PLNT3140 INTRODUCTORY CYTOGENETICS

MID-TERM EXAMINATION

1 p.m. to 2:15 p.m. Thursday, October 20, 2011

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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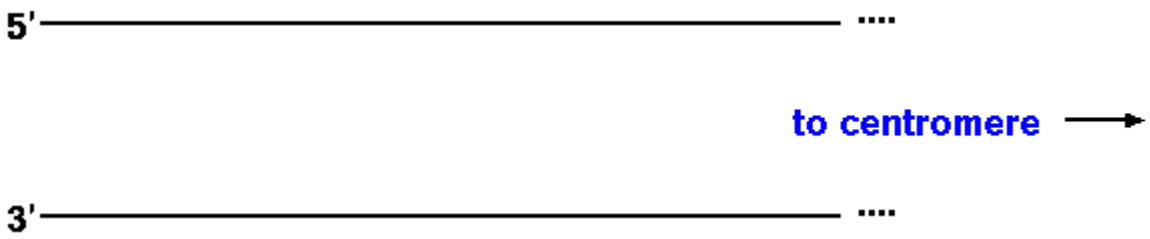
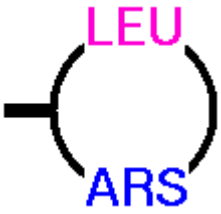
1. (10 points) The diagram below shows both original strands at one end of a linear chromosome. Redraw the diagram below, indicating the newly-synthesized strands after DNA replication, if DNA polymerase was to replicate a linear chromosome, without telomerase activity. Show leading and lagging strands, and label 5' and 3' ends.



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.



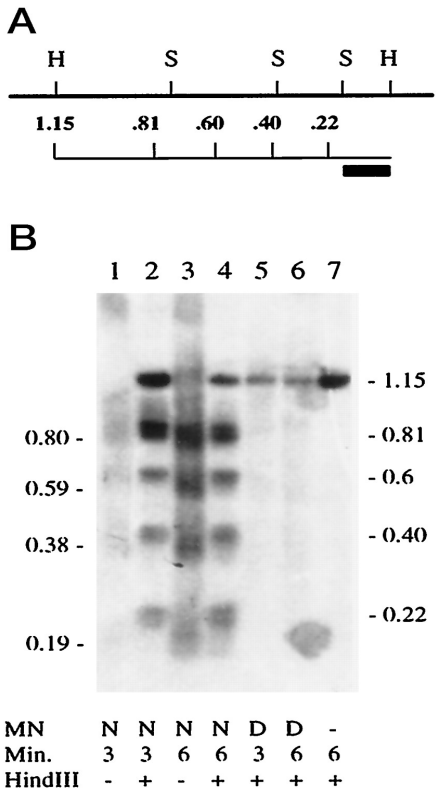
The construct was transfected into yeast with the Leu$^-$ genotype. The yeast were propogated on media supplemented with Leucine for several generations. After that time, individual clones (ie. cells from isolated colonies) were replated on minimal media. A few clones grew on the minimal media while 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) Describe how colchicine can be used to cause eukaryotic cells to divide synchronously in cell culture.

4. (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?

5. (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?

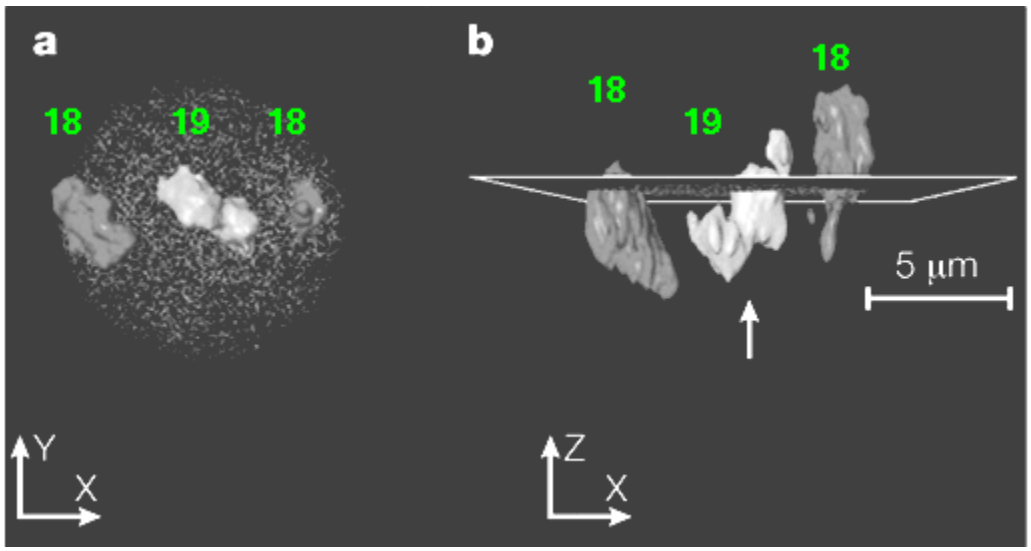
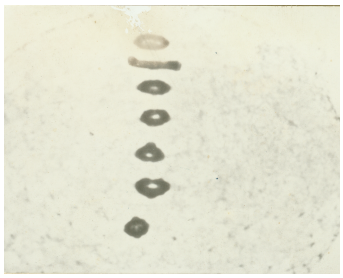
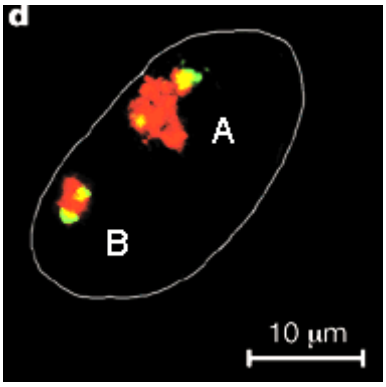


Figure 1:

6. (10 points) Explain why chromosomes at meiotic metaphase I often have a "doughnut" shaped appearance, as shown in the figure.



7. (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.



8. (5 points) Many eukaryotic organisms go through a haploid phase eg. plant gametophyte generation. How does going through a haploid phase benefit the species, from an evolutionary point of view?

9. (20 points) In your exam booklet, constuct a table like the one below, and fill in each cell in the table with at least one example.

	PROKARYOTES	EUKARYOTES
TAXONOMIC GROUPS		
CELL-BIOLOGY		
GENOME STRUCTURE		
GENE EXPRESSION		
CELL CYCLE		

10. (15 points) The protocol for C-banding, discussed in class, is given below.

a. Roots are harvested, pretreated and fixed in 3:1 95% ethanol:glacial acetic acid for at least 24 h. The roots are softened in 45% acetic acid or in 0.5% aceto-carmine. Slides are prepared by the squash method and the cover glass is removed using dry-ice method. Chromosomes adhere to the slide surface.

b. Dehydration. Typically slides are placed in 95 to 100% ethanol for 1 hour.

c. Denaturation. Treatment with barium hydroxide for 5 to 15 min at elevated temperature 50-55° C .

d. Renaturation. The slides are then washed with distilled water and transferred to incubation at 60° C in saline sodium-citrate solution SSC (NaCl). Incubation periods and temperature are variable.

e. Staining. Slides are then stained with Geimsa stain and checked periodically to see how the stain is progressing. When the optimal staining has been achieved, the slides are rinsed in distilled water to remove the excess stain, air-dried, stored in xylene overnight, air dried again and the cover slip is mounted using Canada Balsam, etc.

Briefly describe the purpose of :

i) The acid treatment a

ii) The alkali treatment in c

iii) The renaturation step in d ie. why does this step affect centromeric DNA differently and the rest of the genome?

11. (10 points)

a) (8 points) The components of the microscope could be said to be organized into two sections, labeled A and B on the figure. For each section, describe in one sentence the common purpose to which all components in that section contribute.
b) (2 points) In section B, there is one component that, from the viewpoint of function, really belongs in the category for section A. Which one is that, and why?