**PCR…What is it & How does it work?**

**The Problem:**

You have located the gene for the production of insulin. The only way we can use this gene is to amplify the DNA it is contained in. When we amplify we are really just creating many copies of this DNA. So how can we make these copies from our small sample in the laboratory? This possibility was not available until 1983 when a scientist, Kary Mullis, came up with the concept of PCR. Consequently, this revolutionary development won him the 1993 Nobel Prize! Now it’s up to us to use PCR in solving this and many other problems in molecular biology.

**The Materials:**

In order to be successful at carrying out PCR we must first understand what is needed in

order to make the reaction work. Using your knowledge about DNA and Replication, try

to guess what some of the necessary ingredients are based on the following statements.

1. Something to copy that contains the insulin producing gene:

Your guess\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

2. Something that will unzip the double helix strand and copy it:

Your guess\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

3. Something that initiates the copying process at both ends (5’ and 3’) of the unzipped strand. These must contain specific sequences to start the copying process at the correct location. (This one you may have to look up)

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

4. Something that is available to the copier that is the “raw material” that makes up the new strand:

Your guess\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**The Method:**

Before beginning the experiment we must prepare the necessary ingredients and have a clear understanding of the process that is about to take place in our vials. Follow the links below to answer the adjacent questions.

1. **The Ingredients:** Click on the link and list of ingredients that will go into our vial in preparation for PCR. <http://www.yourgenome.org/facts/what-is-pcr-polymerase-chain-reaction>

a. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

b. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

c. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

d. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

e. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Compare this list to your original guess. How did you do?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

2. **The PCR Steps:** Click on the link and list the steps involved in a cycle of PCR. Provide the term for each step and a brief description of what the term means.

<http://www.dnai.org/b/index.html> (Click on “Amplifying” and watch the 2D animation)

Step 1: (Term)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ (Temperature °C)\_\_\_\_\_\_\_\_\_\_\_

What does it do?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Step 2: (Term)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ (Temperature °C)\_\_\_\_\_\_\_\_\_\_\_

What does it do?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Step 3: (Term)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ (Temperature °C)\_\_\_\_\_\_\_\_\_\_\_

What does it do?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Lets watch this process in action . Remember, this all takes place in your vial which is placed in a machine called a thermocycler. The thermocycler simply regulates the temperature changes!! Click on this link to watch <http://www.dnai.org/b/index.html> (Click on Amplifying and watch the video)

**The Experimental Results:**

Now that you have the background knowledge necessary to carry out PCR you can try it for yourself. Click on either or both of these links to carry out the next experiment in a virtual test tube…

<https://www.dnalc.org/resources/animations/pcr.html>

<http://learn.genetics.utah.edu/content/labs/pcr/>

Answer the following questions:

**Experiment 1:**

a) Ingredients added:

• DNA template

• Primer B

• Primer A

• DNA Polymerase

• Nucleotides

b) Number of DNA target Strands produced after 1 cycle \_\_\_\_\_\_\_\_\_\_\_\_

Number of DNA target Strands produces after 4 cycles \_\_\_\_\_\_\_\_\_\_\_\_

Predict the number DNA target Strands produced after 24 cycles \_\_\_\_\_\_\_\_\_\_

In Experiment 2 predict what will happen if only one primer is added.

**Experiment 2:**

a) Ingredients added:

• DNA template

• Primer B ONLY

• DNA Polymerase

• Nucleotides

1) Prediction/Hypothesis:

**The Applications:**

To succeed at PCR understanding what is happening inside the test tube is as important as being familiar with the kinds of laboratory equipment. Click on the following link <http://www.hhmi.org/biointeractive/bacterial-identification-virtual-lab> to experience PCR as laboratory technician trying to identify a sample of mystery bacteria. Follow the directions provided below.

1. Click on the “Bacterial ID Lab”

2. Read the introduction and provide the following information:

a) What is name of the piece of DNA used to identify bacteria? \_\_\_\_\_\_\_\_\_\_\_\_\_\_

b) How will we know what kind of bacteria is in our sample? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

3. Click to enter the lab. Next, click on the drawer containing the gloves to begin.

4. Click on “PCR Amplification” located at the bottom left of the screen.

5. Click on “Reference” at the top of the screen and identify as many of the tools you can. List them below. You may end up adding to this list as we continue.

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\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_

6. As you follow the directions on your screen, try to answer the following questions:

a) What is contained in the PCR Master Mix?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

b) What is in our Positive control vial?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

c) What is in our Negative control vial?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

d) What is in our Experimental vial?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

e) What is the purpose of the thermocycler?\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

7. You’ll notice that there are several other steps beyond PCR that will allow us to analyze our PCR product in order to identify the sample bacteria. PCR is just one important step needed to make the rest of the investigation possible!

a. What technique could you use to separate and identify the individual DNA pieces just created through PCR?