Gene 5102-99
Midterm Examination
October 8, 1999

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

I. Interpretation (35 points total)

   A. Facts and observations:
      Chromosomal DNA of a panda was sheared to two different average lengths: 4,000 bp and 300 bp. Each preparation was then fractionated kinetically into repeated DNA and unique sequence DNA. This was done by incubating the denatured DNA to the appropriate C_{t} value followed by separation of single stranded DNA from double-stranded DNA using hydroxyl apatite (HAP) chromatography. Samples of each of the four fractions were spotted on a nylon membrane and hybridized with a β-globin cDNA probe. Results are shown in the table below.

<table>
<thead>
<tr>
<th></th>
<th>4000 bp avge. DNA</th>
<th>300 bp avge. DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeated</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Unique</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

+++= strong hybridization of probe; + = weak hybridization of probe

Assignment:

**Explain the shift from unique to repeated fractions as the average size of the DNA is made longer (15 points)**
B. Facts and observations:
DNA was extracted from a growing culture of a fungus. The DNA was denatured. Aliquots of the denatured DNA were subjected to electrophoresis on two separate agarose gels. Each gel was Southern blotted to nylon membranes. One membrane was probed with a single-stranded DNA synthesized from a phagemid clone containing an insert of gene YFG. The other membrane was probed with a single-stranded DNA complementary to the first probe (made from a phagemid with the insert in the opposite direction).

Assignment:
How does a phagemid differ from a plasmid? Why was a phagemid preferred as template for this application? (5 points)

Both probes bound strongly to DNA several kbp in length and to very large DNA. However, only the first probe bound to DNA in the 100 to 500 bp range.

Assignment:
Using your knowledge of DNA replication, explain the difference in probe binding to the small single-stranded DNA fragments. (15 points)
II. Basis for principles (30 points)

A. Principle:
   DNA is the material of heredity.

Assignment:
   Give an experimental observation that supports the above assertion. A complete answer will include more than the name of a technique. It will briefly describe the technique and the result of its application. (15 points)

B. Principle:
   Each chromosome consists of only a single double-stranded DNA molecule.

Assignment:
   Describe one experimental observation supporting the above conclusion. A complete answer will include more than the name of a technique. It will briefly describe the technique and the result of its application. (15 points)
III. Experimentation (35 points)

Pretend that you are studying an organism whose chromosomes are large enough to be distinguishable from one another by chromosome banding techniques. Genetic maps for many different kinds of molecular markers are available for this organism.

Assignment A:
Choose one kind of molecular marker (you may wish to look over the rest of this section before making your choice). What marker type did you choose? (5 points)

For the marker type you chose, assume that 3 markers (A, B and C) mapped to the same linkage group and that their order on the linkage map was A-B-C.

Assignment B:
Describe the experimental steps and special materials (probes, libraries, panels, arrays, etc.) needed to place these markers on a molecular map of the chromosome. (10 points)

Assignment C:
What order do you expect to find markers A, B, and C on the molecular map? (2.5 points)
Assignment D:
Describe the experimental steps and special materials (probes, libraries, panels, arrays, etc.) needed to place the molecular markers on a map of bands and interbands of the morphological chromosome. (10 points).

Assignment E:
What order do you expect to find markers A, B, and C on the morphological map? (2.5 points)

Assignment F:
Do you expect the ratio of the distance from A to B to the distance from A to C to be the same in all three maps (genetic, morphological, molecular)? Why or why not? (5 points)