I. Interpretation (35 points)

Facts and observations:
1. A maize line (P) that produces purple tassels was crossed with a near isogenic line (C) that produces light brown tassels. 401 F1 progeny plants were examined. 400 produced purple tassels (Sibs). One (Sport) produced light brown tassels.

2. The gene responsible for the purple-white difference is known and a plasmid clone of a 3.0 kbp gene fragment from the P line is available. The fragment contains an EcoRI site, but no sites for BamHI or SalI.

3. DNA was extracted from leaf tissue of each the P line, the C line, the Sibs and the Sport. The DNAs were restricted with EcoRI (E), BamHI (B) and SalI (S), singly and in all possible combinations. The resulting DNA fragments 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 3.0 kbp cloned gene fragment.

The bands detected are shown in the following tables:

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<th>E</th>
<th>B</th>
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<th>ES</th>
<th>EB</th>
<th>SB</th>
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<tbody>
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<td>P line</td>
<td>6.0</td>
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<td>4.0</td>
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<td>2.0</td>
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<tr>
<td>C line</td>
<td>6.0</td>
<td>10.0</td>
<td>7.5</td>
<td>4.5</td>
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<td>4.0</td>
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<tr>
<td>Sport</td>
<td>13.0</td>
<td>17.0</td>
<td>12.0</td>
<td>9.0</td>
<td>8.0</td>
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</tbody>
</table>
Assignment A:
For each the P line, the C line, the Sibs and the Sport, identify whether the plants are homozygous or heterozygous for the purple color gene and for RFLP's detected by this probe and one or more of the restriction enzymes.  (5 pts)

Assignment B:
Derive a restriction map for enzyme sites in the P line DNA.  Note that not all sites responsible for making the fragment will be mappable (3 E, 2 S, and 1 B site should be located relative to one another).  (10 pts)

Assignment C:
What is the difference between the restriction map for this segment of C line DNA and of the P line?  (5 pts).

Assignment D:
Can you decide whether this difference (C vs. P maps, see assignment C) is responsible for the tassel color difference?  Why or why not?  (5 pts).

Assignment E:
For each enzyme and enzyme combination, predict the banding pattern expected for DNA from Sibs.  (5 pts).

Assignment F:
From the Sport DNA restriction data, deduce, as precisely as possible, what happened genetically to produce the Sport phenotype (5 pts).

II. Basis for principles (35 points)

Principle:
Phenomena that played crucial roles in generating evidence that nucleic acids were the genetic material are critical parts of current molecular cloning protocols.

Assignment:
Support the above statement. A complete answer should include a description of the use of two phenomena in the historical discovery of nucleic acids as genetic material and in molecular cloning.
III. Experimentation (30 points)

Facts and observations:
1. An indexed array of cosmids derived from a single eukaryotic chromosome has been characterized by profiling of restriction fragments. For profiling, HindIII (recognition sequence AAGCTT) fragments were end-labeled and the products digested with Mbol (recognition sequence GATC). Fragments were separated by gel electrophoresis and the sizes of the labeled fragments were recorded and analyzed by computer.

2. The computer analysis resulted in four contigs for the single chromosome. Analysis of some cosmids failed to detect any labeled fragments.

3. You suspect that the failure to join the four contigs into one large contig is due to the presence of satellite DNA in three places in the chromosome.

Assignment:
Your task is to design one approach to test your suspicion. (25 pts)

Describe the results expected if the failure to join the contigs is due to satellite DNA. (5 pts)

Describe the results expected if the failure to join the contigs is not due to satellite DNA. (5 pts)

IV. Help the instructor

This exam took me ____ minutes to complete.

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