Gene 5102-98
Midterm Examination
October 9, 1998

For each item, your tasks are highlighted in **bold face**.

I. Interpretation (35 points total)

Facts and observations:
A. Paul & R. J. Ferl (Plant Cell 10:1349) recently reported the following experiment.

1. They isolated nuclei from maize tissue.
2. They incubated the isolated nuclei with varying concentrations of DNase I, an endonuclease active on DNA.
3. They put the DNase I-digested nuclei in agarose plugs.
4. They treated the plugs with proteinase K and sodium dodecyl sulfate to remove proteins that bind to DNA.
5. They put the plugs in an agarose gel electrophoresis apparatus capable of running a pulsed field gel electrophoretic (PFGE) separation called CHEF. Under CHEF conditions, fragments >800 kbp show length-independent migration.
6. After electrophoresis, they stained the gel with ethidium bromide to observe the DNA.

The result is shown in Fig. 1. on the last page (tear off, if you wish)

1. They also isolated high molecular weight DNA, free of proteins, from the same tissue.
2. They digested the DNA with varying concentrations of DNase I, as they did the nuclei.
3. The digested DNA was also analyzed by CHEF as described above.

The result is shown in Fig. 3.

**Assignment A:**

Why was a PFGE separation method used in place of direct agarose gel electrophoresis? (5 points)
Assignment B:
From inspection of the figures, describe the differences between the products generated by increasing DNase I concentration treatment of nuclei and those generated by similar treatment of naked DNA. (5 points)

Assignment C:
Which aspect of chromatin structure (nucleosomes, supernucleosomes, loops, arms, etc.) is most likely responsible for the differences you described above? Explain your reasoning. (10 points)
Paul and Ferl also did Southern transfers of gels such as those in Fig. 1. One such transfer was hybridized with a labeled fragment containing the *Adh1* gene. Another was hybridized with a labeled fragment containing the *GRF1* gene. In the *Adh1* hybridization, a diffuse probe-reactive band was seen at 90 kbp. In the *GRF1* hybridization, the probe-reactive material was about 100 kbp.

**Assignment D:**
Describe the Southern transfer process (5 points).

**Assignment E:**
What further insights into chromatin structure do these results provide? (10 points)
II. Basis for principles (30 points)

A. Principle:
In most cases of \textit{in vivo} DNA replication, DNA strand separation and new strand synthesis are coupled.

**Assignment A:**
Describe one experimental observation (or series of related observations) supporting coupling. (15 points)

B. Principle:
ARS are DNA sequences that, when inserted into yeast insertion plasmids (YIPs), convert those plasmids to yeast replicating plasmids (YRPs). ARS are not necessarily origins of replication in the DNA from which they were obtained.

**Assignment B:**
Describe one experimental observation (or series of related observations) supporting the conclusion that ARS are not necessarily origins. (15 points)
III. Experimentation (35 points)

Your laboratory has sequenced a 1 Mbp region of a chromosome of *M. geneticus*. Sequence was obtained from an overlapping series of cosmid clones. Genetic maps of all *M. geneticus* chromosomes are available. Markers on these maps include visible phenotypic traits, RFLP’s, RAPD’s, AFLP’s and allozymes. All reagents and supplies needed to score these markers are available in your laboratory.

Your task is to determine from which linkage group your sequenced region is derived. Put another way, it is to determine which genetic markers correspond to loci on your molecular map.

Assignment A:

Why might you not use some kind(s) of markers for this task and which are they? (5 points)

What kind(s) of markers would you use for this task? (5 points)

Describe one way that you could test whether markers of the kind you have chosen are in the 1 Mbp stretch OR whether your 1 Mbp has sequences corresponding to the markers you have chosen? (12 points for any satisfactory method; 3 points for high efficiency methods; 10 points for a clear description of the expected results of your approach and how those results will be interpreted).
Figure 1. Limited DNase I Digestion of Maize Nuclei Converts the Genomes into a Set of Fragments with a Median Length of 45 kb.

(A) Maize nuclei incubated with increasing concentrations of DNase I. Lane 1 is the control, no treatment; lanes 2 to 7 contain 0.01, 0.02, 0.03, 0.1, 0.2, and 0.5 µg/mL DNase I, respectively. Lane 8 contains molecular markers labeled in kilobases.

(B) Densitometric scans of selected lanes from (A). The black line is the DNase I control. The gray lines illustrate how the parent band is converted to the domain-sized fragments with an increase in DNase I concentration; increasingly lighter gray indicates increasing concentrations of DNase I. The legend denotes shades for concentrations of 0.02, 0.05, 0.1, and 0.5 µg/mL DNase I. The positions of key molecular length markers (dotted lines) are indicated beneath the graph.

Figure 3. Naked DNA Shows a Different DNase I Digestion Pattern than De Nucleated Digests.

(A) The first lane (C) illustrates that genomic DNA prepared by dextran sulfate and CsCl density gradients is mechanically fragmented into lengths < 100 kb. Lanes 2 to 4 contain 3 µg of the control DNA incubated with 0.1, 0.5, and 1.0 µg/mL DNase I, respectively. Lane 5 contains molecular length markers indicated in kilobases.

(B) The control lane (C) was derived from an excised parent-genomic DNA band resolved on a preparative CHEF gel of untreated nuclei (see Methods). Lanes 2 and 3 show the results of digesting similar plugs containing the parent-genomic DNA band with 0.05 and 0.1 µg/mL DNase I, respectively. Lane 4 contains molecular length markers indicated in kilobases.