Extra information for analyzing the gel from Lab #10-11

Calculation of the sizes of PCR DNA fragments using the DNA size standards:

The DNA size markers were phage DNA cut with the restriction enzyme *Hae*III. The size of each fragment is known (from largest going smaller: 1353 bp, 1078 bp, 872 bp, 603 bp, 310 bp, 281 bp, 271 bp).

- 1. Measure the distance that each marker fragment migrated from the well.
- 2. On <u>log paper</u> (or using a graph program that you can select the Y axis to be <u>logarithmic</u>), plot the size of the marker DNA fragment vs. the distance that it migrated from the well. You need only graph marker DNA fragments 1353 bp, 1078 bp, 872 bp, 603 bp, 310 bp because the size of your sample bands will be within this range.



Distance from the well

- 3. Measure the distance that each sample PCR DNA fragment migrated from the well.
- 4. Using the distance that you measured in step 3 and the standard curve that you generated in step 2, determine the size of your PCR DNA fragment.

Estimating the number of repeats in each PCR DNA fragment:

The total size of the PCR DNA fragment on the gel is the sum of the following:

From 5' end of primer #1 to repeat #1=		116 bp
Repeat #1	=	14 bp
(n-1)(16 bp) where $n = #$ of repeats	=	X bp
From the end of the last repeat	=	<u>32 bp</u>
to the 5' end of primer #2		
Total size	=	162 + X

1. Solve for X (size of the total repeat region except for repeat #1):

162 + X	= estimated size of PCR DNA fragment	
Х	= estimated size of PCR DNA fragment - 162	2

2. Solve for n (number of repeats):

$$(n-1)(16 bp) = X$$

 $n = X/16 + 1$