PCR: Polymerase Chain Reaction Made Fun!!

Background Information on PCR:

A surfing ex-hippie from California named Cary Mullins wanted a way to generate large amounts of DNA from a single copy. He succeeded in his endeavors in amplification of DNA and won the Nobel Prize.

Cary Mullins initially began by using the "three graduate student" student method of having each student do one of the following:

- Denature
- Annealing
- Extending

This took a very long time and the results were not always accurate. He had to think of another way. He invented the Thermocycler for PCR. This fantastic little machine made amplifying DNA extremely less complicated and turned the world of Microbiology around 360 degrees in the wonderful results that can be obtained in a few hours instead of days, weeks or even months.

Why Polymerase Chain Reaction is Important:

Polymerase Chain Reaction (PCR) works by amplifying DNA sequences. Even if you have an extremely small amount of a sample DNA molecule to begin with, you can generate millions of copies of that DNA. As a result there are many uses for PCR. The PCR can be used in clinical diagnosis, reducing the valuable time before a diagnosis is made in a shorter time frame. Viruses such as meningitis, hepatitis, and even the flu can be diagnosed by using PCR. Thus knowing what is wrong with the patient, a doctor can prescribe the correct medicine to the patient faster hopefully insuring a quicker recovery.
PCR can be used in genetic analysis for paternity cases, crime scenes, and to determine the heredity factor of a particular gene. PCR can be used for genetic analysis to determine the source of a bacterial infection or even in the diagnosis of the AIDS viruses using PCR primers specific for sequences found only in the genes of that particular virus. PCR is used in many other ways such as genetic engineering and for forensic analysis. There are countless possibilities for this wonderful invention called Polymerase Chain Reaction.

**What is needed to Begin Polymerase Chain Reaction (PCR):**

Begin with a segment of DNA that needs to be amplified in order to generate many copies. What is needed to accomplish the goal?

1) A few molecules of DNA, which are called the “template” that includes the DNA segment that is to be amplified which called the “target sequence”. Just a tiny bit of DNA is needed to accomplish our goal.

2) Two PCR primers are needed. These are short pieces of single stranded DNA that match the sequence at either end of our target DNA segment. The primers are needed for synthesis to start.

3) An enzyme is needed to make the copies of DNA. The PCR procedure involves high temperature steps so the use of a heat resistant DNA polymerase is required. This would be extracted from heat resistant bacteria living in hot springs at temperatures up to 90 degrees Celsius. Most often Taq polymerase from Thermus aquaticus is used.

4) A supply of nucleotides is needed for the polymerase to use when making new DNA.

5) Last needed is a PCR machine to keep changing the temperature. The PCR process requires cycling around through different temperatures. The PCR machine makes this task easier and more efficient. The machine is called a Thermocycler.
How The Polymearse Chain Reaction (PCR) Works:

Cycling through with PCR:
1) To separate the strands of DNA, we need to heat our template to 90 degrees Celsius for a minute or two.

### PCR - STRAND SEPARATION 90°C

2) Although the primers are present from the first step, they cannot bind at the high temperature of 90 degrees Celsius. Therefore our next step is to drop the temperature to around 50 to 60 degrees Celsius, which will allow the primers to anneal to the complementary sequences on the template strands. The longer the primer, the more specific will be the binding.

### PRIMERS ANNEALING 50°C- 60°C
3) Next, we maintain the temperature at approximately 70 degrees Celsius for a minute or two to allow the polymerase to elongate new DNA strands starting at the primers. **DNA synthesis occurs from the 5' to the 3' for both new strands.

We now have two partly double stranded pieces of DNA.

- NOTE: The two new strands of DNA are not as long as the original DNA template, they are missing a piece at the end where synthesis began. However, they are double stranded over the region where our target sequence is located!
- This cycle of events is repeated over and over. Clear results can be seen from the DNA up to 40 cycles.
* Purpose:
The Polymerase Chain Reaction (PCR) will show how scientist can take a tiny segment of DNA and make enough DNA to for testing purposes.

*Teachers Notes:
This activity involves the Polymerase Chain Reaction or PCR. The students will work on the project as a class. The students can do an at-home project on PCR to go along with the in class discussion fro an individual grade.

*Class Time Needed:
The activity can easily be done in one to two class periods. The activity contains two sections: lesson/instruction and piecing together the PCR.

*Preparation Time:
The PCR is made of Fun-noodles, a Styrofoam floating toy found at discount stores. The teacher will need to choose two different colors of Fun-noodles and cut the Fun-noodles in pieces of twelve-inch sections and then divide each section in half-length wise. You will need approximately sixteen twelve-inch sections of each color, which can be done from three whole Fun-noodles of both colors. The teacher will then need to take half of the pieces of each color and put them aside. These twelve-inch sections are to be the denatured half of the DNA fragment. The other half pieces will need to be cut again in nine and three inch sections total. The three-inch sections will become the primers and the nine-inch sections will become the extensions of the DNA nucleotides. To help with primer recognition, you may want to add several more twelve-inch sections of different colors. This should take no more than an hour to prepare.
Fun-noodle PCR Lab:

*Required of Students:

The students will need to bring notebooks and pencils to class. They will need to be able to work with each other as a class. The students can be responsible for doing an at-home project in a smaller scale of the PCR done in class.

*Materials Needed:

1) Eight Fun-noodles (Using two different colors- three of each color)
2) Toothpicks

* Procedure:

- Discuss the principles of PCR with the students. PCR works by amplifying DNA fragment sequences. Even an extremely small amount of a certain DNA molecule to begin with, so little it can scarcely be detected, can generate literally billions of copies by PCR. As a result there are many, many uses for PCR.
- PCR can be used in clinical diagnosis, reducing the valuable time before a diagnosis is made in a shorter frame. Viruses such as meningitis, hepatitis, and even the flu can be diagnosed by using PCR. Thus knowing what is wrong with the patient, a doctor can prescribe the correct medicine to the patient faster hopefully insuring a quicker recovery.
- PCR can be used in genetic analysis for paternity cases, crime scenes, and to determine the heredity factor of a gene. PCR can be used, as in this activity, for genetic analysis to determine the source of a bacterial infection.
- PCR is used in many other ways such as genetic engineering and for forensic analysis. There are countless possibilities for this wonderful invention called Polymerase Chain Reaction or PCR.
The fundamentals of PCR begin with a segment of DNA that is to be amplified in order to generate many copies. For this to occur we need to follow three steps:

1) Denaturation: First, an excess of primer, (three-inch Funnoodles—I used green and purple Funnoodles), which are typically a synthetic sequence of 20-30 nucleotides, is mixed with the DNA fragment (two different colors of twelve-inch Funnoodles held together with toothpicks—I used half green and half purple to form one double helix of DNA and half pink and half orange to form the second DNA double helix held together with toothpicks) to be amplified. This mixture of primer and fragment is heated (pretend) to approximately 90 degrees Celsius. At this high temperature, the double stranded DNA fragment (two different colors of twelve-inch Funnoodles) dissociates into single strands (Take apart the different colors of Funnoodles)

2) Annealing of Primers: Next, the solution is allowed to cool to approximately 55-60 degrees Celsius (pretend). As it cools, the single fragment strands of DNA (pink and orange twelve-inch Funnoodles) reassociate into double strands. However, because of the large number of primer (three-inch green and purple Funnoodles), each of the fragment DNA (twelve inch green and purple Funnoodles) will pair up with the complementary primer (one three-inch green primer pairs up with one twelve-inch purple DNA fragment and one three-inch purple primer pairs up with one twelve-inch green DNA fragment held together with toothpicks, remembering to observe one at 5' and of DNA and the other at the 3' end of DNA) leaving the rest of the fragment single stranded (approximately nine-inches).

3) Primer Extension: Now a very heat-stable type of DNA polymerase, called Taq polymerase is added along with a supply of all four nucleotides, (nine-inch sections of green and purple Funnoodles). Using the primer (three-inch sections of the green and purple Funnoodles that are already attached to the twelve-inch opposite color of DNA fragment Funnoodles with toothpicks), the polymerase copies the rest of the fragment as if it were replicating DNA (attach the nine-inch green or purple that matches the three inch primer to the opposite color fragment of twelve-inch DNA fragment). You now have two copies of the DNA fragment. Repeat this procedure twice with all new copies of the DNA fragments. If one fragment of DNA reproduces in one cycle to form two copies of the DNA fragment, then two DNA fragments will reproduce in the next cycles to make four copies and four DNA fragments will reproduce to form eight copies of the DNA fragment. This can continue to occur for forty to sixty cycles without losing integrity, forming millions of copies of the original one fragment of DNA.
*You may allow the students to form their own PCR experiment at home using such things as plastic colored straws; colored Popsicle sticks and colored sponges.

*Methods of Evaluation:

1) Each student will be evaluated as to class participation during the PCR procedure.

2) Each student will be evaluated on the take home project on PCR. It will be graded according to correctness and turned in on time. Rubric attached.
Credits:

1) University of Mississippi Medical Center-
   A) Dr. Rockhold, Pharmacology Department, for implementing the Base Pairs Program, a summer workshop, with other doctors, most notably Dr. Donna Sullivan, that encourages teachers of all disciplines to continue education for the students. The emphasis being in science.
   B) The Microbiology Department for allowing me to follow them around for 6 weeks, getting a “hands-on”, “minds-on” experience during an AAI fellowship.

2) AAI for allowing the opportunity to continue to learn through a summer fellowship.

PCR Project

Grading Rubric:

1. Turned in on time
   *Early*
   ____________ (+20)

2. Completed sequence
to eight copies of DNA
   ____________ (Possible +35)

3. Correct sequence of
the target DNA, primers,
& newly made DNA
   ____________ (Possible +30)

4. Mix of DNA, primers
& nucleotides
   ____________ (Possible +10)

5. Name on Project
   ____________ (+5)

****Total Grade****
