C. elegans Prelab Worksheet #1: PCR

Hypothesis: The three enhancer gene mutations that we have identified are working together with a mutation in *C. elegans mks-1* to cause a dye filling defect. We hypothesize that if we can reintroduce a wild type copy of each enhancer gene in our mutant *C. elegans* strains, we can rescue the dye filling defect.

Week #1 Objective: Use PCR to generate wild type fragments of our three enhancer genes from wild type C. elegans genomic DNA. **Everybody is expected to work as a team towards the overall success of the project.**

Method: We will be using the standard PCR protocol for the DNA polymerase Phusion (New England Biolabs). The protocol calls for the following recipe, **prepared on ice.** The primers you will need are listed in the table below and depend on your group number.

10 µl	5X Phusion High Fidelity Buffer
1 µl	10 mM dNTPs
2.5 µl	10 µM Forward Primer
2.5 µl	10 µM Reverse Primer
1.25 µl	*C. elegans* Genomic DNA (212 ng/µl)
0.5 µl	Phusion DNA Polymerase
32.25 µl	Nuclease-free water
50 µl	Total Reaction Volume

For a standard PCR reaction, there are three major steps: **denaturation**, **annealing**, and **extension**. The temperature for the annealing step is dependent on the length and composition of your primers. You are given this temperature below. The duration of the extension step is dependent on the size of the fragment you are trying to make. You will need to calculate your extension time based on the rule that Phusion can generate 1000 bp (1 kb) of DNA every 30 seconds.

	Group 1	**Group 2**	**Group 3**
C. elegans Strain	YH2086	YH2103	YH2105
Enhancer Gene	*K07F5.6*	*unc-33*	*F16F9.1*
Primers	BY5806 (F) BY5807 (R)	BY5808 (F) BY5809 (R)	BY5810 (F) BY5811 (R)
Annealing Temp	58° C	63° C	65° C
Fragment Size	~1.6 kb	~8.7 kb	~2.7 kb
Extension Time			

Customize the following thermocycling protocol for your specific gene fragment:

	Temperature	Duration	
Initialization	98° C	30 seconds	
Denaturation	98° C	10 seconds	Repeat for 35 cycles
Annealing	____	30 seconds	
Extension	72° C	_____	
Final Extension	72° C	5 minutes	
Hold	4° C		