Biology Project Lab (BIOL 2309)
PCR HOMEWORK

Due: bring a hard copy at the beginning of class (check Course calendar for exact date)

This assignment counts toward your "HW & Assignment Sheets" grade.
Credit: 5 points, if completed and submitted on time.

Name: _____ **Date: _____**

Objective:

- To explain the key steps in setting a PCR reaction
- To prepare a PCR protocol
- To set-up and perform a PCR reaction

*Note: Some of the questions and columns contain "**complete in class**" phrase. These will be completed in class.*

Part 1: The PCR reaction

Watch this JoVE video on PCR (if you are not on campus it will ask you to log in with your husky account), and answer the following questions:

1. How long does the annealing step typically last?

2. Once the PCR cycles are complete, you may not be able to take out the PCR tubes right away. What temperature is typically programmed for the hold step?

3. How will you prevent contamination of your samples with foreign DNA or nucleases?

4. While setting up the reaction, where are you going to keep the tubes? Why?

5. What DNA template are you going to use to set up your negative control? Why?

6. Which component are you going to add last to the PCR reaction tube? Why?

Part 2: PCR protocol

To complete part 2, keep in mind the following information:

- When setting up the PCR reaction and conditions, the primary component to pay attention to is the enzyme. You will be using *PfuUltra* II Fusion HS DNA Polymerase (Agilent, cat # 600670). You can find the information you need to complete this homework on the Agilent website: www.agilent.com
  *Note: the PCR reaction components for this enzyme may differ from the generic reaction described in the video. Adhere to the **Agilent protocol** for the specific enzyme we will use.*
- The *thuE* primers we have in the freezer are each in stock concentration of 100 µM. The primers have Tm of 63.9ºC and 62.8ºC.
- The dNTPs mix is in stock concentration of 100 mM.
- The Pfu reaction buffer is in stock concentration of 10X.

1. In Table 1 below, fill in the **two columns on the left** as part of your homework. We will finalize the protocol in class and you will fill in the third column right before you set up your reaction. You will use this table as your protocol for setting up your PCR reaction.

   Genomic DNA (template) to be used **(complete in class)**: _____

   Table 1. PCR reaction mixture.

Component (concentration) (complete as HW)	**Suggested amount per 50 µl reaction (complete as HW)**	**Used amount per 50 µl reaction (complete in class)**

2. You will also have to set up the PCR machine and you need to finalize the reaction conditions. Complete table 2 for your homework. We will finalize table 3 in class.
   Note: For the "Ta" below, write down the exact temperature you think should be used, e.g., 45ºC.

   Table 2. PCR reaction conditions **(complete as homework).**

Segment	**Number of cycles**	**Temperature**	**Duration (min or seconds)**
1			
2			
		Ta	
3			

Table 3. PCR reaction conditions **(complete in class).**

Segment	Number of cycles	Temperature	Duration (min or seconds)
1			
2			
		Ta	
3			

3. What was the most challenging part of this homework? Please explain.

4. List the literature references you used.