ANALYSIS OF RFLP DATA BY SEMI-LOG PLOT

BACKGROUND INFORMATION FOR ANALYSIS OF DATA

You will learn how to quantify RFLP data from DNA samples separated on an agarose gel by calculating base-pair length from a standard line that you have generated from a sample (the ladder or marker) whose fragment lengths are already known. You will then apply the same process to data from Dr. Comstock's laboratory to characterize the RFLP pattern for elephants from four national parks in Africa.

MATERIALS:

lab handout
semi-log paper
map of Africa
millimeter ruler (optional)
pencil

When we start working with DNA, one of the first things we can learn about it is the size of the DNA. Size is such a basic piece of information that unknown DNA fragments are often first named just by their size.

It's possible to make a reasonable guess of the sizes of bands on a gel by comparing them by eye to a marker band. Since we know what size the marker's bands are supposed to be, when the band whose size we're trying to determine lines up pretty close, we've probably got a band of similar size.

A much more accurate method, however, is to graph our results on semi-log paper. Because of the wide range of DNA sizes that are resolved on our gels, we use a logarithmic scale on the vertical, or y-axis so we can fit in all the different sizes. On the horizontal, or x-axis, we plot the distance each of those bands has traveled from the wells (in centimeters or millimeters). This is where the name semi-log comes from: semi means half, and log is for logarithmic. One of the two (or half) of the axes is logarithmic.

The marker DNA gives us a ruler for determining the size of our unknowns. We first plot what the markers have done in our gel. We use that information to determine the sizes of our unknown DNA fragments. In a way, we're making our own unique ruler that is accurate for this gel only.

Say we have a very simple gel. We've run a marker that gives us three bands, at 20kb (kilobases, or thousand bases), 5.3 kb and 2.9 kb. We've also run an unknown sample. We can make a safe guess that the lower marker band is the 2.9kb band (why do we know that?), the next one is 5.3kb and that the upper one is 20kb. Here's what the gel looks like:

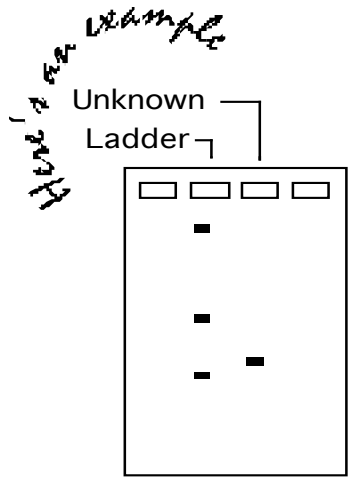


Figure 1. Gel showing DNA fragment bands from an unknown and ladder (marker) sample.

Since all DNA should migrate in the same way (i.e., assuming the same conformation (shape) of the DNA and uniform charge), we can eyeball the size of the unknown band. It looks like it's between 3.5kb and 4kb. This is just a rough guess, though. That guess might be good enough, but what if we need a more accurate size estimate? We do a semi-log plot.

We first measure how far our bands have traveled from the wells. We can measure either from the center of the wells to the center of the bands or from the lower edges of the wells to the lower edges of the bands. It doesn't matter but we have to be consistent. Our 20kb marker band is 18 mm from the well, 5.3kb band is 62 mm from the well, and the 2.9kb band is 75 mm. See Figure 2 for the actual semi-log plot of our results.

Mark a scale on the horizontal, X-axis which can contain these three sizes, plus the starting point of zero. Next, look at the vertical, Y-axis. The scale can be confusing. Each line, marked 1 or 10, where the lines seem to have collapsed represents a 10^1, or a ten-fold increase. Zero is not on the scale. So, the bottom one is 10^2, or 100. The next is 10^3, or 1000. Next is 10^4 or 10,000, and the final one on this scale, marked 10, is 10^5, or 100,000 base pairs. If we look at the example, and play around with figuring out where a number should go, it'll start making sense.

Now we need to draw a straight line passing as near as possible to all of the points indicating our markers. At the ends of our line, the line may curve. That's fine and is a result of very large or small fragments moving very slowly or very fast. See the example of an actual semi-log plot from a gel with many fragments (Figure 3).

All we know for sure about our unknown band is how far it traveled, so we measure that, just as we did for the marker bands. Our unknown band traveled 72 millimeters. Starting on your semi-log plot at the distance traveled (x axis, 72 mm), move straight up until you intersect with your drawn line. Now go straight left until you hit the y axis, the size scale. Read the size of your unknown band. You should come up with 3,500 or 3.5kb. You've now made a fairly accurate estimate of how large that unknown band is.

To review:

1) Identify the marker bands by size.
2) Measure the distance that each band has traveled.
3) Draw the scales for distance and band length on your semi-log graph paper.
4) Plot the location of each band (distance traveled and size).
5) Draw a straight line passing as close as possible to all the data points.
6) Based on the distance the unknown band has traveled, estimate its size using the 'standard' line drawn from the marker data.

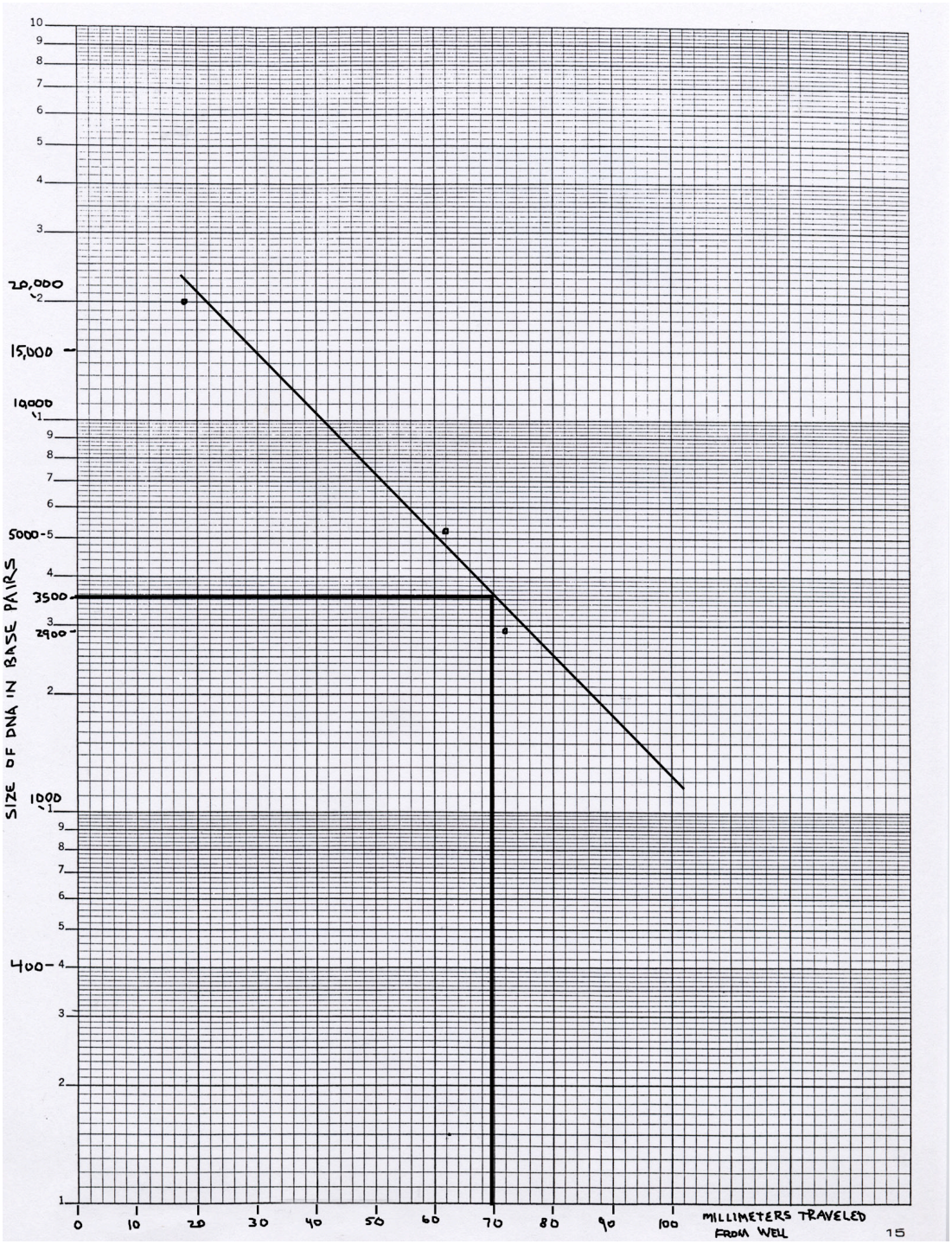

SIZE OF DNA IN BASE PAIRS
20,000
15,000
10000
5000
3500
2900
1000
400
MILLIMETERS TRAVELED FROM WELL
0
10
20
30
40
50
60
70
80
90
100
15

Figure 2

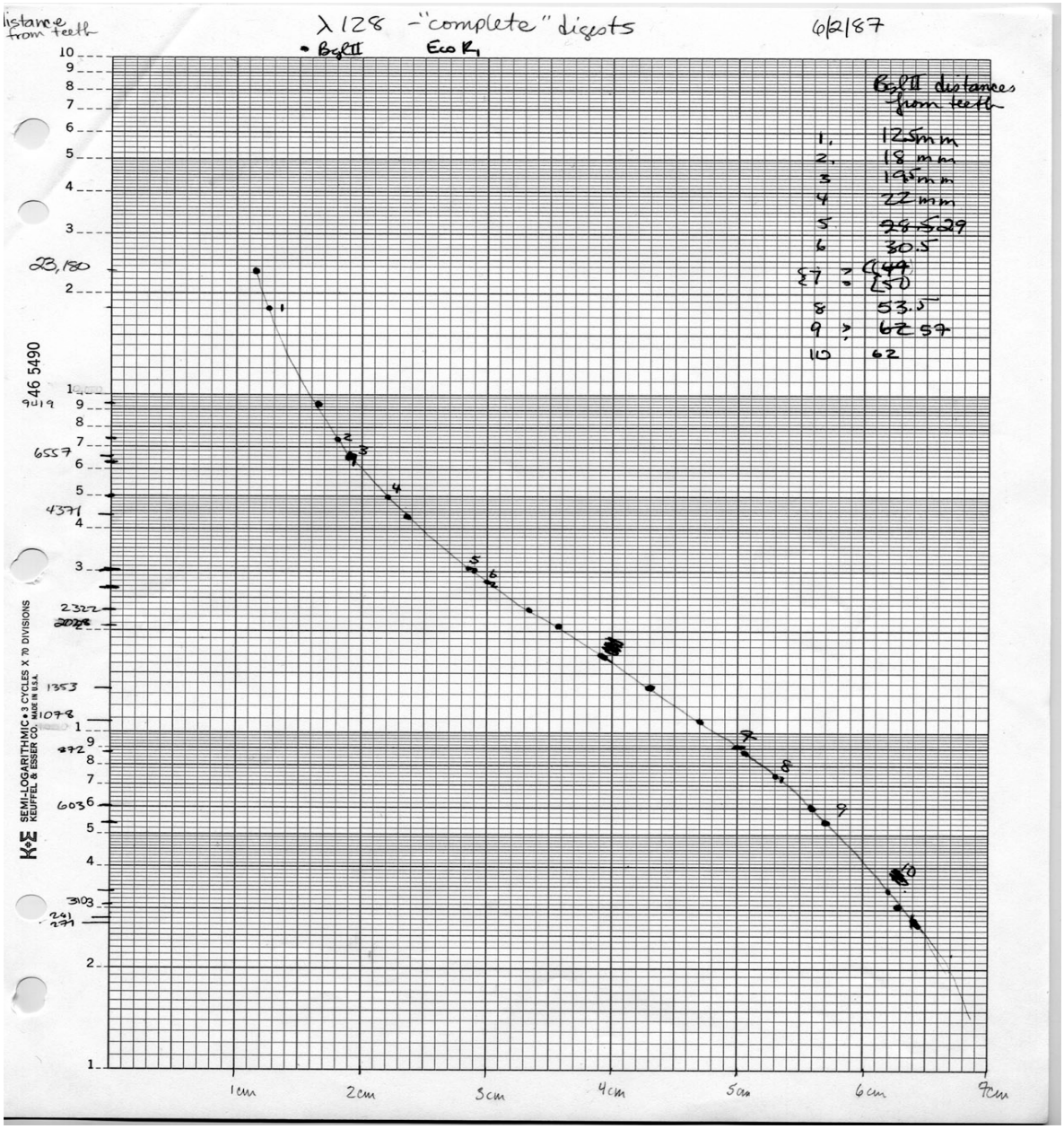
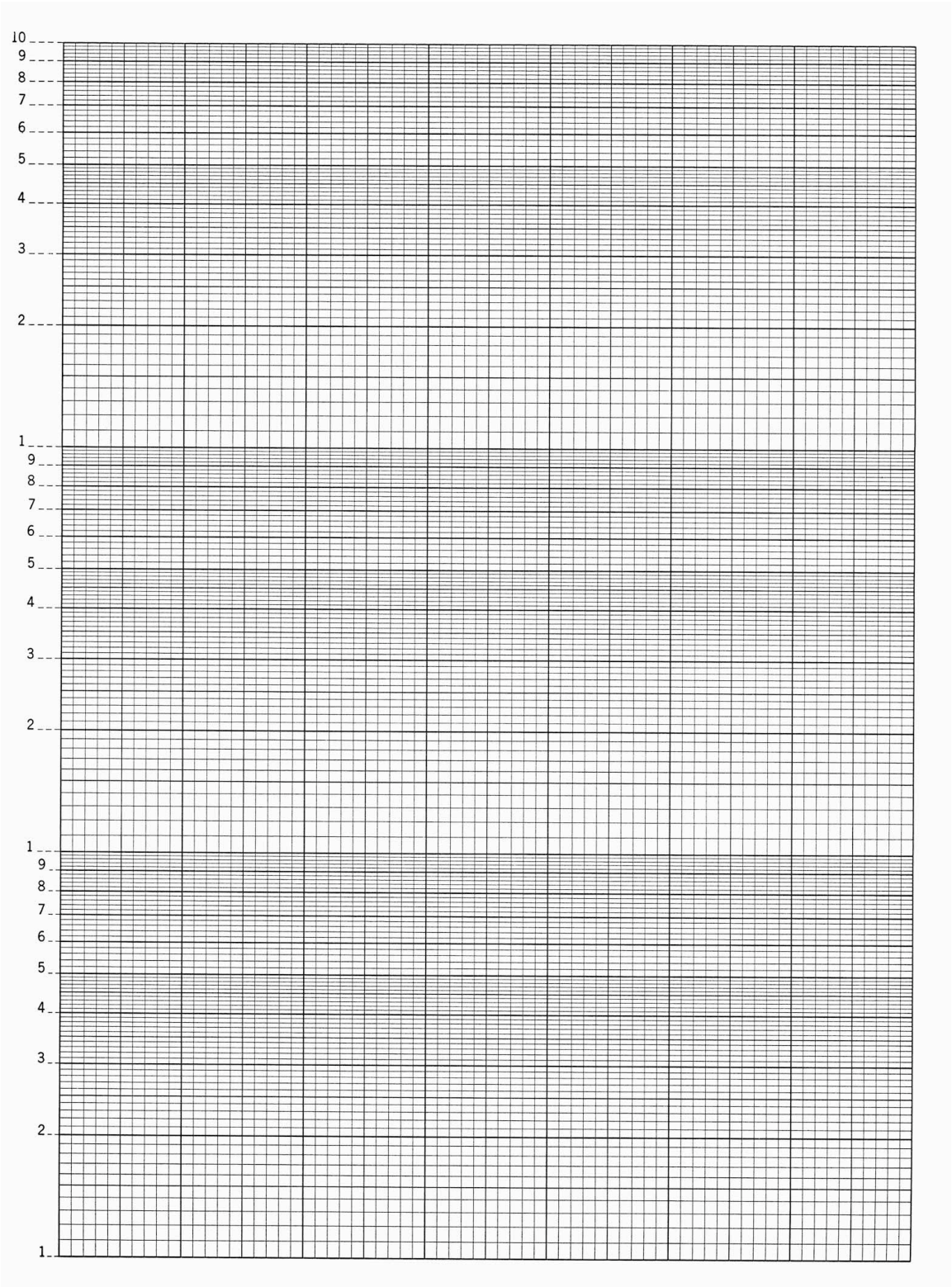


Figure 3 – example of semi-log plot of a standard curve


10
9
8
7
6
5
4
3
2
1
9
8
7
6
5
4
3
2
1
9
8
7
6
5
4
3
2
1

TO ANALYZE THE COMSTOCK GEL, PLEASE DO THE FOLLOWING:

Using the photocopy below of the gel from Kenine Comstock's lab, create a semi-log plot to determine the size (base pair length) of the unknown bands. The known base pair lengths of the marker are written along the right side of the gel and their corresponding band is indicated by a connecting line.

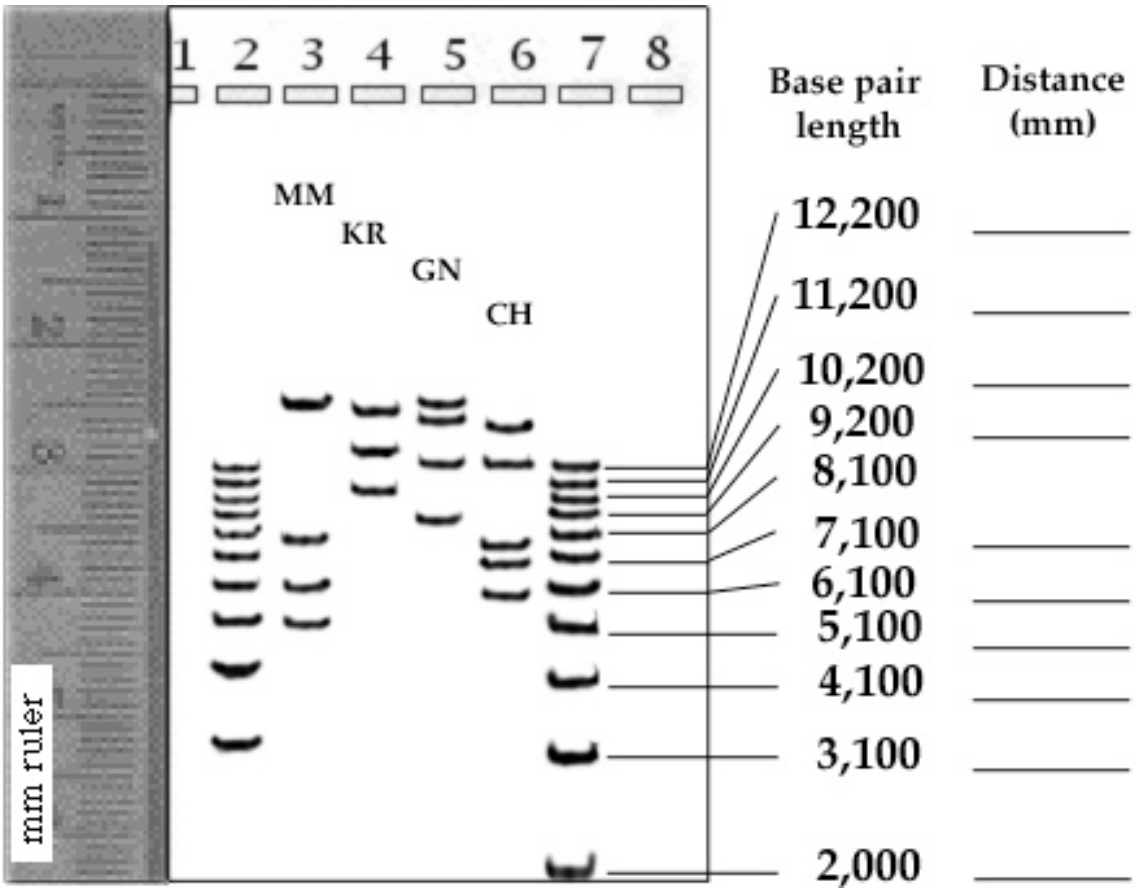
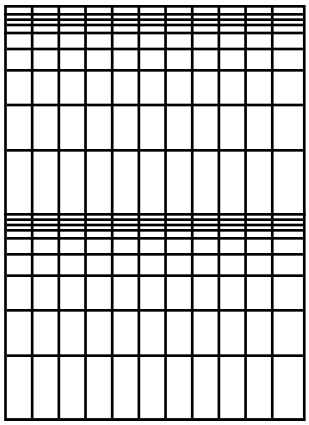


Figure 4. This agarose gel shows RFLP bands for one elephant from each of four African national parks. (MM= Masai Mara, KR=Kruger, GN=Gonarezhou, CH=Chobe). Marker IV 1kb+ is used as a ladder in lanes 2 and 7. Base pair lengths for the ladder are listed to the right of the gel.

1. For the ladder (lanes 2 or 7), measure the distance in millimeters from the well to each band. Use the magnified ruler in Figure 1 or a real millimeter ruler.
2. Record the distance measurements in the column at the far right of Figure 1.

3. Set up a graph on semi-log paper with 'distance traveled' (in millimeters) on the horizontal (X) axis and 'base-pair length' on the vertical (Y) axis. Orient the paper as shown at right. Label the axes.



4. Plot the distance and base-pair length data from the ladder lane onto the semi-log paper. Draw a straight line through the data. This 'standard line' should come as close as possible to all the data points.

5. For each DNA band in each lane, measure its distance (in millimeters) from the well. Use the standard line constructed from the marker lane to estimate as precisely as possible the sample band's length.
   a. Enter the semi-log graph from the 'X' axis at a chosen distance traveled.
   b. Move vertically on the graph until you intersect the standard line.
   c. Move horizontally from the standard line until you intersect the Y axis.
   d. Determine your base-pair length at that intersection with the Y axis.

6. Enter the distanced traveled and fragment length data for each sample below in Table I. Begin with the longest fragment as Fragment 1 and continue listing fragments in order of decreasing size. Note that not every sample will have the same number of RFLP fragments.

Table I. RFLP data for elephants from four national parks in Africa: distance traveled by each band (mm) and base-pair length (l).

Fragment	Masai Mara		Kruger		Gonarezhou		Chobe	
	mm	l	mm	l	mm	l	mm	l
1								
2								
3								
4								
5								
6								

TO ANALYZE YOUR OWN GEL, PLEASE DO THE FOLLOWING*:*

1. Record the length of each of the fragments of your known ladder or marker next to the appropriate band on the acetate sheet or drawing of your gel.
2. Generate a semi-log plot, same as was done for the Comstock gel (follow steps 1-5 above).
3. Record the lengths of the unknown bands from your gel on the Data Submission Form (DSF).

4. Compare the number and size of fragments from the ivory DNA to that of the Comstock gel database as well as to the samples you ran on your gel.
5. Record the most likely location or source of your ivory, based on DNA fingerprinting or banding patterns (fragment numbers and sizes) observed on your DSF.

DIRECTIONS FOR DATA SUBMISSION DS571

- Record the national park for the samples from Dr. Comstock's laboratory and the base-pair lengths of the RFLP fragments from Table I in list form in the upper portion of the handout called 'Data Submission Sheet.'
- Use the map of Africa you are given to find and record on the data submission sheet the countries in which the four parks are located.
- Using information from the teacher's overhead transparency, record the vegetation regime that prevails at each park (forest or savannah) on the Data Submission Form.

QUESTIONS TO FOLLOW ANALYSIS OF DATA

1. What causes RFLP patterns in lanes 3 to 6 of the Comstock gel to look different?

2. How are the RFLP patterns in lanes 3 to 6 of the Comstock gel similar? How are they different?

3. What are the two ways you have to compare RFLP data? What are the advantages and disadvantages of each? Which do you prefer?

4. Elephant populations from the four national parks represented in Dr. Comstock's data appear to have different RFLP patterns? Does this surprise you, or would you expect this? Why?

5. Why do you think that the RFLP patterns seem to be different between parks, but the same within parks?

6. Can you imagine situations in which more than one RFLP pattern might be found in a single park? How about situations in which one RFLP pattern might be found in more than one park?

7. Is knowing the RFLP pattern from one elephant in a park enough to conclude that all elephants in the park share the same pattern? Are two elephants enough? Three? How many would you test to decide that only one RFLP pattern is found in each park?

8. What risks to their survival do you think African elephants face today? Are all of the risks due to humans? What non-human risks might affect elephants' survival?

9. Hunting for ivory is not the only human activity that threatens the African elephant's survival. Clearing land for farming and harvesting trees decorative and insect-resistant lumber both affect elephant habitat. Which two populations of African elephants from your set of Dr. Comstock's samples are most at risk from logging and agricultural activity?

10. Do you think the risks for a park elephant in Africa are different from country to country?

POLITICAL MAP OF AFRICA