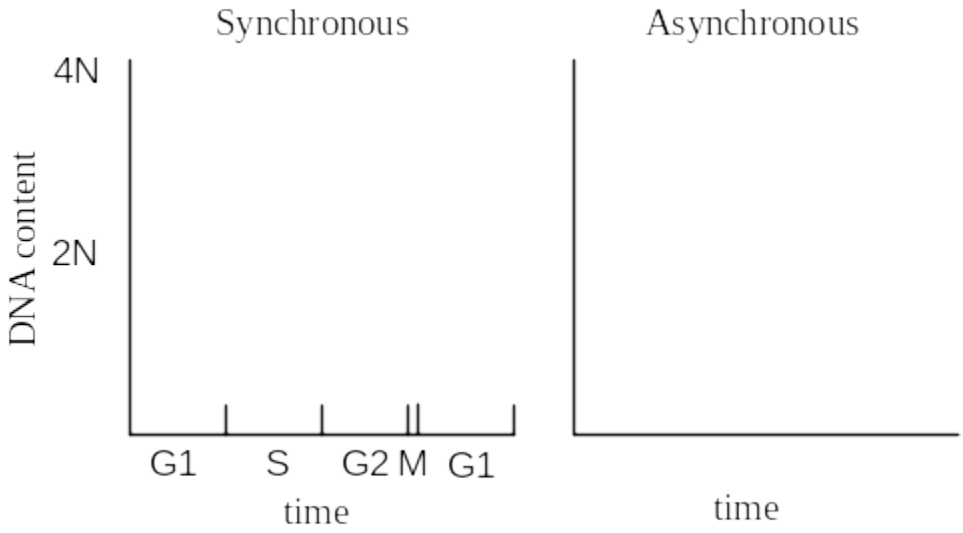
In your exam book, make a list I - V, and choose the letter that best matches each statement.

I.
II.
III.
IV.
V.

10. (10 points) We have previously discussed evidence that high-level chromosome folding requires at least 2 levels of folding, above the level of the chromatin domain. G-banded barley chromosomes are shown below. Explain how the 2-level model would be supported by the G-banded chromosomes shown. Which chromosomes lend support to that model?



11. (10 points) One batch of cells was grown in Synchronous culture, so that all cells are going through the cell cycle at the same time. Another batch of cells was grown in asynchronous culture, so that cells divide at different times. DNA content was measured in cells from each batch. For the synchronous cells, times for G1, S, G2 and M are given. Draw similar graphs, indicating the amount of DNA in each of the two cultures at different times.



12. (5 points) Consider the hypothesis that the way chromosomes fold at different locations along their lengths is ultimately determined by the DNA sequences in each region. Give at least 2 observations that have we discussed in class which support this hypothesis.

13. (10 points) Histones are considered the most highly conserved proteins in nature. For example, out of 102 amino acids, Histone H4 has only 2 amino acid differences between pea and cow. What evidence regarding histone structure have we discussed in class that would help to explain the high degree of amino acid conservation histone proteins over long evolutionary time scales?

14. (5 points) Prokaryotes get by with a single origin of replication per chromosome. Eukaryotic chromosomes each have many origins of replication. What is it about eukaryotic chromosomes that requires having more than one replication origin, and how do multiple origins solve this problem?