

DNA BAND SIZES SEMI-LOG PLOTTING

ESTIMATING SIZES OF DNA BANDS BY SEMI-LOG PLOT

When we start working with DNA, say a new DNA clone or plasmid, 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.

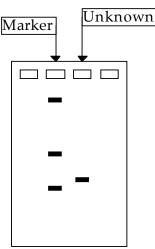
It's possible to make a reasonable guess of the sizes of bands on a gel by comparing them by eye to a marker band. Since you know what size the marker's bands are supposed to be, when the band whose size you're trying to determine lines up pretty close, you've probably got a band of similar size.

A much more accurate method, however, is to graph your results on semi-log paper. Because of the wide range of DNA sizes that is 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 which is accurate for this gel only.

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Say we have a very simple gel. We've run a marker which gives us three bands at 20 kb (kilobases, or thousand bases), 5.3 kb and 2.9 kb. We've also run an unknown sample. Here's what the gel looks like:



We can make a safe guess that the lower marker band is the 2.9 kb band (why do we know that?), the next one is 5.3 kb and that the upper one is 20 kb. 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 3.5 kb and 4 kb. 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.

See the next page for the actual semi-log plot of our results. We first measure how far our bands have traveled from the wells. You 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 you have to be consistent. Our 20 kb marker band is 18 mm from the well, 5.3 kb band is 62 mm from the well, and

the 2.9 kb band is 75 mm. 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 you 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 line connecting the points indicating our markers. You may not be able to draw a straight line; that's OK, just make your best guess of a straight line which includes all the points. At the ends of your line, the line may curve. That's fine and is a result of very large or small fragments moving very slowly or very fast.

All you know for sure about your 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, and we come up with 3,500 or 3.5 kb. You've now made a fairly accurate estimate of how large that unknown band is.

MARKER SIZES

 λ (lambda) is ~48,502 bp, depending on the strain. It has 12 bp single-stranded cohesive (*cos*) or sticky ends with complementary sequence that can bind that each other within the same molecule or in another λ molecule. At room temperature, these *cos* ends do like to stick together resulting in changed DNA band sizes. (To prevent this, samples can be heated gently before running on the gel.)

* means that these are end fragments-they may be faint on gels if the samples are not heated before running.

λ EcoR I (Marker I)

- 1) 21,226* left end
- 2) 7,421
- 3) 5,804
- 4) 5,643
- 5) 4,878
- 6) <u>3,530*</u> right end 48,502

<u>λ Hind III digest (Marker II)</u>

27,500 present when sample not heated before running 1) 23,130* left end 2) 9,416 3) 6,551 4) 4,361 right end 5) 2,322 6) 2,027 7) 564 } faint 8) 125 } " <u>λ EcoR I + Hind III (Marker III)</u> 1) 21,226* left end

- 2) 5,148*
- 3) 4,913
- 4) 4,268
- 5) 3,530* right end
- 6) 2,027
- 7) 1,904
- 8) 1,584
- 9) 1,375
- 10) 947
- 11) 831
- 12) 564} faint
- 13) 125} faint

pBR 322 = 4361 base pairs, a circle

ø XRF Hae III digest

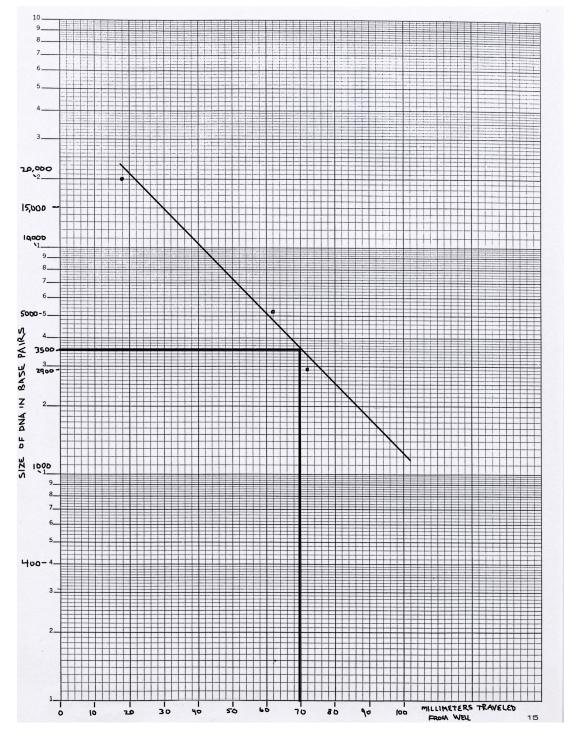
- 1) 1,353
- 2) 1,078
- 3) 872
- 4) 603
- 5) 310
- 6) 281, 271} doublet
- 7) 234
- 8) 194
- 9) 118
- 10) 72} faint

<u>1 kb Plus ladder (Invitrogen)</u>

1)	12,000
2)	11,000
3)	10,000
4)	9,000
5)	8,000
6)	7,000
7)	6,000
8)	5,000
9)	4,000
10)	3,000
11)	2,000
12)	1,650
13)	1,000
14)	850
15)	650
16)	500
17)	400
18)	300
19)	200
20)	100

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AGAROSE GEL ELECTROPHORESIS OF DNA



EXAMPLE

Put onto semi-log plot:

- 1. Identify the marker bands, by size
- 2. Measure distance each band traveled
- 3. Draw the scale onto your semi-log paper
- 4. Plot the location of each band (size and distance traveled)
- 5. Draw a best-fit line connecting the dots

Based on the distance your unknown band traveled, estimate its size using the line you drew using the markers.

AGAROSE GEL ELECTROPHORESIS OF DNA

