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### Identification of Bacterial Species Using Colony PCR

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# Identification of Bacterial Species Using Colony PCR

Kaiti Walker

Ouachita Baptist University

# Introduction

## Founder

As he drifted down Highway 128 in California in his Honda Civic, Kary Mullis had an epiphany, one that would revolutionize the world of biology. In 1985, polymerase chain reaction, or PCR, was invented by Kary Mullis. Mullis came up with the idea while working as a chemist for Cetus Corporation in Emeryville, California (Smithsonian).

## DNA Replication

In cellular DNA replication, the entire strand of DNA is duplicated. Because DNA is double stranded, the two strands must be separated by the enzyme DNA helicase making two template strands. Primase then comes in and makes a primer on each template strand using complementary base pairing. This primer provides the free 3' OH group. DNA polymerase can then bind to the primer and replicate the DNA strand using free nucleotides. After replication is complete, there are two identical copies of the original strand of DNA.

## Polymerase Chain Reaction

PCR amplifies a specific, desired segment of DNA. That is what makes the idea so unique; any desired DNA segment can be amplified millions of times in a single experiment. Several components are needed for a PCR reaction: DNA containing the segment desired for amplification; DNA polymerase; an excess amount of dNTPs, or free nucleotides; and an excess amount of primers. DNA polymerase is the enzyme that builds a new strand of DNA by using dNTPs and creates a complementary strand of DNA using base pairing. In PCR, a set of forward and reverse primers designed by

the researcher are used. Primers are, perhaps, the most important part of a PCR reaction. They are short segments of nucleic acid that allow for the flanking of a specific region of the DNA. They attach at the ends of the amplified sequence. The primer is what primase would normally synthesize. They tell the polymerase where to bind to start the replication process by providing the necessary free 3' OH group and double stranded region. A set of primers will specify the exact region of DNA to be replicated. Primers that can be designed by the researcher make the idea of PCR both useful and unique.

### **PCR Reaction**

The reaction occurs in several steps to unwind and replicate the region of interest (Fig 1). The first step is denaturation. During this step, the reaction mixture is heated to 94-98°C, breaking the hydrogen bonds between complementary nucleotides and causing the two strands of DNA to separate. This creates two template DNA strands for synthesis. After denaturation, the reaction temperature is lowered to allow for annealing. During the annealing process, the primers bind or anneal to the DNA template strands. This step usually occurs at a temperature of 55-65°C. The temperature for this step is very important; if the temperature is too high, the primers will not attach, but if the temperature is too low, there is a chance the primers will not bind perfectly or will bind in the wrong place. The last step is elongation. The reaction is heated to 72°C to allow synthesis of the DNA. DNA polymerase uses the free nucleotides to create a strand complementary to the template strand of DNA. Because there are two template strands of DNA, at the end of the cycle, two copies of the target sequence are created. By repeating this cycle over and over, there is an exponential increase in the number of

copies of the target DNA segment ("Biology Animation Library-Polymerase Chain Reaction").

### **Invention of the Thermocycler**

When Mullis first invented PCR, the process was very long and tedious due to the temperature switching. So, an instrument was invented by Mullis and his colleagues to cycle among the different temperatures, and "Mr. Cycle" was created. Although this invention significantly sped up the process, another issue remained—the heat needed to denature the DNA caused the DNA polymerase to break down. To solve this problem, more DNA polymerase had to be added at the start of each cycle. This issue led scientists on a search for something that could stand high temperatures of the denaturation step of the PCR reaction. They decided on the DNA polymerase from the thermophilic bacterium *Thermus aquaticus*, which lives optimally at 70°C (Brock, 1994). The bacterium's polymerase could withstand the higher temperature used in the PCR process and replaced the original polymerase isolated from *E. coli*. Scientists no longer had to add new polymerase for each cycle. In 1985, the DNA Thermal Cycler was created to satisfy the need for an instrument that could cycle the temperatures rapidly, a feature Mr. Cycle did not have. Within a few years, PCR became an important tool in diagnostic labs across the country. Mullis received the 1993 Nobel Prize in chemistry for his accomplishments in PCR (Smithsonian).

### **Gel Electrophoresis**

Gel electrophoresis is often used to view the results of a PCR reaction. An agarose gel is used when visualizing PCR products. It contains pores that allow the molecules to move through it. The pore size is dependent on the agarose

concentration—the higher the percentage of agarose, the smaller the pores will be. A higher percentage is better for smaller segments, while a lower percentage is better for larger segments. This method uses charge to separate particles based on size. The smaller molecules can move faster and farther through the gel separating the DNA by size (“Agarose gel electrophoresis of DNA”). The PCR products, along with loading dye, are injected into the wells of a gel (Fig 2). The loading dye is used to visualize the sample as it moves through the gel allowing the experimenter to know when to turn the apparatus off so that the DNA does not run completely off the gel (Fig 3). (“Biology Animation Library-Gel Electrophoresis”).The gel electrophoresis apparatus (Fig 4) runs a charge through the gel, pushing the DNA from the negative to the positive end because the phosphate backbone of DNA gives it an overall negative charge. A DNA ladder consisting of known band lengths is also run in the gel for comparison (“Biology Animation Library-Gel Electrophoresis”).

### **DNA Visualization**

DNA is visualized in the gel using a DNA binding stain. The stain can either be incorporated into the gel itself or applied post-gel electrophoresis. Stains can be viewed using a UV light. Ethidium bromide was the most commonly used until it was found to be extremely toxic because it can easily soak through the skin and bind to the DNA. The ethidium bromide is an intercalating agent, meaning that it inserts itself in between the base pairs of DNA. This can cause mutations such as frameshifts (a different protein is made), chromosomal recombination (different combinations of the alleles), or a stop in cell division leading to developmental problems (Oswald, 2007). Although ethidium bromide is still used, with appropriate safety precautions, many other stains

have been created in order to visualize the DNA in the gel (“Biology Animation Library- Gel Electrophoresis). A transilluminator is used to see the stained DNA bands.

### **16S rRNA gene**

In all bacteria and archaea, there is a gene that codes for the 16S rRNA which is on the 30S ribosomal subunit. This subunit, along with the 50S ribosomal subunit, make up the ribosome. mRNA is then fed through these subunits to make the protein. Although all bacteria have a gene to code for the 16S rRNA, the DNA sequence of the gene is species specific. This fact makes it possible to use the sequence of the gene to definitively identify a bacterial species, rather than guessing based on observed characteristics. Another reason to use the 16S rRNA for identification purposes is that the gene is about 1500 base pairs. It is a fairly short gene and can be amplified quickly and easily (“16s rRNA gene”).

### **My Research**

My research consisted of two separate parts, both involving the PCR methods. In part one I was trying to identify bacteria isolated from a chicken coop. In part two I was trying to identify bacteria isolated from the bluff. In both parts, I used a technique called colony PCR. This concept uses a single colony of bacteria to complete the PCR reaction. One pipet tip from a single colony is selected from the plate and mixed into a tube containing ultrapure water. A small amount of this bacterial mixture is then added into the PCR reaction. In the first part of my research, in the spring of 2014, I used bacteria isolated by the Fall 2013 microbiology class. The original samples were taken from a local chicken coop, and from there, each of the six groups isolated a single bacterial species. Many metabolic tests were run on each bacterium. The results of the

metabolic tests were used to identify the isolated species by using a guide book. Time did not permit for the bacteria to be sent off for sequencing to see if each group had predicted the correct species of bacterium. Plates streaked with each group's bacterium were stored in the refrigerator. The goal of my part of the project was to take each group's bacteria, amplify the 16S rRNA gene using PCR, run the product through a gel to check for the presence of DNA, and send the samples off to be sequenced.

Because PCR had not previously been done on these bacteria, I had to pick two primer sets to be used. I found two articles that both used the 16S rRNA amplification for bacterial identification and decided to try the primers from both papers. The first article discussed a project that used DNA extraction procedures and PCR conditions for DGGE (denaturing gradient gel electrophoresis) analysis of bacterial populations in freshwater samples (Yamaguchi, 2013). Their goal was to study microbial changes in the water caused by industrialization and urbanization. They used 16S rRNA forward primer 933F (5'-GCA CAAGCGGTGGAGCATGTGG-3') and reverse primer 1387R (5'-GCCCGGGAACGTATTCACCG-3') (Yamaguchi, 2013). The second set of primers I used came from an experiment that was identifying bacteria found in the gut of broiler chickens (Torok, 2008). The data were then used to correlate the composition of the microbial communities in the gut to the amount of energy that is able to be metabolized. The primers used by these scientists were universal 16S primers, forward primer 27F and reverse primer 907R (Torok, 2008). The sequences of these primers are 5'-AGAGTTTGATCMTGGCTCAG-3' and 5'-CCGTCAATTCMTTTRAGTTT-3' ("16S Ribosomal DNA").

In the second part of my research, in the spring of 2015, I used bacteria isolated by the Fall 2014 microbiology class from soil samples collected from the bluff near Ouachita's campus. Each group isolated several species of bacteria and tested them for the production of antibiotics. If the group had a bacterium that produced antibiotics, further metabolic testing was done in an attempt to provisionally identify the species. PCR, using the 8F, 1492R primer set, was then done to amplify the 16S rRNA gene for confirmatory bacterial identification. If the reaction was successful, the DNA samples were purified and sent off for sequencing. Each group's bacteria were stored in the refrigerator until needed. Each group was able to use PCR to obtain the sequence to identify the species of their isolated bacteria, except for two groups—Hall and Feather. The Hall bacterium is very waxy, which could have been the reason it did not work. The goal for my part of this project was to try several method adaptations to see if the PCR will provide enough DNA for the bacterial species can be identified. Unlike the first part of my research, in this part I used a pre-made PCR bead. The bead contained all the ingredients necessary for the reaction except for the primers and source DNA. The primers for the second part are universal 16S forward and reverse primers—forward primer 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1492R short (5'-ACGGCTACCTTGTTACGACTT-3') ("16S Ribosomal DNA"s). This was the same primer mix used in the Fall 2014 microbiology class.

## **Research Spring 2014: Chicken House Samples**

### **Materials**

10 mM dNTPs (New England BioLabs® Inc.)	Nuclease-free water
40X TAE Buffer	PCR tubes
5X Crimson Taq Reaction Buffer	Primer set 1 (27F, 907R)
6X Gel Loading Dye	Primer set 2 (933F, 1387R)
Agarose	Quick Load® 1 kb DNA ladder (New England BioLabs® Inc.)
Colonies of each type of bacteria	Quick Load® 100 bp DNA ladder (New England BioLabs® Inc.)
Crimson Taq Polymerase	Thermocycler
EZ-Vision® in-gel stain	Transilluminator
Microcentrifuge tubes	TSA plates
Micropipettes: 0.1-10 µL, 2-20 µL, 20-200 µL	

## Methods

### Bacteria

In order to have fresh bacteria for the PCR reaction, new streak plates had to be made. The new plates were made on tryptic soy agar (TSA). TSA is a growth medium that provides the nutrients that bacteria needs in order to grow. The plates were incubated at 37°C for 24 hours. New streak plates were made every few days to keep the bacteria fresh until it was time to start the PCR reaction.

### PCR Master Mix

For each of the two primer sets, 3 µL of the forward primer and 3 µL of the reverse primer were added to 24 µL of nuclease-free water to make the primer set

mixtures. A master mix was then created for each of the primer sets. The master mix consisted 100  $\mu\text{L}$  of 5X Crimson Taq Reaction Buffer, 10  $\mu\text{L}$  of 10 mM dNTPs, 1  $\mu\text{L}$  of the primer set mixture, 2.5  $\mu\text{L}$  of Crimson Taq polymerase, and 386.5  $\mu\text{L}$  of nuclease-free water, mixed in a 1.5 mL microcentrifuge tube. For each of the six bacteria types being tested, two PCR tubes were prepared, one containing 25  $\mu\text{L}$  of primer set 1 master mix, the other containing 25  $\mu\text{L}$  of the primer set 2 master mix. Each bacterium was added to the appropriate PCR tube by taking a micropipette tip, poking a colony, and sloshing it into the reaction mixture. The control contained no bacteria. Each of the tubes were put into the thermocycler and run on the program labeled "Kaiti16S". This program consisted of 95°C for 30 seconds; then 35 cycles of 95°C for 30 seconds, 53°C for 45 seconds, and 68°C for 70 seconds; then 68°C for 5 minutes. Products were stored at -20°C until it was time to run the gels.

## **Gels**

1% agarose gels were made by mixing 97.5 mL of deionized water, 2.5 mL of 40X TAE Buffer, and 0.8 g of agarose in a 250 mL beaker. The mixture was microwaved for 90 seconds to melt the agarose. Then, 1 drop of EZ-Vision® in-gel stain was added to the mixture. Once the beaker was cool enough to hold with bare hands, the gels were poured into the molds, allowing 25 mL per gel. In a new PCR tube, 3  $\mu\text{L}$  of the thawed PCR product and 3  $\mu\text{L}$  of 6X loading dye were mixed together. Two gels—one for each primer set—were placed into the same gel electrophoresis apparatus. Then, the apparatus was filled with buffer made from 10 mL of 40X TAE buffer combined with 390 mL of deionized water. The wells were then loaded with 5.5  $\mu\text{L}$  of the PCR product/loading dye mixture. 10 microliters of the 1 kb ladder was loaded

in the first well and 10  $\mu\text{L}$  of the 100 bp ladder was loaded in the last well. The gels were run at 70 volts until the loading dye was  $\frac{3}{4}$  of the way down the gel. The gels were then viewed using a transilluminator.

## Results and Discussion

When viewed with the transilluminator, the gels showed no trace of DNA product (Figs 5 & 6). The bands that were visible are known as primer dimer. This means that rather than the primer annealing to the bacterial DNA, it stuck to itself. Therefore the target 16S rRNA gene was not amplified at all. This could have been due to lab errors when pipetting each of the ingredients into the master mix. However, there was not enough time left in the semester to redo the experiments to see if the errors could be corrected.

## Research Spring 2015: Bluff Samples

### Materials

1X TAE Buffer	Microcentrifuge tubes
6X Gel Loading Dye	Micropipettes: 0.1-10 $\mu\text{L}$ , 2-20 $\mu\text{L}$ , 20-200 $\mu\text{L}$
Agarose	NanoDrop
Colonies of each type of bacteria	Nuclease-free water
DNA Clean and Concentrator kit (Zymo Research)	PCR tubes
Ethidium bromide	Primer mix (8F, 1492R short)
GelRed Nucleic Acid Stain (Phenix Research Products)	(idtDNA)

PuReTaq™ Ready-To-Go™ PCR

Thermocycler

Beads (GE Healthcare)

Transilluminator

Quick Load® 1 kb DNA ladder

TSA plates

(New England BioLabs® Inc.)

Tween® 20 (Amresco)

## Methods

### Bacteria

In order to have fresh bacteria, new streak plates were made. In addition to the Hall and Feather bacteria that did not previously produce much product, two previously identified bacteria, the Teel and Lewis bacteria, were chosen as positive controls because they produced a lot of product. The method adaptations were applied to these control bacteria as well to ensure that the adaptations would not negatively affect the PCR reaction. New streak plates were made on tryptic soy agar (TSA) and incubated at 37°C for 24 hours to provide fresh bacteria for the PCR reactions.

The first step was to run the PCR as it was previously run in the Fall 2014 microbiology class to ensure that the Hall and Feather bacteria did not produce much product and that the Lewis and Teel bacteria still produced a lot of product. Each PCR bead was dissolved in 23 µL of nuclease free water. The bacterial mixture was made in separate PCR tubes for each of the four bacteria samples by adding 50 µL of nuclease free water to one pipet tip of bacteria. These components were mixed by pipetting up and down. To the tube containing the dissolved PCR bead, 2 µL of the 8F, 1492R primer mix was added. One microliter of the bacterial mixture was then added to the correctly labeled PCR tube. The reaction mixtures were then run on the thermocycler on the program “MICRO2014”. This program consisted of 94°C for 10 minutes; then 30

cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 110 seconds; then 72°C for 10 minutes. When the program was finished, the samples were stored at -20°C until needed.

## **Gels**

The next step was to run the samples through a gel. One percent agarose gels were made by mixing 97.5 mL of deionized water, 2.5 mL of 40X TAE Buffer, and 1.0 g of agarose in a 250 mL beaker. The mixture was then microwaved for 90 seconds to melt the agarose. Then, 2 µL of ethidium bromide was added to the mixture. Once the beaker was cool enough to hold with bare hands, the gels were poured into the molds allowing around 25 mL per gel. The PCR products were then thawed to run through the gel. In a new PCR tube, 5 µL of the PCR product and 1 µL of 6X loading dye were mixed together. The gel was placed into the apparatus and the apparatus was filled with 1X TAE buffer that was diluted from 10X stock TAE buffer. The wells were then filled with all 6 µL of the PCR product mixture. Ten microliters of 1 kb ladder was placed on one side. The gels were run at 125 volts until the loading dye was  $\frac{3}{4}$  of the way down the gel. The gels were then viewed using a transilluminator. Results are described below.

## **PCR Method Adaptations to “MICRO14”**

Two method adaptations were tried in order to get more product from the PCR reaction. The first adaptation was to increase the preheat time from 10 minutes to 15 minutes in order to break down the waxy exterior of the Hall bacterium and liberate the DNA. The program was entered into the thermocycler and labeled “MICRO14L.” This program consisted of 94°C for 15 minutes; then 30 cycles of 94°C for 30 seconds, 58°C

for 30 seconds, and 72°C for 110 seconds; then 72°C for 10 minutes. The second adaptation was the addition of Tween, a detergent, also to break down the waxy exterior of the Hall bacterium. Tween is a detergent known to work well with PCR. The Teel bacterium was run as a positive control. The PCR products were then run through a 1% agarose gel containing 1 µL of GelRed stain at 125 volts. A 1 kb ladder was run as a comparison. One microliter of 20% tween was added to give a final concentration of 0.8%. The PCR was run on the “MICRO14” program. The Teel bacteria with tween was run as a control. In this set of reactions, the Teel bacteria was also run with both of the Spring 2014 primer sets rather than the 8F, 1492R to test if those primers still worked in case the 8F, 1492R primer ran out. The PCR products were run at 125 volts on a 1% agarose gel using the GelRed as the stain. A 1 kb ladder was run as a comparison. Gels were viewed using a transilluminator. Results are described below.

### **PCR Product Purification**

In order for the PCR product to be sequenced, it had to be purified. The DNA Clean and Concentrator kit by Zymo Research was used. First, the amount of PCR product had to be measured. My samples had 14 µL of product. Then, 7 volumes of DNA Binding Buffer was added to each sample—98 µL for each sample. Each sample was then loaded into a Zymo-Spin Column in a collection tube. They were centrifuged at 16,000 rpm for 30 seconds and the flow through was discarded. Then, 200 µL of DNA Wash Buffer was added to each column and centrifuged again for 30 seconds. The flow through was discarded. The DNA Wash Buffer step was repeated, and the flow through was discarded. The Zymo-Spin Column was placed in a sterile 1.5 mL tube. 25 mL of ultrapure water was added to the column and centrifuged for 30

seconds to elute the DNA. The columns were discarded and the tubes were stored at -20°C. The NanoDrop was then used to determine the concentration of DNA in order for the samples to be sequenced.

## **Results and Discussion**

For the PCR products amplified using the “MICRO14” program with no method adaptations, the positive control bacteria showed a large amount of DNA, except for the Hall bacteria (Fig 7). The PCR products were about 1500 base pairs long, which was expected. The control showed no DNA which was expected. The Feather bacterium also showed a large amount of DNA. This was unexpected due to the fact it did not produce a lot of DNA product in the Fall 2014 PCR reaction. However, this could have been caused by student error during the PCR process in Fall 2014. Because the bacteria produced a lot of DNA during this reaction, the Feather bacteria was not used to develop new PCR methods from that point forward, only the Hall bacteria. The increase in preheat time and the addition of Tween were both chosen for method adaptations because of the waxy nature of the Hall bacteria. I thought the longer preheat time would give the bacteria more time to break down the waxy exterior, and in turn allow for the primers to better anneal. The Hall bacteria showed a large amount of DNA following the longer preheat (Fig 8). The Teel bacteria also produced lots of DNA showing that the longer preheat time did not negatively affect the PCR reaction. However, the control also showed a presence of DNA. The reasons for this are unknown. The PCR products were between 1000 and 1500 base pairs. I thought that the Tween would break down the bacteria and allow for the PCR reaction to be more successful. The Tween also worked. The gel shows a high presence of DNA for the

Hall bacteria (Fig 9). The Tween did not have a negative effect on the Teel bacteria. The PCR products were between 1000 and 1500 base pairs in length. This gel also contained the Spring 2014 primers used with the Teel bacteria. Primer set 2 (933F, 1387R) worked, but primer set 1 (27F, 907R) did not work. If the 8F, 1492R primer set runs out, primer set 2 will be used instead. The purified PCR products were then sent off for sequencing. Four samples were sent: the Hall bacteria with the longer preheat time, the Teel bacteria with the longer preheat time, the Hall bacteria with the tween, and the Teel bacteria with the tween.

## Conclusions

After the PCR products were purified, they were sent to UAMS for sequencing. When the sequences came back, a BLAST search was used to find similar sequences in the 16S rRNA gene of bacteria in the database. When the Hall bacterium sequence was BLAST searched, the results showed that it was in the genus *Streptomyces*. I was not able to definitively select a species, but it is for sure some sort of *Streptomyces*. This genus is commonly found in soil (Manteca, 2009). It makes sense that the Hall bacterium is in this genus. When the Teel bacterium was BLAST searched. The results showed that the sequence is present in many species of the *Serratia*. Member of the genus *Serratia* are characteristically red. The Teel bacterium was also red when grown at room temperature. The species was not able to be identified, but the genus was definitive. Along with the nucleotide sequence, a chromatogram was also sent (Fig 13). Chromatograms are the visual graph of the sequence. Fluorescently labeled nucleotides are used and the fluorescence is measured. The chromatogram is the graph resulting from the measurements.

## Other Results

### Dilutions

Along the way, several different rounds of PCR reactions were completed. Before the longer preheat and the addition of tween, a series of dilutions of the Hall bacteria were tested. The sample of 1 pipet tip of bacteria mixed in 50  $\mu\text{L}$  nuclease-free water was labeled as “undiluted.” A series of ten-fold dilutions were created from that. Each PCR tube contained 45  $\mu\text{L}$  of nuclease-free water and 5  $\mu\text{L}$  of the previous dilution. This made dilutions of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ , and  $10^{-4}$ . The Teel bacteria was run “undiluted” with the primer set made in November 2014 and another sample with the primer set created in February 2014 to ensure that the primers are both working the same. The samples were run on the “MICRO2014” program and stored at  $-20^{\circ}\text{C}$ . When run through a 1% agarose gel, the dilutions showed almost no presence of DNA. The Teel bacteria still showed a large amount of DNA (Fig 10).

### Round 2

To ensure the results were accurate, a second round of PCR reactions were run with either the longer preheat or the addition of tween. However, I did not let the PCR beads reach room temperature before adding each of the elements. The gel still showed bands, except they were not as bright as the previous test (Fig11). This could have been due to the PCR beads still being cold; therefore, the reaction did not work as well. Because of this error, I did not count this repetition as successful.

### Round 3

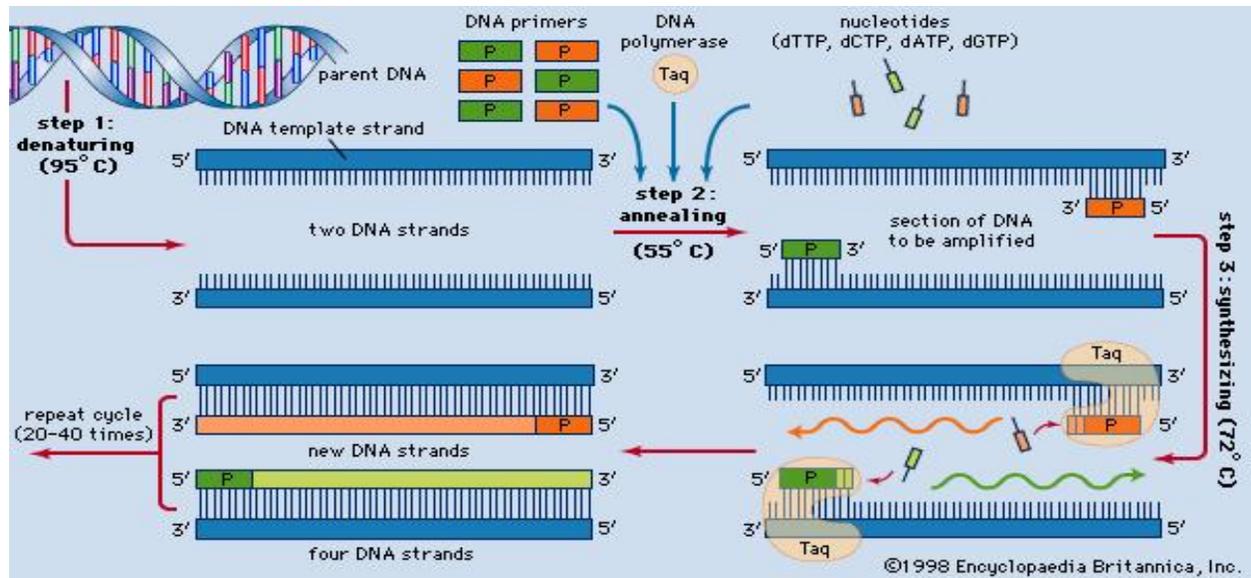
Before starting a third round of PCR reactions to confirm that both the longer preheat and the addition of tween worked, I realized there was not enough 8F, 1492R

primer mix to complete the set of reactions. So, I used primer set 2 from Spring 2014. After the PCR products were run through a 1% agarose gel and viewed using a transilluminator, the gels did not look the same as before (Fig 12). The bands on this gel were closer to 500 base pairs, not 1000 to 1500 base pairs. This could have been due to primer set 2 cutting a different segment than the 8F, 1492R primer set. I decided not to count this set of reactions because it was the only one run with a different set of primers, and it is not certain that it did not cut a shorter region of DNA.

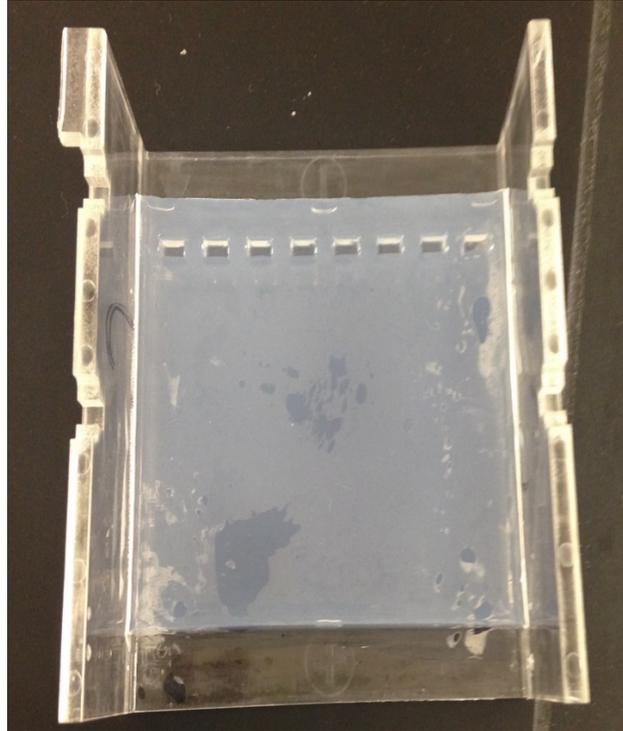
## **Reflection on Research Experience**

I think that every student should have to conduct research in order to graduate. Not only does conducting research put you ahead in the graduate school application process, compared to those who haven't conducted research, it teaches techniques that will be useful in your field of study. In doing research, I learned techniques that I would not have learned through a lab in another class. Before doing research, I had never had the chance to learn about PCR. I didn't know what it could be used for or how it worked. I had never poured a gel or used a gel electrophoresis apparatus. I am in my last semester of college, and even though I have had many labs, I did not get to learn these techniques in them. My overall experience of the research process was very positive. I thoroughly enjoyed the time I got to spend in the lab, and the time I got to spend with Dr. Plymale, my research advisor. She was one of the major reasons I enjoyed doing research. Time in the lab was spent working on the project as well as plenty of smiles and laughter. When I would make a mistake, she corrected me. Although I'm sure I frustrated her at times, she didn't let it show. She continued to be positive the whole time, which in turn made a very positive experience for me. On the

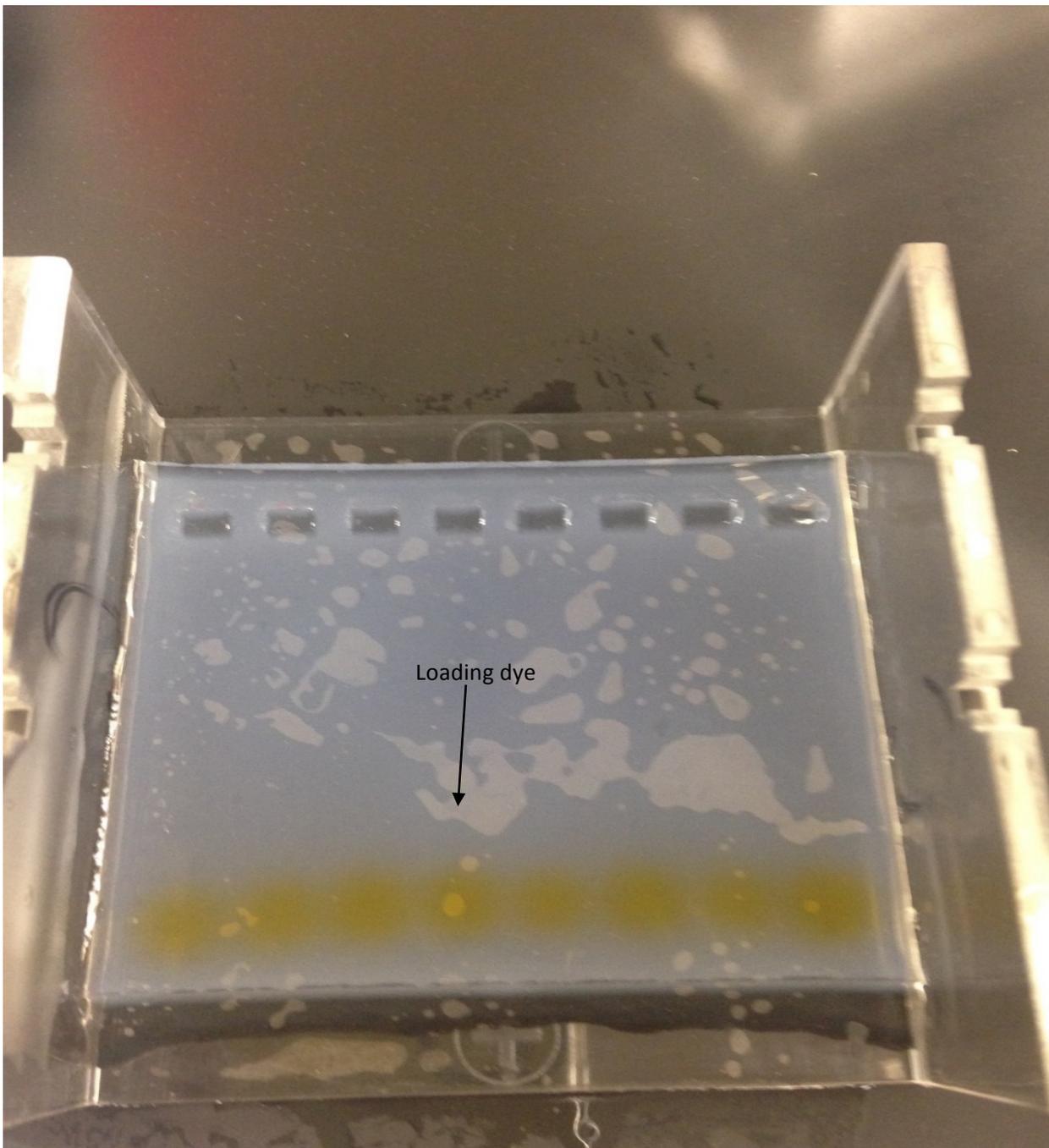
other end, research for anyone can bring about frustration. I learned that you can't always expect things to work the first time; sometimes a process has to be repeated twice or maybe even three or four times before it will work. Research taught me to be a little more patient and to not rush through a procedure because it could end up skewing the results. I truly am appreciative of Ouachita and the School of Natural Sciences for the experience given to me through research.



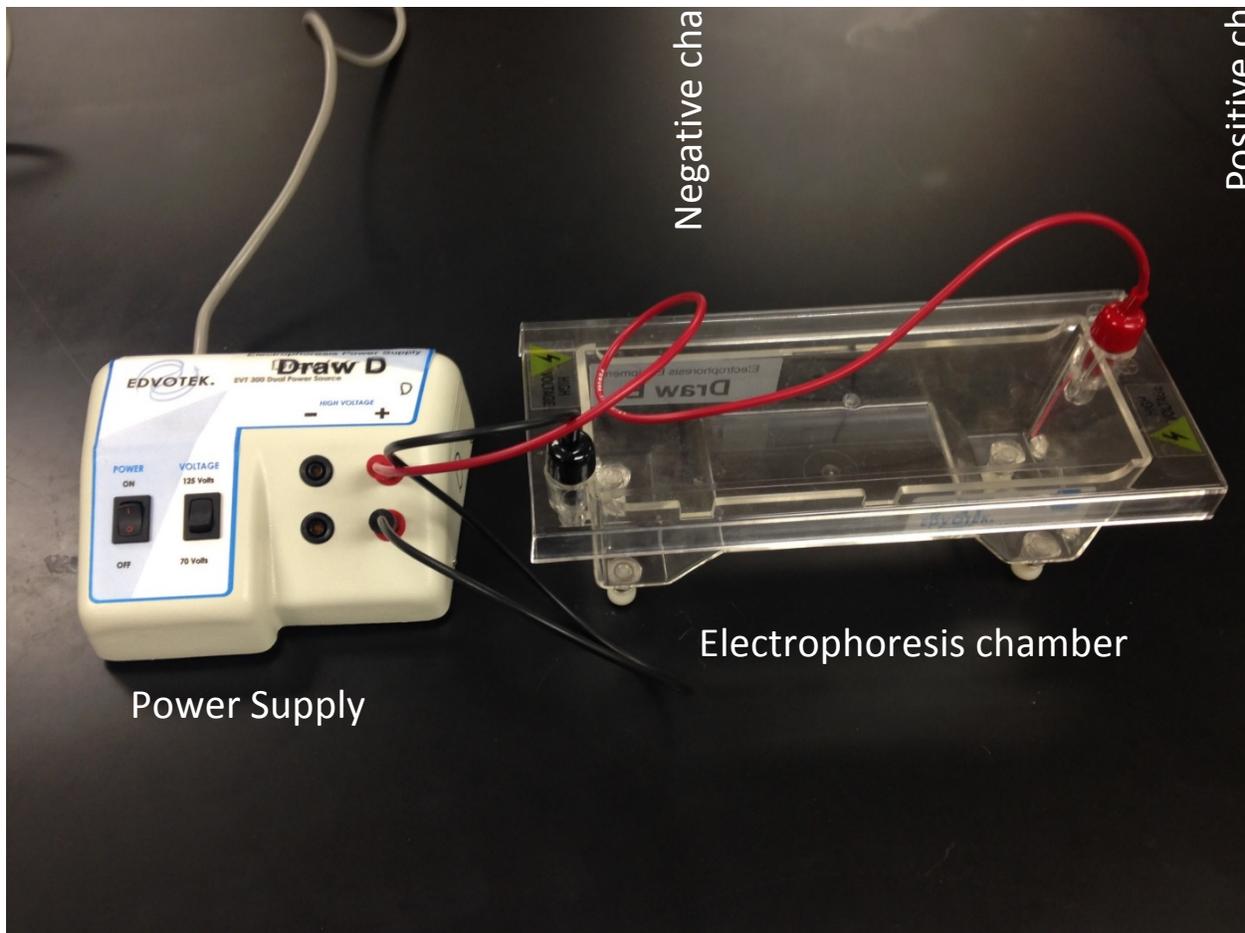
**Figure 1:** A diagram showing the process of PCR and how it takes one copy of DNA and makes hundreds of thousands of copies ("Do Not Like New Test Results; If I Had a Super Power for One Day, I Would...").



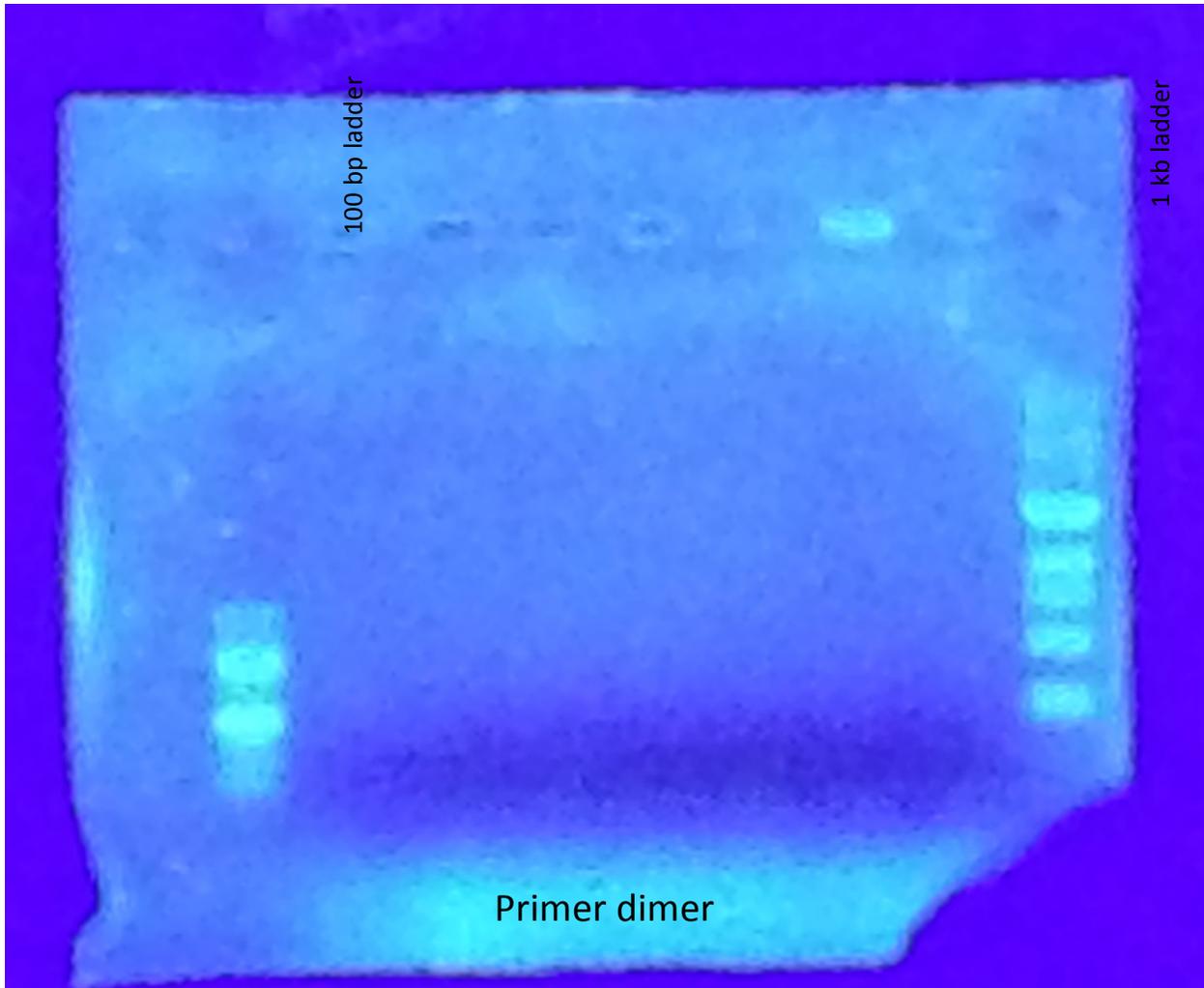
**Figure 2:** The image on the left shows what a gel looks like right after it is poured. The gel is still clear. The comb will produce the wells that the PCR products will be loaded into. This image on the right shows what a solidified gel looks like. The gel is completely solidified when it is a whitish color as seen in this picture.



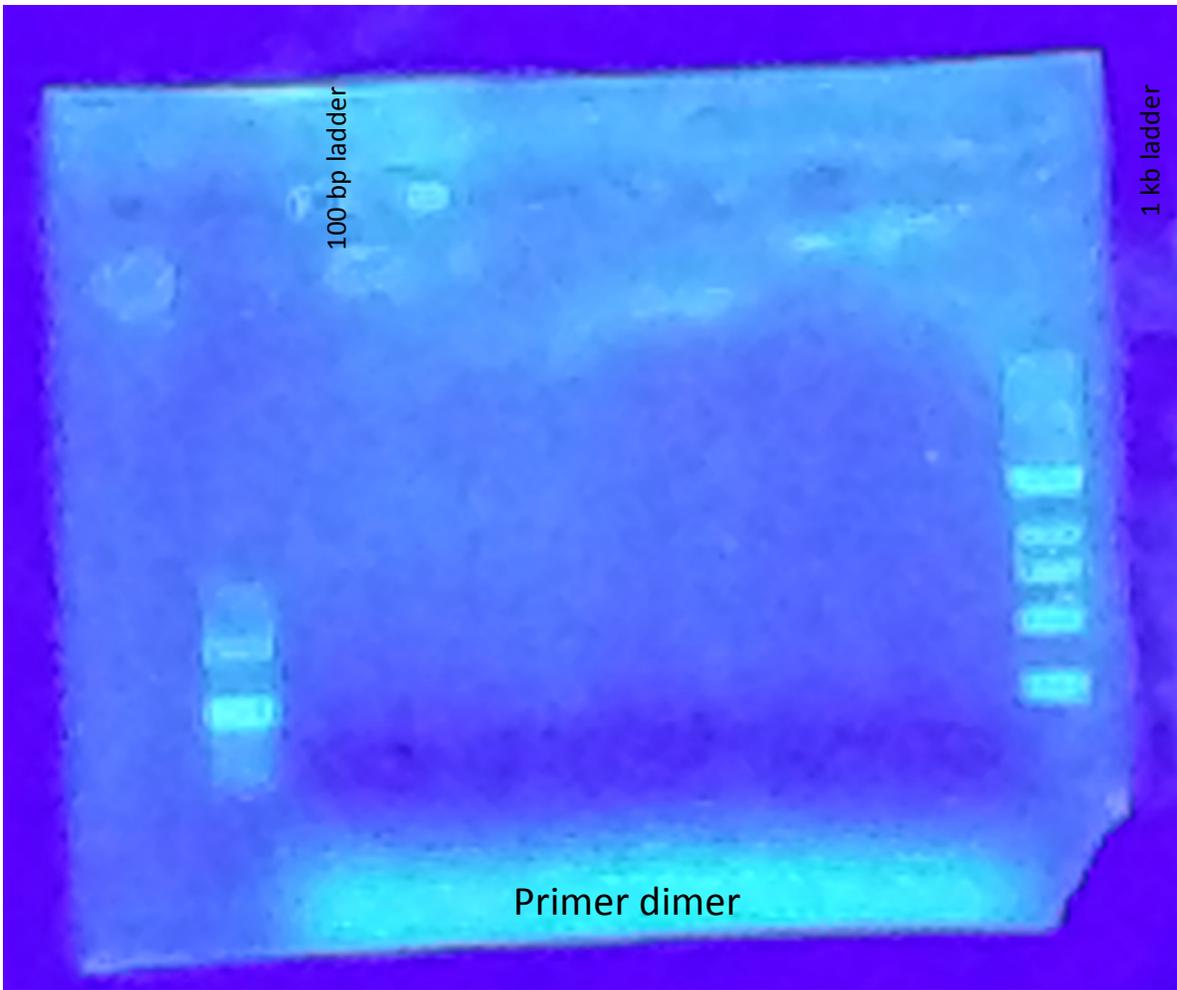
**Figure 3:** This figure shows how far to run the samples through the gel. Once the loading dye is close to the end, the apparatus needs to be turned off.



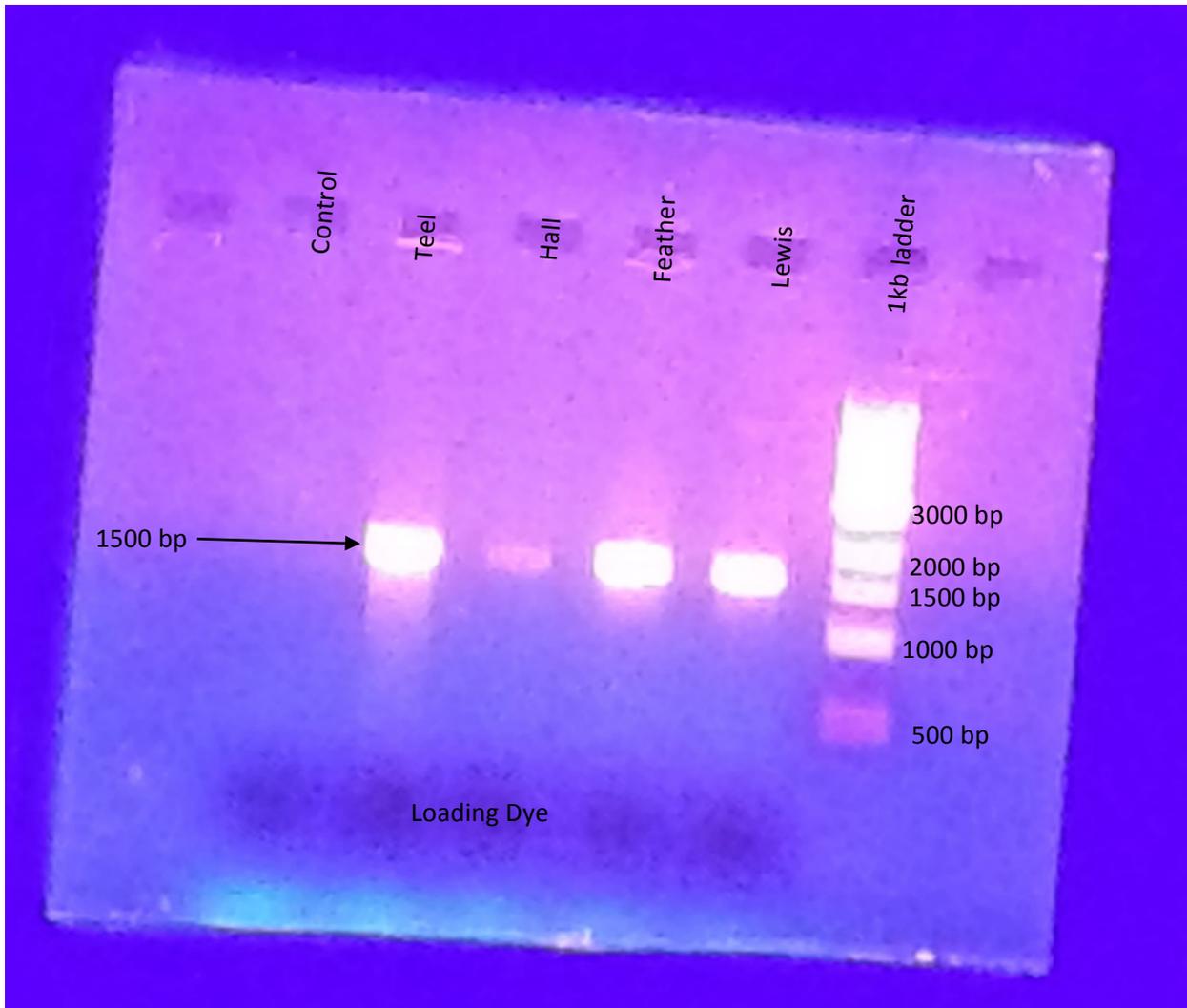
**Figure 4:** This figure shows the components of a gel electrophoresis apparatus. The DNA will run from the negative end to the positive end due to the negative nature of the DNA. The negative nature of DNA is due to the phosphate backbone. This negative nature is attracted to the positive end and repelled from the negative end.



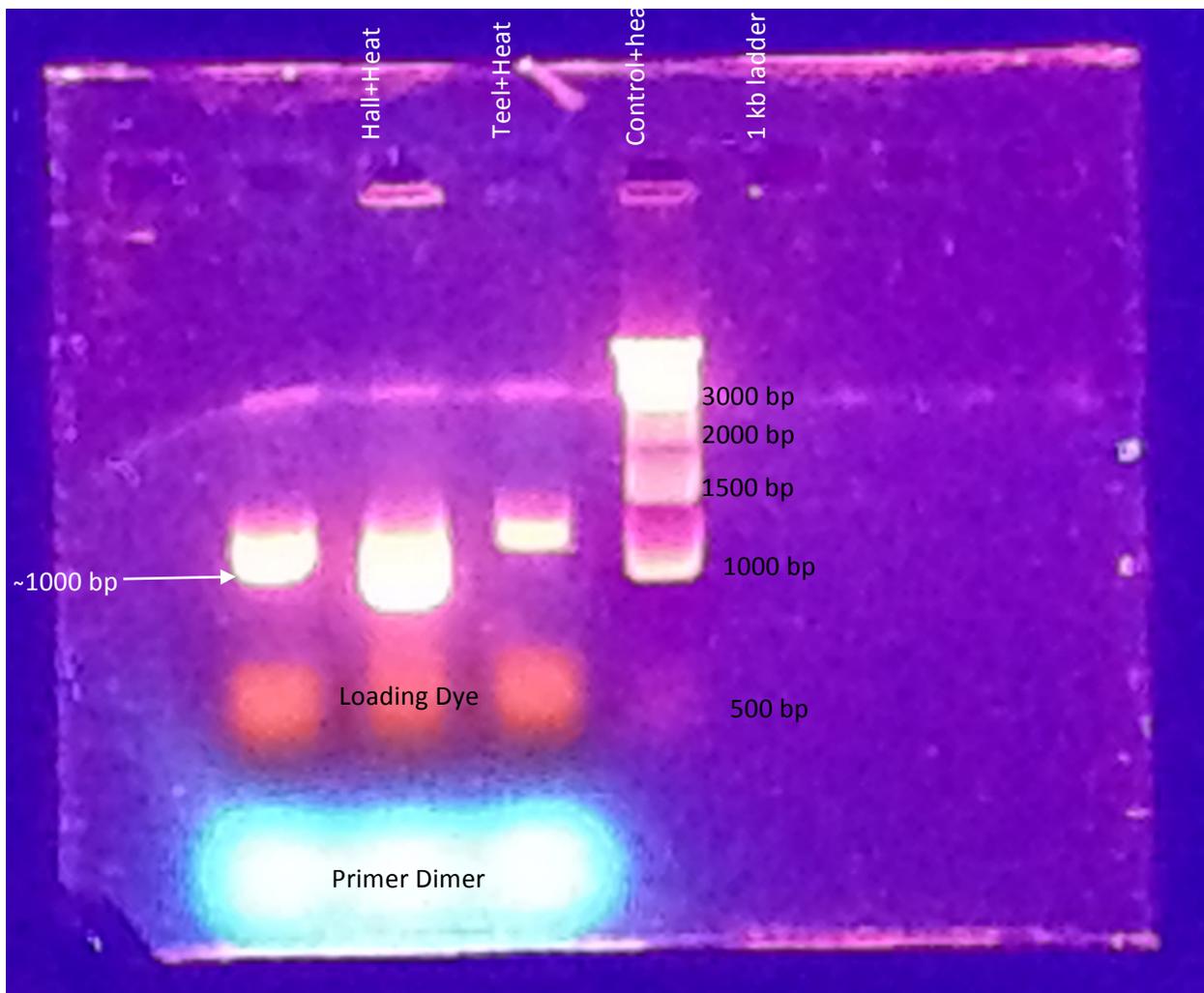
**Figure 5:** This figure shows the gel results using primer set 1. There is no trace of DNA in any of the lanes.



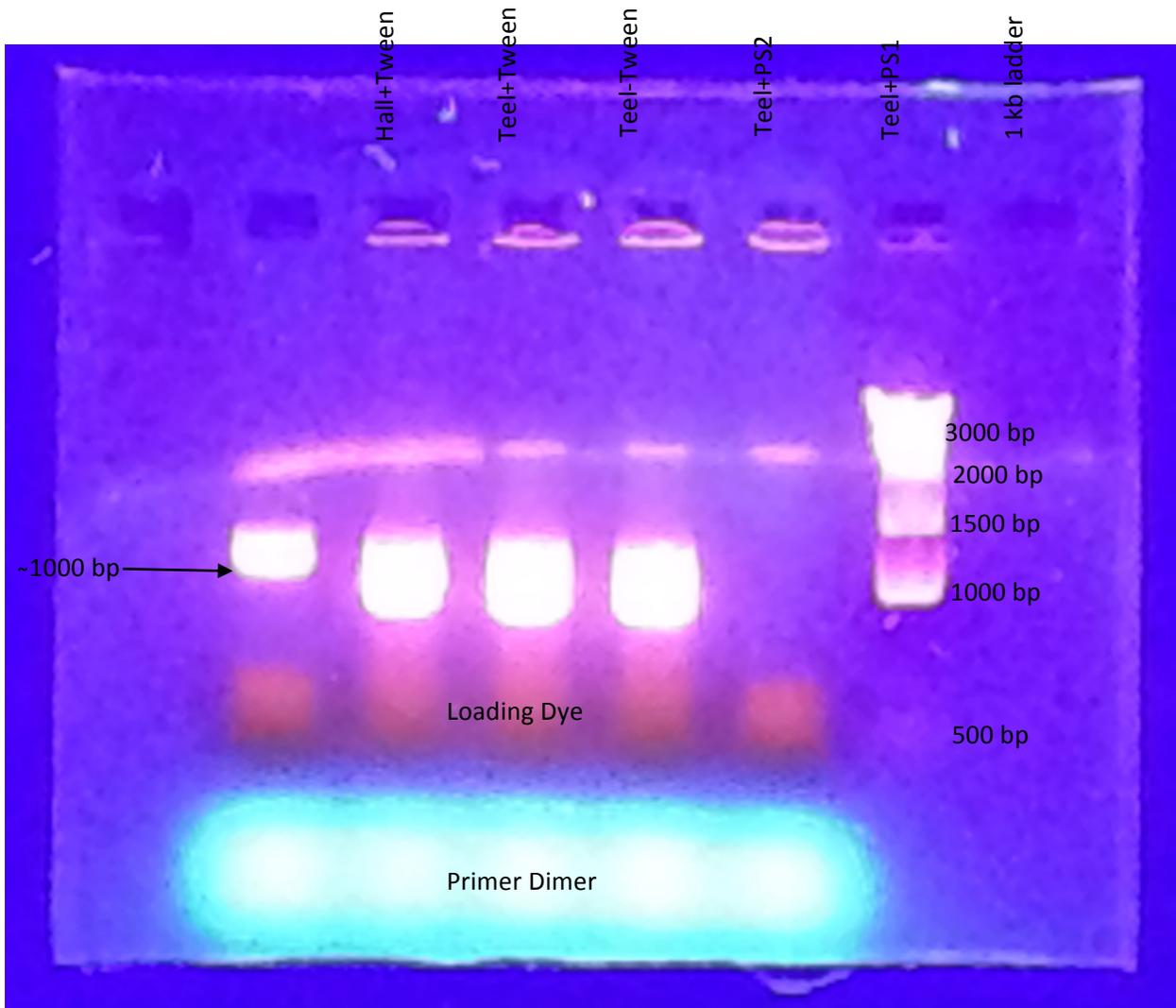
**Figure 6:** This figure shows the gel results using primer set 2. There is no trace of DNA.



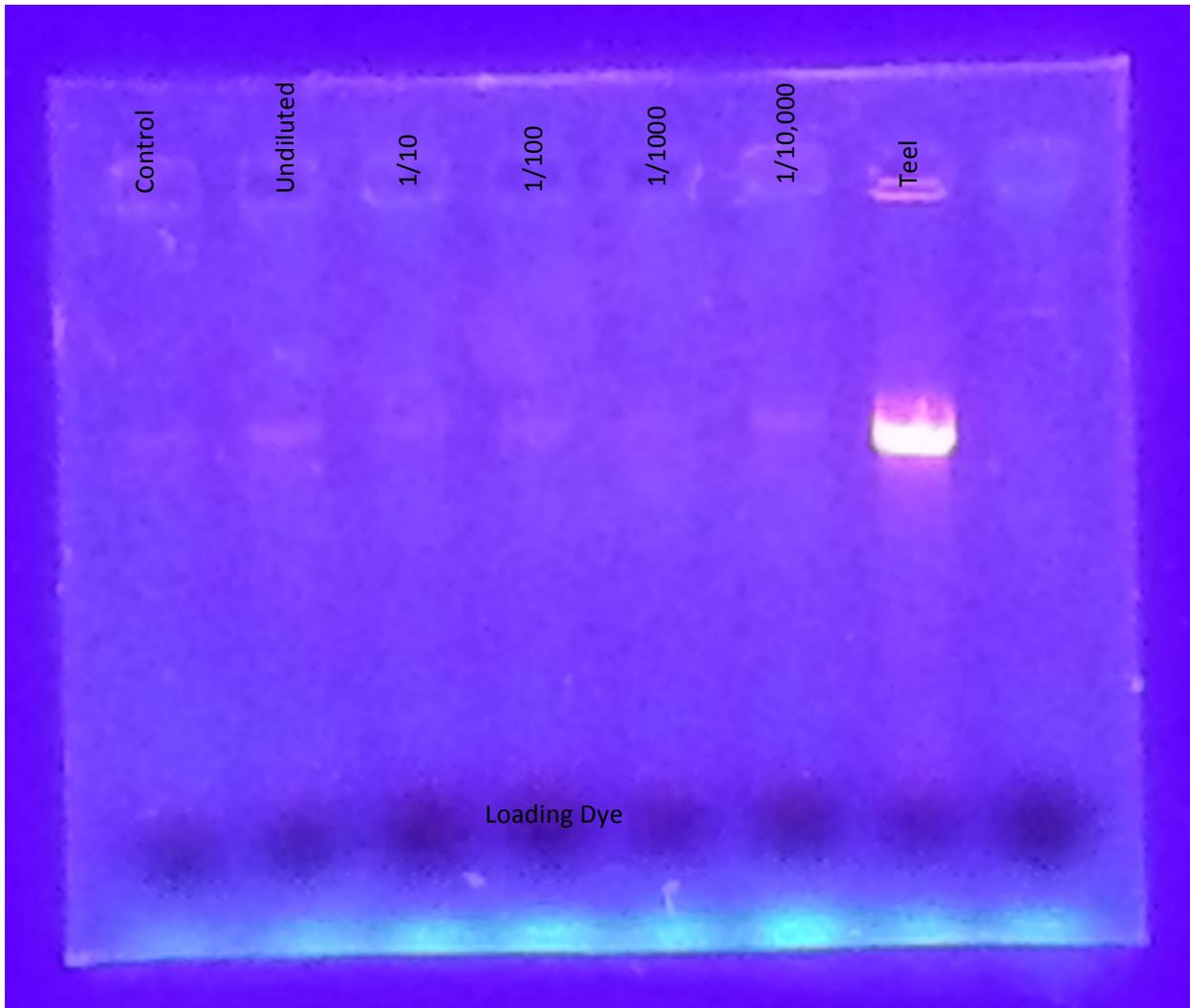
**Figure 7:** This figure is the gel with the samples run with no method adaptations. The Hall bacteria still does not show a large amount of DNA. The Feather bacteria, however, did show lots of DNA. The control showed no presence of DNA. Teel and Lewis were run as the positive controls because they previously worked. Hall and Feather were run as experimental variables because they previous did not work.



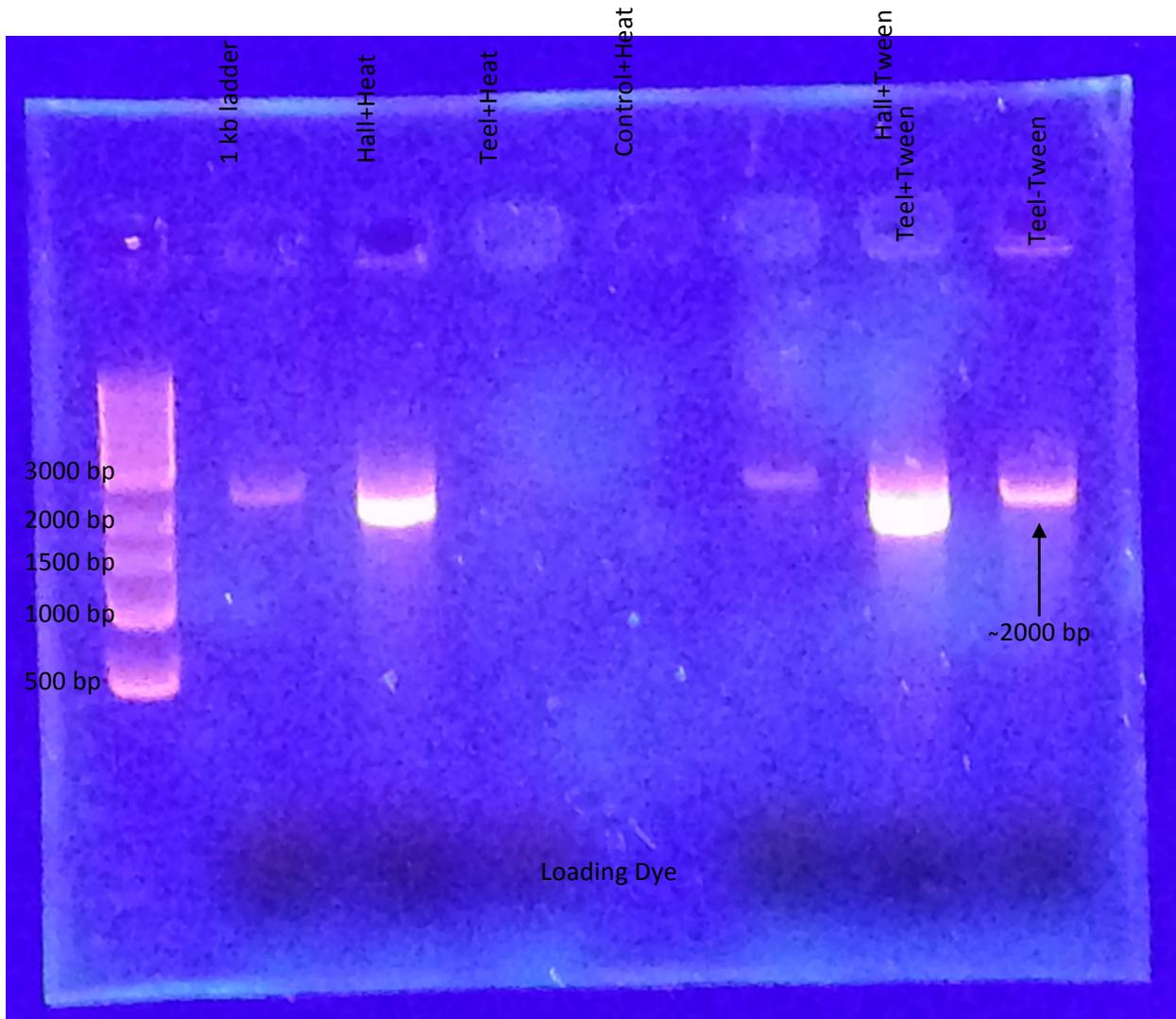
**Figure 8:** This figure shows the gel with the PCR products run with the 15 minute preheat time rather than 10 minutes. The Hall bacteria showed lots of DNA. The Teel bacteria was run as a control for the longer and the control contained no bacteria. However, the control also showed the presence of DNA. The reason for that is unknown.



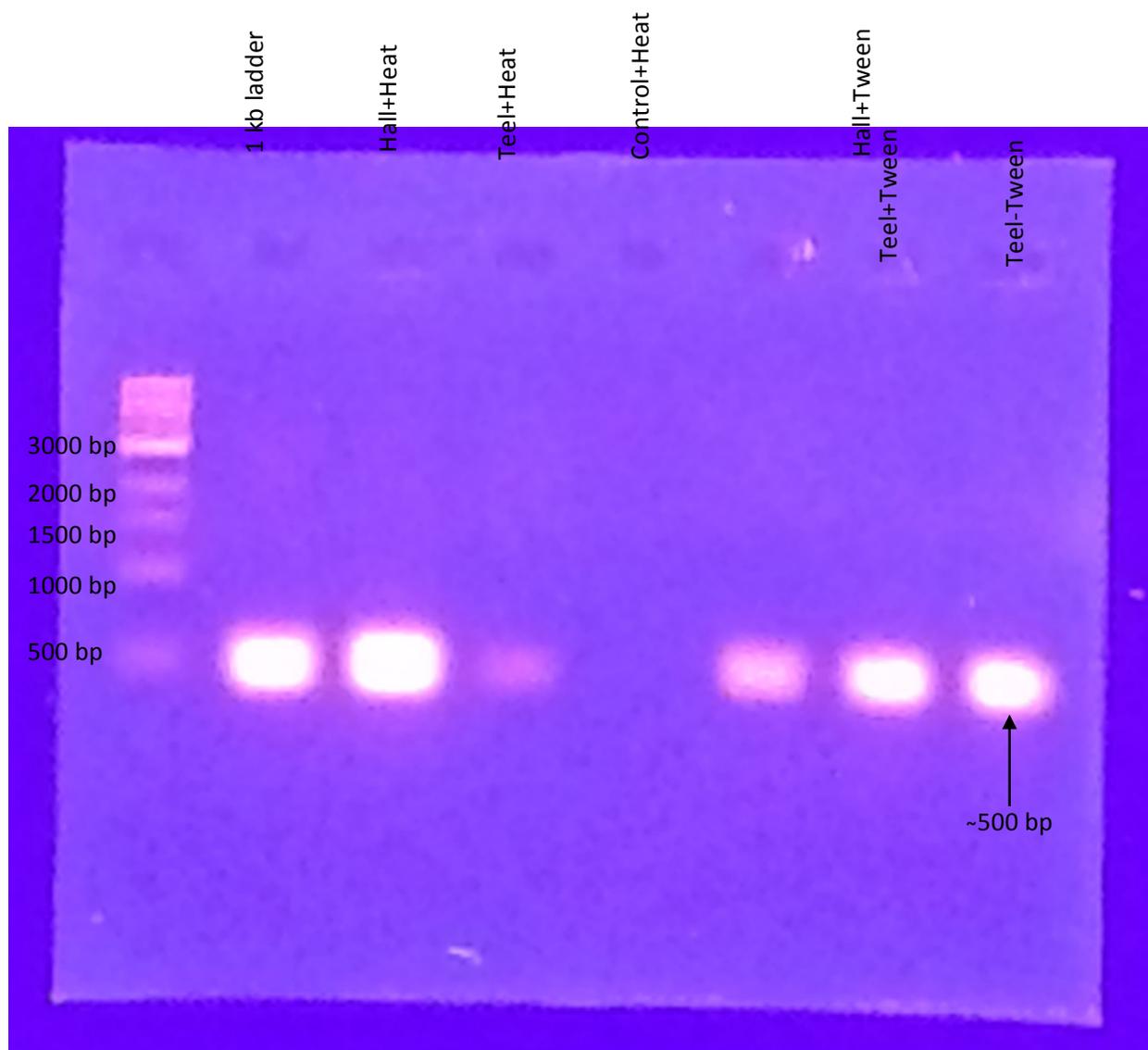
**Figure 9:** This figure shows the gel results from the addition of tween. There is lots of DNA for the Hall bacteria as well as for the Teel bacteria meaning it did not negatively affect the Teel bacteria. This gels also shows that primer set 1 (PS1) did not work, but primer set 2 (PS2) still worked.



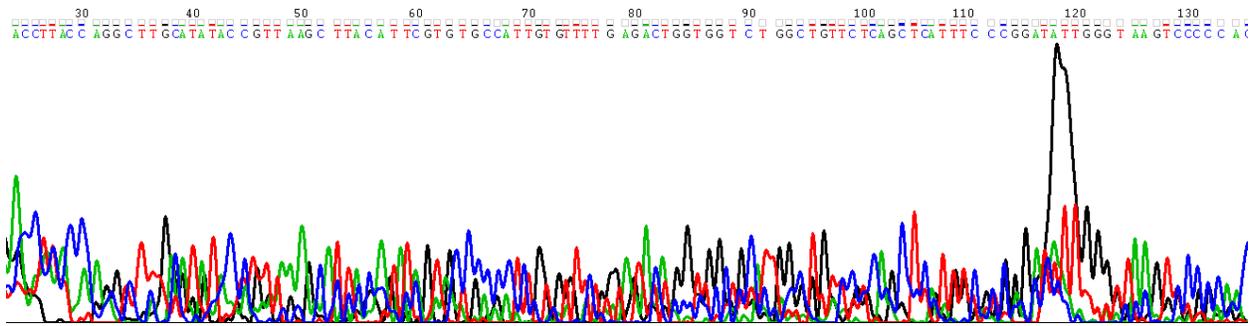
**Figure 10:** This figure shows the gel from the various dilutions on the Hall bacteria. None of the dilutions made a difference. There is hardly any DNA present in any of the lanes. However, the Teel bacteria continues to show lots of DNA. The control contained no bacteria, and showed no DNA, as expected.



**Figure 11:** This figure shows the results of the second round of PCR reactions for the longer preheat time and the addition of tween. The PCR beads were still cold, and that could account for why the bands are not as bright.



**Figure 12:** This figure shows the gels results from the third round of PCR reactions for the longer preheat time and the addition of tween. Primer set 2 was used because there was not enough 8F, 1492R primer mix to complete the set of reactions. However, a large amount of DNA is seen in both of the Hall bacteria samples.



**Figure 12:** This figure is part of one the chromatograms for the Hall bacterium. This is the visual representation of how the sequence is written.

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