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

I. Interpretation (35 points)

Facts and observations:
1. *M. geneticus* is a hypothetical mammal. A 10 kbp fragment of the transcribed portion of *M. geneticus* ribosomal DNA (rDNA) was cloned in a bacterial plasmid vector.

2. Total DNA of *M. geneticus* was sheared to fragments of 300 bp average size, denatured and allowed to renature at various Cot values. At each Cot used, the double-stranded DNA was probed by hybridization with the cloned rDNA fragment.

3. Little, if any, hybridization occurred to double-stranded DNA obtained after renaturation to an extent that allowed 20% of the DNA to renature. Extensive hybridization was seen with double-stranded DNA from a renaturation that was 60% complete. No additional hybridization was detected when the Cot was raised so that 95% of the DNA renatured.

Assignment A:
What can you conclude about rDNA genes of *M. geneticus*? Be as specific as possible (10 pts).

4. Total DNA of *M. geneticus* was digested separately to completion with *EcoRI* and with *BamHI*. Another aliquot of the DNA was digested to completion with both enzymes.

5. The products of DNA digestion were separated by gel electrophoresis and transferred by the Southern method to derivatized nylon. The nylon was probed by nucleic acid hybridization with the labeled 10 kbp cloned gene fragment.

The bands detected are shown in the following table:

<table>
<thead>
<tr>
<th></th>
<th>EcoRI</th>
<th>BamHI</th>
<th>both</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.0 kbp</td>
<td>11.0 kbp</td>
<td>7.0 kbp</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>9.0</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0</td>
<td></td>
</tr>
</tbody>
</table>
Assignment B:
Construct a restriction map of the rDNA region of *M. geneticus*. (10 pts)

Assignment C:
Is the map circular or linear? (5 pts)

Assignment D:
Why is the total size of reactive *Eco*RI fragments greater than the 10 kbp fragment used as a probe? (5 pts).

Assignment E:
Based on the kinetic hybridization result and the restriction mapping, describe the organization of rDNA genes in *M. geneticus* (5 pts).

II. Basis for principles (35 points)

Principle:
Not all heritable information is in the form of DNA.

Assignment:
Provide one example that supports the above statement and explain how it supports the statement (10 points).

Principle:
Some proteins can serve as molecular markers for the genetic chromosome.

Assignment:
Provide one example that supports the above statement and explain how it supports the statement (10 points).

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

Assignment:
Provide one experimental support of the above statement and explain how it supports the statement (10 points).

Principle:
PCR can be used in the analysis of VNTR markers.

Assignment:
Explain how PCR can be used in this manner (5 points).
III. Experimentation (30 points)

Facts and observations:
1. A mutation in the human Kras oncogene causes a predisposition to bladder and liver cancer.
2. The mutation is a single base change (G to C) from the sequence GAGGTC to the sequence GAGCTC.
3. The mutation causes a change of the 12th amino acid of the encoded protein from glycine to arginine.
4. GAGCTC is a recognition site for the restriction endonuclease, SacI. SacI sites upstream and downstream of the mutation site are conserved in all humans tested.

<table>
<thead>
<tr>
<th>consvd. SacI</th>
<th>mut. site</th>
<th>consvd. SacI</th>
</tr>
</thead>
<tbody>
<tr>
<td>upstream</td>
<td></td>
<td>downstream</td>
</tr>
</tbody>
</table>

5. The Kras gene has been molecularly cloned and its nucleotide sequence determined.

Assignment:
Devise a method (not involving cloning or nucleotide sequencing) for testing individuals for predisposition to bladder and liver cancer caused by this Kras mutation. (15 points)

Describe the expected results of your test for a non-affected individual, an individual heterozygous for the mutation, and an individual with this mutation in both chromosomes. (15 points)

IV. Help the instructor

This exam took me ____ minutes to complete.

Submit completed exam to U. Melcher (NRC 354) for grading.