Paperclip PCR Instructions
(PCR = Polymerase Chain Reaction)

Purpose:
In this exercise, you will perform the role of the polymerase chain reaction (PCR) thermocycler and the enzyme DNA polymerase, in three PCR cycles. You will use colored paper clips that represent primers and free nucleotides to synthesize strands of DNA. Keep in mind the complementary base pair rules: adenine (A) is complementary to thymine (T) and cytosine (C) is complementary to guanine (G).

Materials:

Adenine = Green paper clips	**bind to**	**Thymine = Red** paper clips
Cytosine = Blue paper clips	**binds to**	**Guanine = Black** paper clips
Labeling tape	Sharpie or other ink pen	

Procedure:

1. Gather your materials.
2. Build the two primers required by hooking the paperclips together according to the color key above under Materials:
   - Build 7 "Forward" primers = AAT [if they are not provided already assembled]
   - Build 7 "Reverse" primers = GCA [if they are not provided already assembled]
3. Have one pair of students move to the opposite side of the lab bench you will be working on.
4. Build your first strand of template DNA (Strand 1, or the 'top' strand of DNA) by hooking the paperclips together according to the color key above [if it is not provided]:

   5' - **A G C A A T C G G T T A G C** – 3'

5. Use a small piece of labeling tape and a pen to label the 5-prime (5') end of DNA Strand 1 and Primer 1, as seen in Figure 1. The 3-prime ends of DNA Strands 1 and 2 are also labeled in Figure 1 on the next page.
6. Build the second strand of DNA (Strand 2, or the 'bottom' strand of DNA) complementary to the top strand [if it is not provided].

   3' - **T C G T T A G C C A A T C G** – 5'

7. Use a small piece of labeling tape and a pen to label the 5-prime (5') end of DNA Strand 2 and Primer 2, as seen in Figure 1.
8. The first step in PCR is DNA melting, or "***denaturation***." In this first step of PCR, the hydrogen bonds between the two strands of nucleotides are broken as the DNA molecule is heated. **To perform this step, slide the two parent chains apart from one another, so that one is in front of each pair of students. Each pair or member of the pair should perform the next steps on their DNA strand(s).**

9. The next step in PCR is "***annealing***," when the temperature lowers a little. In this step, the primers bind to the template DNA strand by complimentary base pairing.

- One primer (which scientists often call the "***Forward***" primer) marks or "***primes***" the beginning of what will be the strand of DNA made from the top strand of DNA.
- The second primer (which scientists often call the "Reverse" primer) primes the beginning of what will be the strand of DNA made from the complement to the bottom strand of DNA.
- **To perform this step, find where the Forward and Reverse primers bind to the template. Remember the base pairing rules! One primer will bind to the 'top' strand of DNA and one primer will bind to the 'bottom' strand of template DNA.**

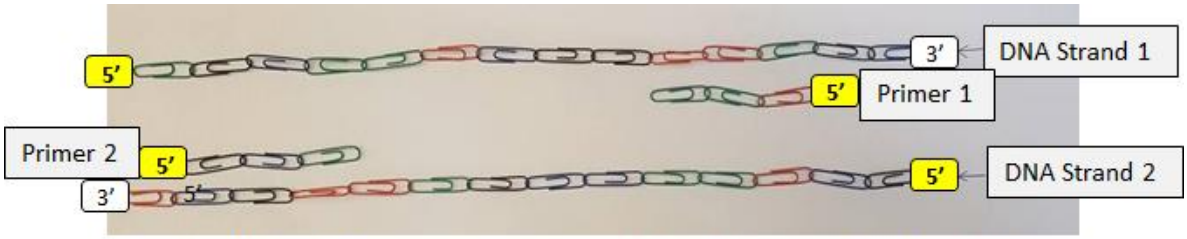

Figure 1: DNA strand 1 (top) and DNA strand 2 (bottom) bind to Primer 1 and Primer 2.

10. The next step is in PCR is DNA synthesis or "***elongation***" from the primer. In this step, the enzyme (DNA polymerase) uses the template strand and base pairing rules to build a new DNA strand that is complementary to the template strand.

- Free nucleotides are added to the new strand, beginning at the primers and going to the end of the strand. Keep in mind that replication occurs in only one direction for each strand (in opposite directions on the two complementary template strands). Since DNA polymerases can travel in only one direction along the parent strand (from 3' toward the 5' end), nucleotides before the primer will not be added.
- **To perform this step, build two new DNA strands (one complementary to the top strand and one complementary to the bottom strand) by hooking the appropriate color paper clips to the ends of the primers, moving towards the LONG end the template DNA.**
- **Label the 5' ends of the new strands of DNA, as shown in Figure 2.**

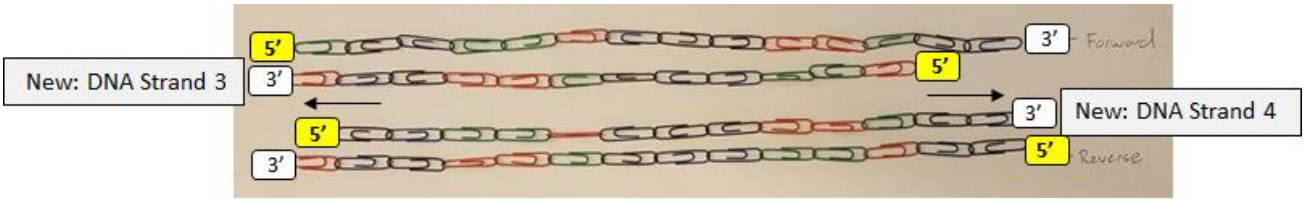

Figure 2: New DNA strands are made by extending the primers, using DNA strand 1 as a template for DNA strand 3 and DNA strand 2 as a template for DNA strand 4.

11. The PCR reaction continually cycles until the required number of "***PCR products***" have been synthesized. In each PCR cycle, the primers attach to the template DNA, both the original DNA and the PCR products made in each cycle (Figure 3, top image). The number of segments doubles with each PCR cycle, and with each cycle, more and more copies of the desired PCR product are made (Figure 3, bottom image). **You have completed one PCR cycle and have two complementary strands. Perform two more cycles: repeat denaturation (Step 6), annealing (Step 7) and elongation (Step 8) in two more cycles.**

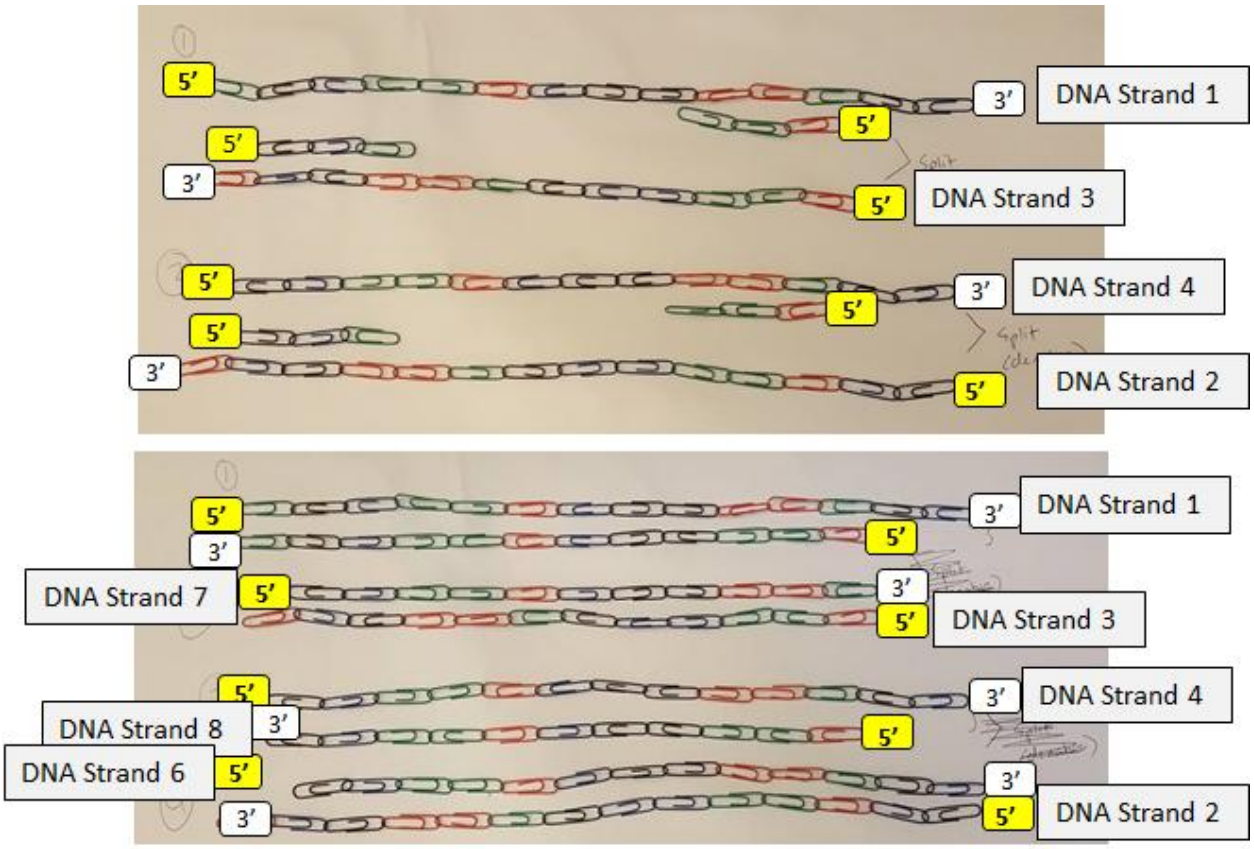

Figure 3: Top Image: Primers ***anneal*** to the DNA templates – both the original DNA strands and the DNA strands made during the previous PCR cycles. Bottom Image: New DNA strands and synthesized by DNA polymerase (***elongation***) from 5′ to 3′, beginning from the 3′ end of each primer.

12. At the end of the third cycle, observe the completed strands synthesized by both pairs of students in your group or both members of your lab pair.

13. Answer the questions on the next page (Page 4).

14. Disassemble all the strands according to the instructions from your teachers and put the clips into their respective containers.

Name:_____Date: _____

1. In what direction are new nucleotides added to a growing strand of DNA?

2. What are the three steps of a PCR reaction and what happens during each step?

3. How many cycles did it take before you had a **DOUBLE STRAND** that consisted of only the desired PCR product, _____ nucleotides long? [Biotech students to fill in]

4. If the PCR process were to stop after 5 cycles, how many **DOUBLE-STRANDED** PCR products would have been produced from **ONE DOUBLE- STRANDED** segment of template DNA?

5. Refer to the question above. How many of these strands would be the desired size DNA, starting with the Forward primer and ending with the Reverse primer?

6. Why are primers used?

7. Why not copy the entire DNA strand?