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Biology 1113
Practice problem
DNA fragment analysis/restriction site mapping

1. Download the excel spreadsheet on Carmen to be used for calculating the size of unknown fragments.
2. Look over the results of the gel (Figure 1). Locate the lane for the size standard. The sizes of the bands are known and marked on the gel. You will use these size standards to develop a predictive relationship between distance traveled on the gel and fragment size. To predict the size of a fragment by how far it traveled we need to work out the basic relationship between size and distance using the size standard. You will graph the distance travelled (x-axis) as a function of size (y-axis) and use a mathematical function to describe this relationship. You may have learned linear regression as a way of making predictive relationships in math class or you may have fit a scatter plot with a trendline. We will use a similar procedure. The equation that best fits your size-distance traveled data can be used to predict the size of unknown fragments from your measures of how far they travel.
3. Measure the distance that each fragment of the size standard traveled from a common point at the bottom of the gel (you should draw a straight line across the bottom from which to measure). Enter these distances into the spreadsheet in the column on the left labeled "**Lambda size marker, Distance (cm)**". Then enter the corresponding fragment sizes in base pairs in the column just to the right. A graph should pop up on the axes below. Note: *Sizing of the smaller fragments will be more accurate than sizing of the larger fragments because when we plot on a log or power scale, small errors in distance translate into large errors in size.*

- **What is the shape of this graph?**



4. Click in the graph to select it. Then, using the design tab, click on "**trendline**". Go to "**More trendline options**" and check the boxes to show the equation and the R^2 value. Select the power function and check the fit. R^2 tells you the proportion of the variance in the data points that is explained by the function. $R^2=1$ is a perfect relationship with all points on the line. Your R^2 should be > 0.95 . The equation can then be used to predict unknown fragment sizes (y) from distances (x).
5. Predict fragment sizes of unknowns by entering the distances traveled by each unknown fragment into the spreadsheet where indicated. Then enter your equation from the trendline into the corresponding cell of the fragment size columns to predict fragment size. Remember to use the "=" to indicate

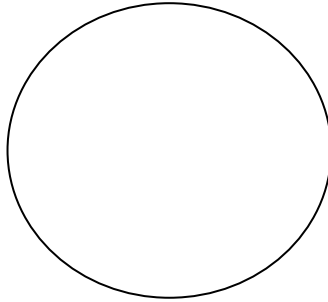
NAME: _____

that a formula follows. Call for help if you do not understand how to enter the function. **Write the predicted fragment sizes above each fragment in your gel picture.** Close the computer and put it away.

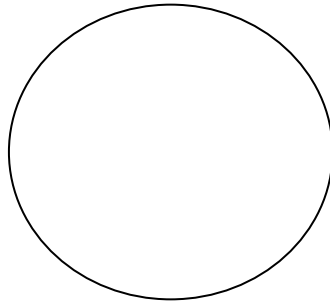
6. Estimate the total length of the DNA plasmid by adding up the fragment lengths from each digest then averaging these sums. You can do this in Excel. It will not be perfect, but the average is better than any one estimate.

- *Length* =

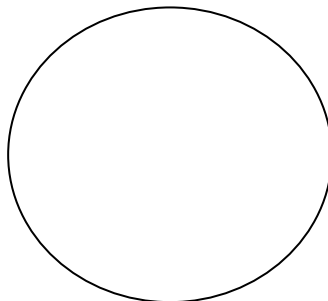
7. **How many *HpaI*** sites are on this circular piece of DNA? Draw their approximate locations on a circle that represents PMap. Label the recognition sites with an *H*. Hint the number or restriction sites =number of fragments in a single digest.



8. How many *PstI* sites are there? Draw the position of any *PstI* sites relative to the *HpaI* sites, which are still in the same relative location that they were above..



9. How many *SspI* sites are there? Draw their position relative to the *HpaI* sites.



NAME: _____

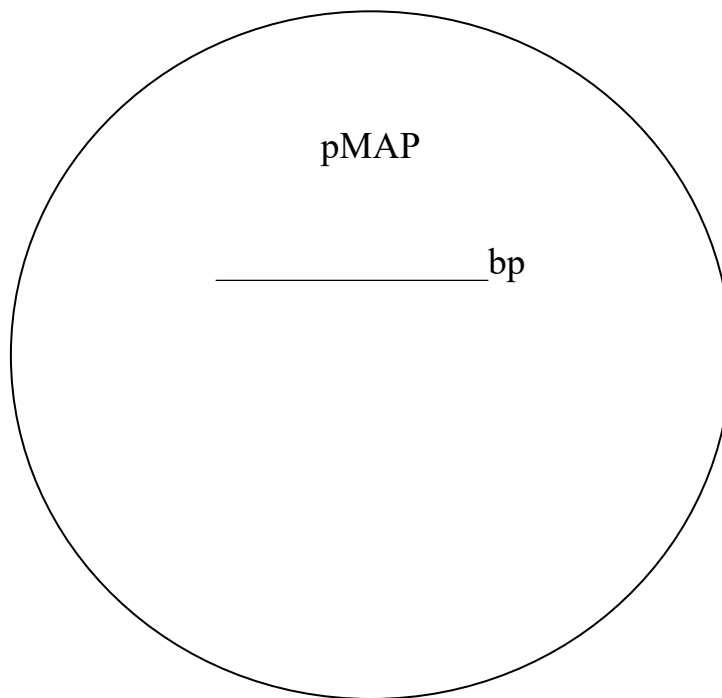
10. Does the smallest *HpaI* fragment remain unchanged after digestion with either *PstI* or *SspI*? (Check the gel) Explain.

11. Which fragments are unchanged from the *HpaI/SspI* digest to the *PstI/HpaI/SspI* digest? Which fragments disappeared? Why did those fragments disappear?

12. Is there a fragment that appears only on the *PstI/HpaI/SspI* digest? What does that mean?

13. Draw the full plasmid map with all restriction enzyme recognition sites present in their relative locations. Make sure you label the restriction sites with the name of the restriction enzyme that cuts there.

NAME: _____



NAME: _____

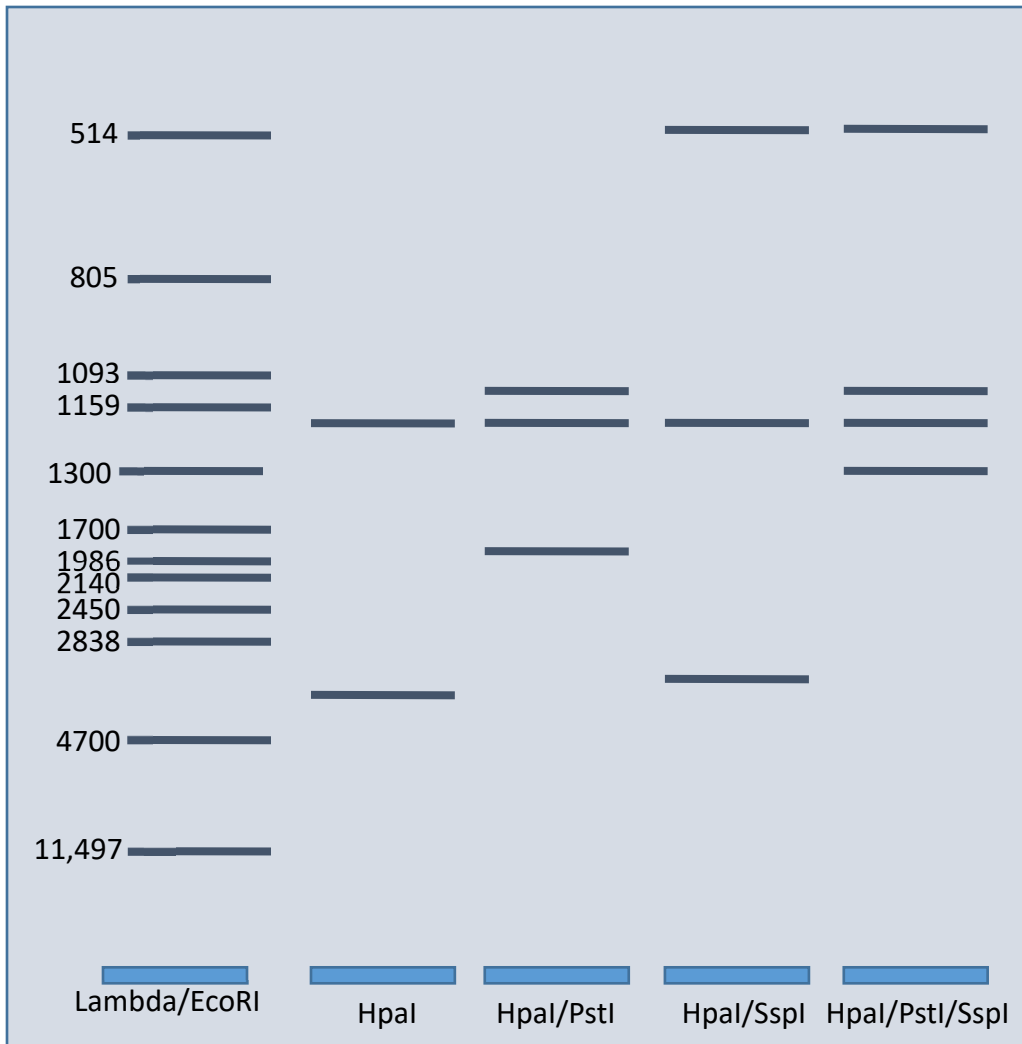


Figure 1. Image of an agarose gel in which the lower edge is the negative end and the upper edge the positive end. Labeled boxes represent wells where DNA was loaded. Horizontal bars represent the relative positions of stained fragments of DNA. The first column, labeled Lambda/EcoRI is a size marker. The numbers represent the length of the fragments in base pairs .