

Name _____ Period _____ Date _____

Quantitative Analysis of DNA Fragment Sizes

If you were on trial or were trying to identify an endangered species, would you want to rely on a technician's eyeball estimate of a match, or would you want some more accurate measurement?

In order to make the most accurate comparison between the crime scene DNA and the suspect DNA, other than just a visual match, a quantitative measurement of the fragment sizes needs to be completed. This is described below:

1. Using a ruler, measure the distance (in mm) that each of your DNA fragments or bands traveled from the well. Measure the distance from the bottom of the well to the center of each DNA band and record your numbers in the table on the next page. The data in the table will be used to construct a standard curve and to estimate the sizes of the crime scene and suspect restriction fragments.
2. To make an accurate estimate of the fragment sizes for either the crime scene or suspect DNA samples, a standard curve is created using the distance (x-axis) and fragment size (y-axis) data from the known HindIII lambda digest (DNA marker). Using both linear and semilog graph paper, plot distance versus size for bands 2–6. On each graph, draw a line of best fit through the points. Extend the line all the way to the right hand edge of the graph.

Which graph provides the straightest line that you could use to estimate the crime scene or the suspects' fragment sizes? Why do you think one graph is straighter than the other?

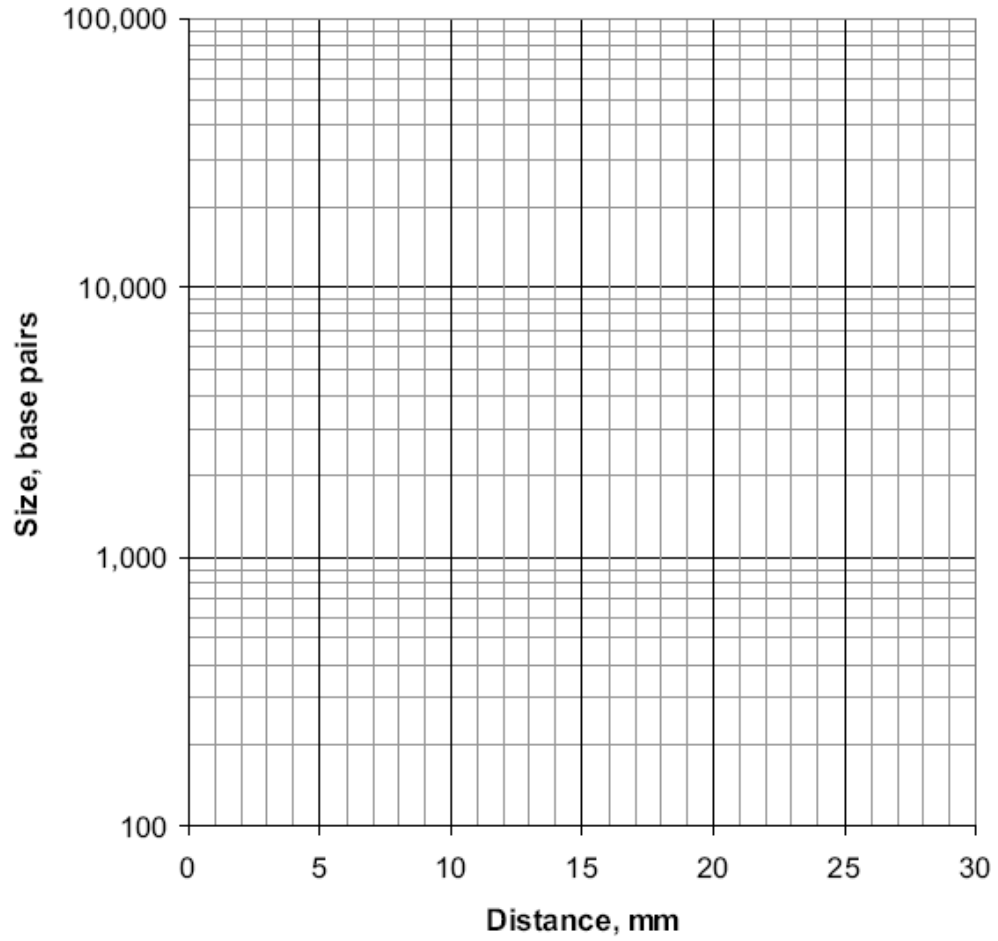
3. Decide which graph, linear or semilog, should be used to estimate the DNA fragment sizes of the crime scene and suspects. Justify your selection.
4. To estimate the size of an unknown crime scene or suspect fragment, find the distance that fragment traveled. Locate that distance on the x-axis of your standard graph. From that position on the x-axis, read up to the standard line, and then follow the graph line over to the y-axis. You might want to draw a light pencil mark from the x-axis up to the standard curve and over to the y-axis showing what you've done. Where the graph line meets the y-axis, this is the approximate size of your unknown DNA fragment. Do this for all crime scene and suspect fragments.
5. Compare the fragment sizes of the suspects and the crime scene.

Is there a suspect that matches the crime scene?

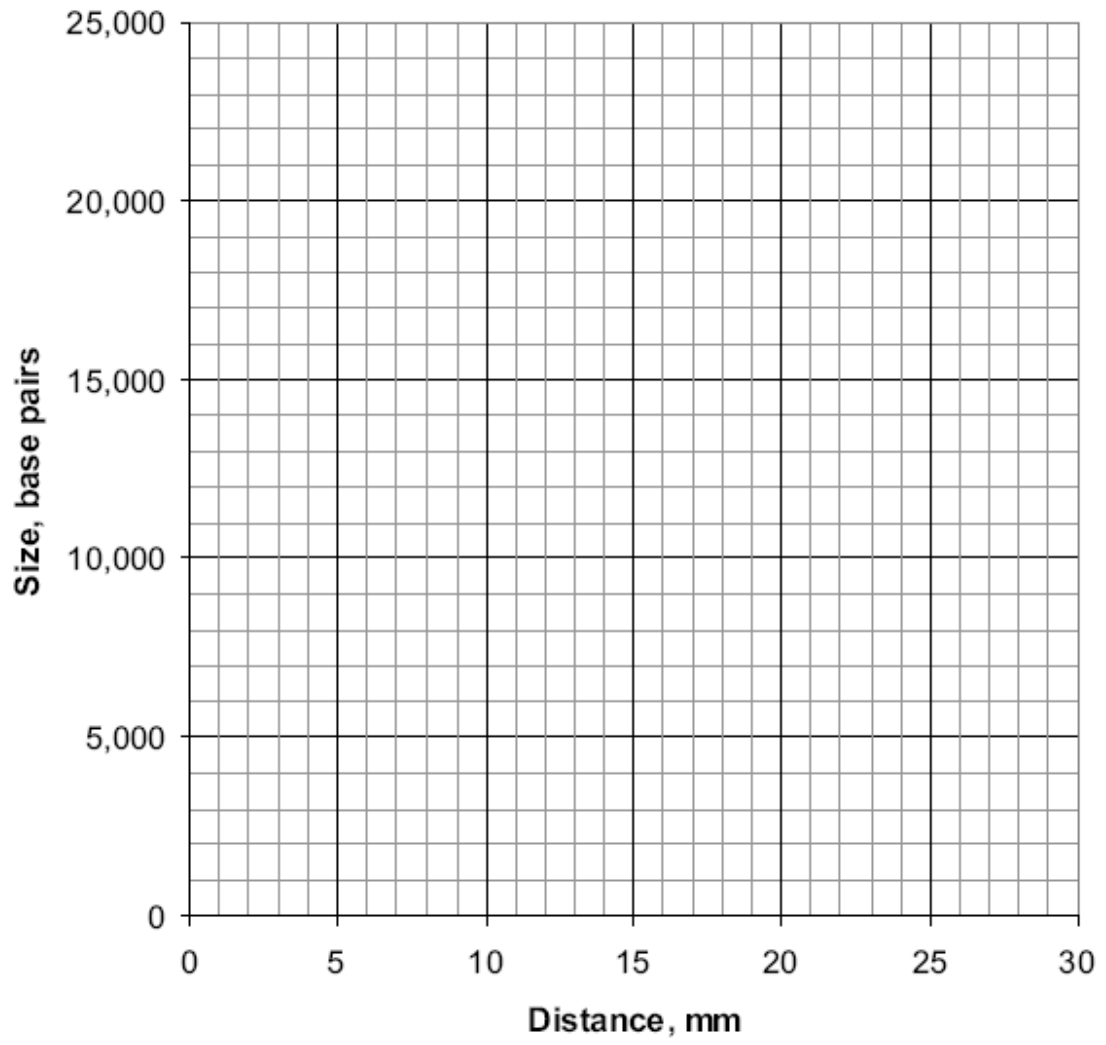
How sure are you that this is a match?

Lambda/HindIII size marker		Crime scene		Suspect 1		Suspect 2		Suspect 3		Suspect 4		Suspect 5		
Band	Distance (mm)	Actual size (bp)	Distance (mm)	Actual size (bp)	Distance (mm)	Actual size (bp)	Distance (mm)	Actual size (bp)	Distance (mm)	Actual size (bp)	Distance (mm)	Actual size (bp)	Distance (mm)	Actual size (bp)
1		23,130												
2		9,416												
3		6,557												
4		4,361												
5		2,322												
6		2,027												

Semilog Graph Paper



Graph Paper



Post Lab: Interpretation of Results

1. What are we trying to determine? Restate the central question.
2. Which of your DNA samples were fragmented? What would your gel look like if the DNA were not fragmented?
3. What caused the DNA to become fragmented?
4. What determines where a restriction endonuclease will “cut” a DNA molecule?
5. A restriction endonuclease “cuts” two DNA molecules at the same location. What can you assume is identical about the molecules at **that location**?
6. Do any of your suspect samples appear to have EcoRI or PstI recognition sites at the same location as the DNA from the crime scene?
7. Based on the above analysis, do any of the suspect samples of DNA seem to be from the same individual as the DNA from the crime scene? Describe the scientific evidence that supports your conclusion.