Restriction Mapping a 4-kb Gene Segment

You have cloned a 4-kb segment of a gene into a plasmid vector (shown in Figure 1 below) and now wish to prepare a restriction map of the gene in preparation for other DNA manipulations. Your adviser left instructions on how to do it, but she is now on vacation, so you are on your own. You follow her instructions, as outlined below:

1. Cut the plasmid with EcoRI.
2. Add a radioactive label to the EcoRI ends.
3. Cut the labeled DNA with BamHI.
4. Purify the insert away from the vector.
5. Digest the labeled insert briefly with a restriction nuclease so that on average each labeled molecule is cut about one time.
6. Repeat step 5 for several different restriction nucleases.
7. Run the partially digest samples side by side on an agarose gel.
8. Place the gel against x-ray film so that fragments with a radioactive end can expose the film to produce an autoradiograph.
9. Draw the restriction map.

Your biggest problem thus far has been step 5; however, by decreasing the amounts of nuclease and lowering the temperature, you were able to find conditions for partial digestion. You have now completed step 8, and your autoradiograph is shown in Figure 2 below.

Unfortunately, your adviser was not explicit about how to construct a map from the data in the autoradiograph. She is due back tomorrow. Will you figure it out in time?

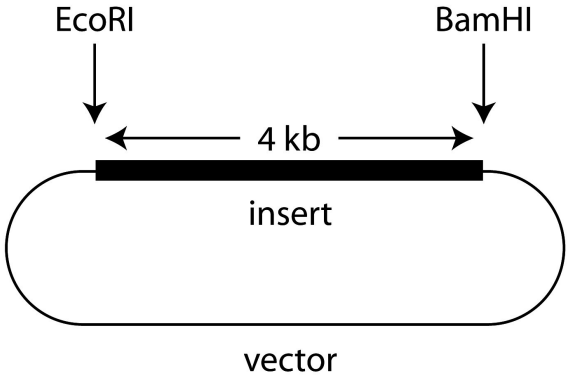

Figure 1: Recombinant plasmid containing a cloned DNA segment.

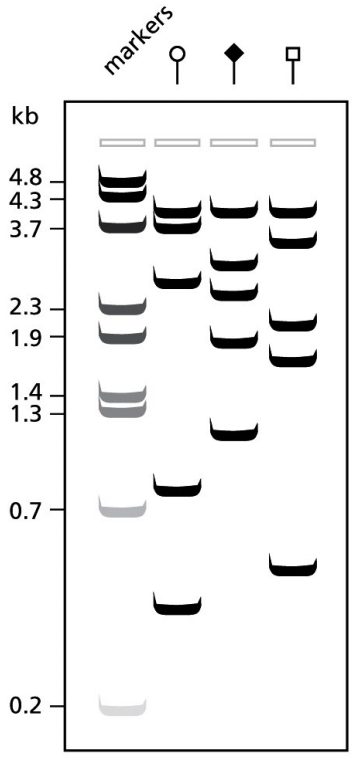

Figure 2: Autoradiograph showing the electrophoretic separation of the labeled fragments after partial digestion with the three restriction nucleases represented by the *symbols*. Numbers at the *left* indicate the sizes of a set of marker fragments in kilobases.