PLNT3140 INTRODUCTORY CYTOGENETICS

MID-TERM EXAMINATION

1 p.m. to 2:15 p.m. Thursday, October 18, 2012

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 15% of the course grade.

Hand in these question sheets along with your exam book.

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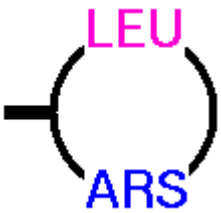
1. (5 points) What is the distinction between the terms "centromere" and "kinetochore"?

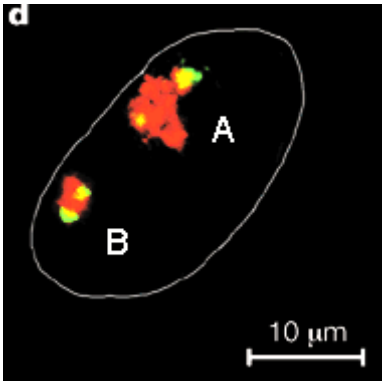
2. (10 points) The construct shown at right contains a Leucine biosynthesis gene (Leu) and an origin of replication (ARS). Random fragments from the yeast genome were cloned into the restriction site, marked by a solid bar.



The construct was transfected into yeast with the Leu$^-$ genotype. To guarantee that transfection worked, cells were first plated on minimal media, so that only those cells successfully transfected with the Leu construct would be selected. The yeast were then propagated on media supplemented with Leucine for many generations. After that time, individual clones (ie. cells from isolated colonies) were replated on minimal media. A few clones grew on the minimal media while most did not. Clones that grew on minimal media were replated onto Leu-containing media for several more generations. Again, individual clones from this culture were replated onto minimal media. This time, all clones grew.

Why is it that some clones failed to grow on the first plating on minimal media? Why did all grow in the second plating on minimal media?

3. (5 points) The accompanying figure shows two human X chromosomes in the interphase nucleus of a human female, visualized using chromosome painting. In humans, genes on one X chromosome are actively transcribed, while genes on the other are not transcribed. The inactive chromosome is often referred to as a Barr Body. Which chromosome in the picture is active and which is inactive? State your reasons.



4. (10 points) In humans, Chromosome 18 is known to have few genes, while chromosome 19 is very gene rich. The image below shows 3D computer reconstructions of interphase nuclei in which territories for chromosomes 18 and 19 have been selectively displayed. (The two copies of chromosome 19 are so close together that they can't be separated visually.)

What conclusions can you draw from these results?

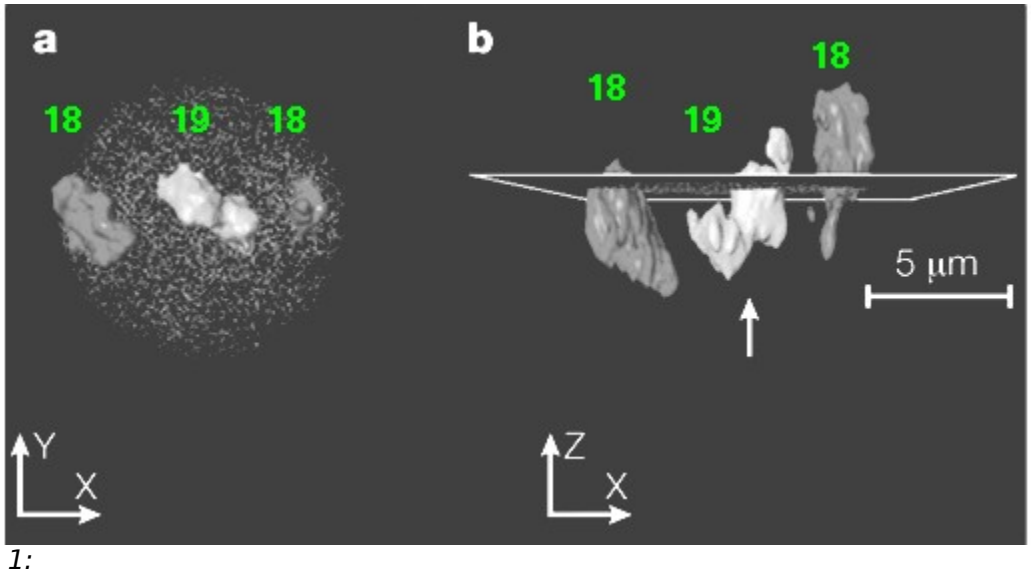


Figure 1:

5. (10 points) Provide a word or phrase for each of the blanks below.

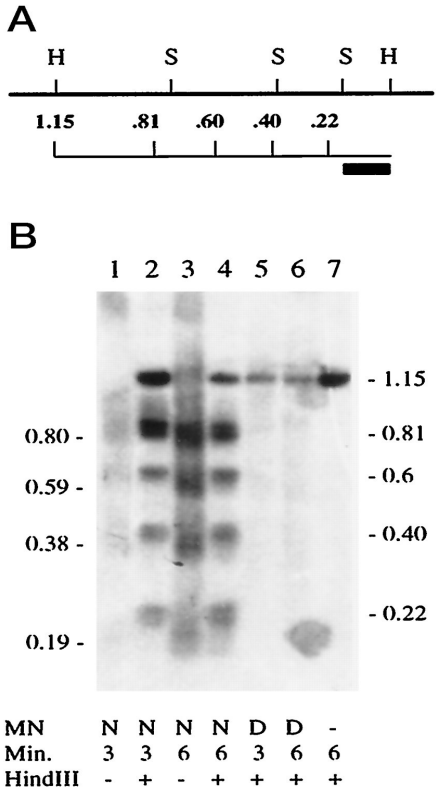
The eukaryotic cell cycle was first defined in experiments in which the rates of _____a_____ were studied, by giving cells in synchronous culture a brief pulse of _____b_____, which was incorporated only during a short phase of the cell cycle. The eukaryotic cell cycle is a compromise around is fundamental conflict between two important processes: _____c_____ and _____d_____. The phase of the cell cycle in which you would be least likely to see high levels of RNA synthesis is _____e_____.

6. (10 points)
The nuclear membrane can be considered a differentiated form of the _____a_____. Chromatin is anchored to the _____b_____ surface of the nuclear membrane.

_____c_____ regulate the transport of _____d_____ from the nucleus to the cytoplasm, and transport of _____e_____ from the cytoplasm to the nucleus.

7. (15 points) The experiment below investigates the chromatin structure in the vicinity of the cyd1 locus in the cylliated protozoan *Tetrahymena thermophila*. The experimental conditions are summarized at the bottom of B. Micrococcal nuclease was added either to purified nuclei or naked DNA (MN), for either 3 or 6 minutes. After these treatments, purified DNA was subsequently digested with HindIII, or not digested (+/-). A southern blot was done with the DNA samples, and blot was probed using the Sau3A/HindIII fragment indicated by the solid bar in A. This method is referred to as indirect end-labeling.

A, restriction map of the macronuclear DNA. H,HindIII; S, Sau3A; bar, probe for the indirect end labeling experiment. The scale below the map describes the fragments (kb) detected in the indirect end labeling experiments in panel B. The linker end of each successive fragment is shown by a vertical line; numbers indicate the distance from the linker region to the HindIII site.
B, indirect end labeling blot. MN, micrococcal nuclease digestion of nuclei (N) or purified DNA (D). Min., minutes of incubation in micrococcal nuclease buffer. Numbers to the left of the blot indicate the median size of fragments generated by micrococcal nuclease in lanes 1 and 3; numbers to the right indicate the sizes of fragments generated by digestion of those fragments with HindIII in lanes 2 and 4.

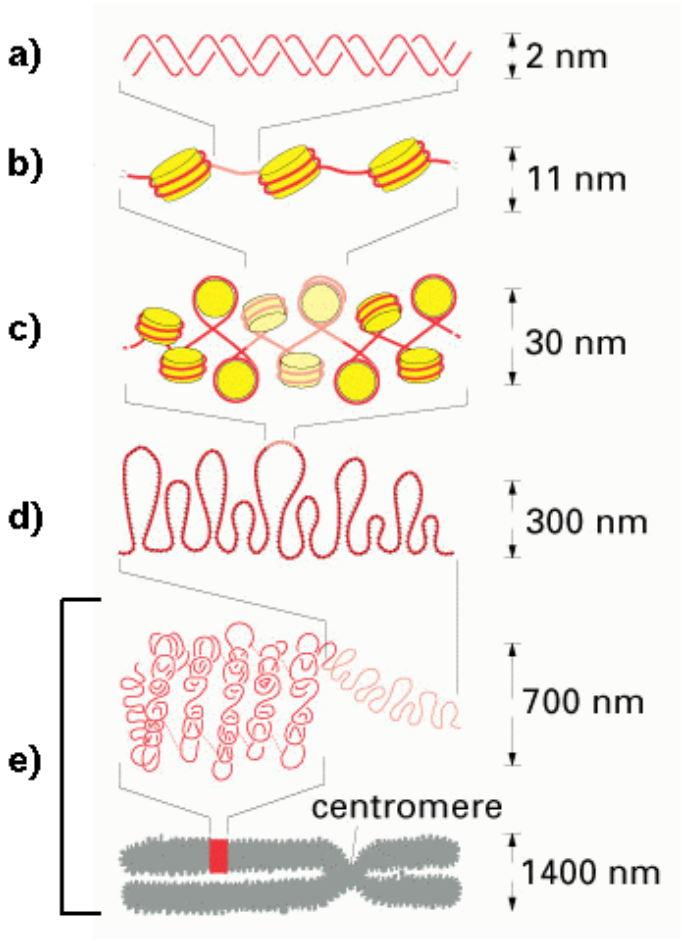


a) (5 points) Explain the difference between the results with isolated nuclei (N) and naked DNA (D), and the nuclease-free control (-).

b) (10 points) In particular, note the results in lane 4, for which the median fragment sizes are shown to the left of the gel. What is the significance of these fragment sizes?

8. (10 points) Give two reasons why root tip cells are an excellent choice of material for observing mitosis in plants.

9. (20 points) Using the accompanying figure as a guide, briefly (1 or 2 sentences each for letters a - e), summarize the levels of chromosome structure



10. (15 points) Knowing what you now know about recombinant DNA methodologies, cite at least five reasons that it is not practical to do genetic engineering of eukaryotic chromosomes by standard in-vitro methods used for manipulating DNA. For example, when we want to clone a piece of DNA into a plasmid vector, we can cut both the vector and insert with a restriction enzyme, ligate the vector and insert together, transform the recombinant DNA into cells, and select for transformants on selective media. We can then determine the size of the insert by isolating plasmid DNA from transformed cells, digesting with a restriction enzyme, and visualizing the insert band in agarose gel electrophoresis. Why can't we do these things with eukaryotic chromosomes?

For clarity when answering this question, break your answer up into sections as in the example below:

a)
b)
c)
d)
e)

11. (5 points) The sequence below shows the Simian Virus 40 origin of replication, as annotated in Sumitra et al. (1986) Mol. Cell. Biol. 6:1663-1670. Recalling what you know about DNA, is there a feature of this sequence which might be particularly important to facilitating formation of a replication fork? Explain your answer.

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                     Early Palindrome    T antigen binding                AT
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GGCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGCGGCCTCGGCCTCTGCATAAATAAAAAAAATTA
CCGGAGGTTTTTTCGGAGGAGTGATGAAGACCTTATCGAGTCTCCGGCTCCGCCGGAGCCGGAGACGTATTTATTTTTTTTAAT
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115 points total