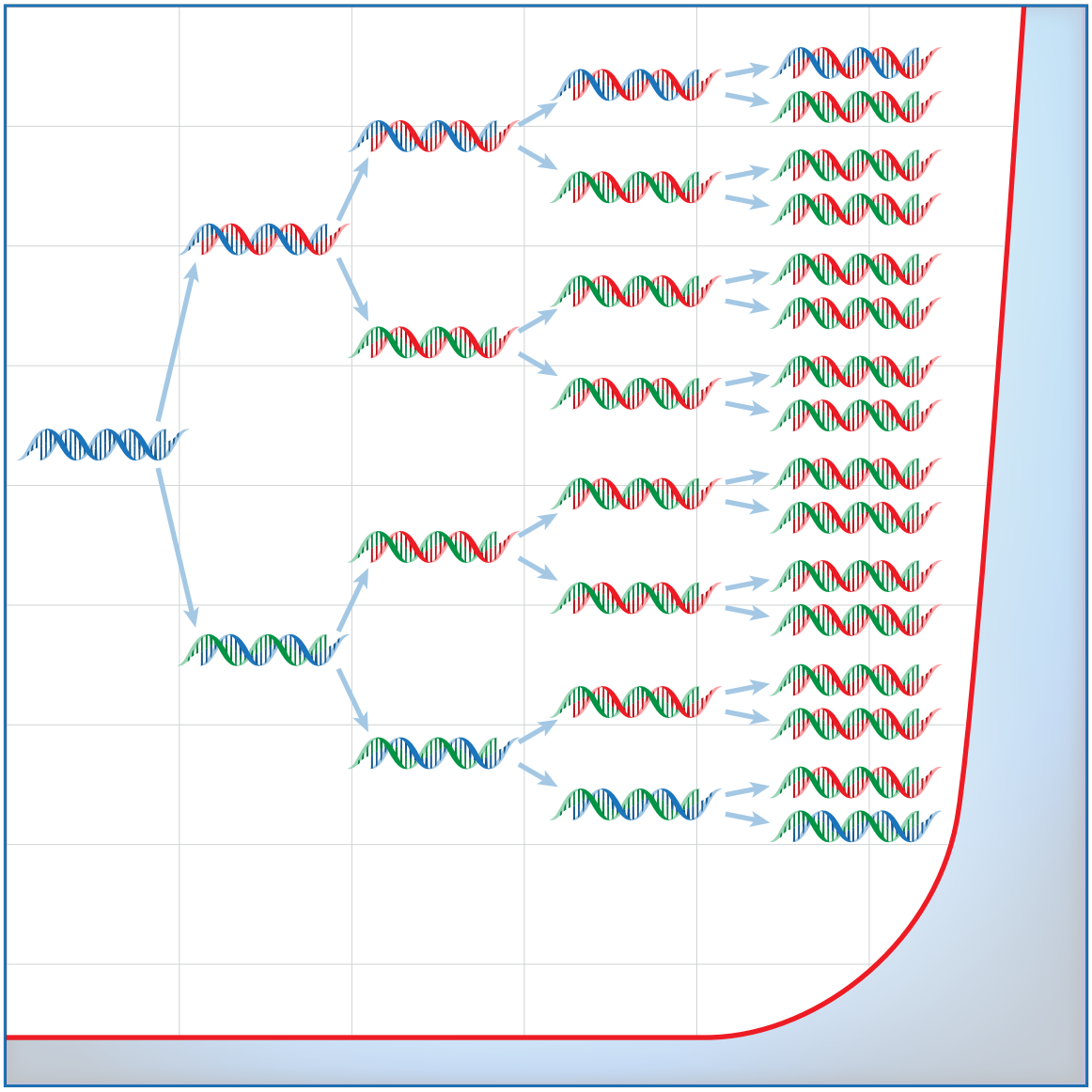
miniOne® SYSTEMS



# Cat# M6005: PCR Cycle Number Analysis
# Student's Guide v031219

## Table of Contents



**Objectives:** How does polymerase chain reaction (PCR) create billions of copies of a piece of DNA in only a few cycles? The mechanism of PCR copies each DNA fragment with every cycle leading to exponential doubling of the number of fragments over time. In this hands-on MiniLab you will gain an intuitive appreciation for the power of exponential amplification by setting up PCR reactions and analyzing the products after a variable number of cycles. You will estimate the minimum number of cycles needed to detect a PCR product on an agarose gel.

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## Laboratory Safety

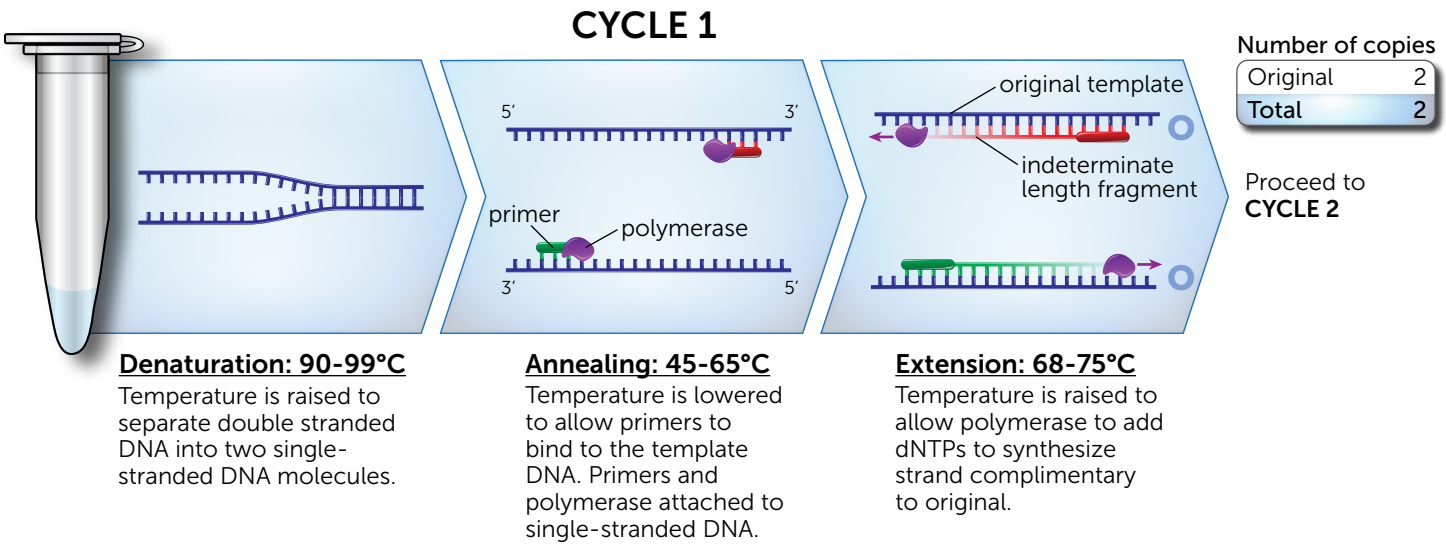
- Wear lab coats, gloves, and eye protection as required by district protocol.
- Use caution when using all electrical equipment such as PCR machines and electrophoresis units.
- PCR machines have surfaces that can be extremely hot. Use caution when opening and closing the lid and when placing and removing tubes.
- Heating and pouring molten agarose is a splash hazard. Use caution when handling hot liquids. Wear eye protection and gloves to prevent burns.
- Wash your hands thoroughly after handling biological materials and chemicals.

## Introduction

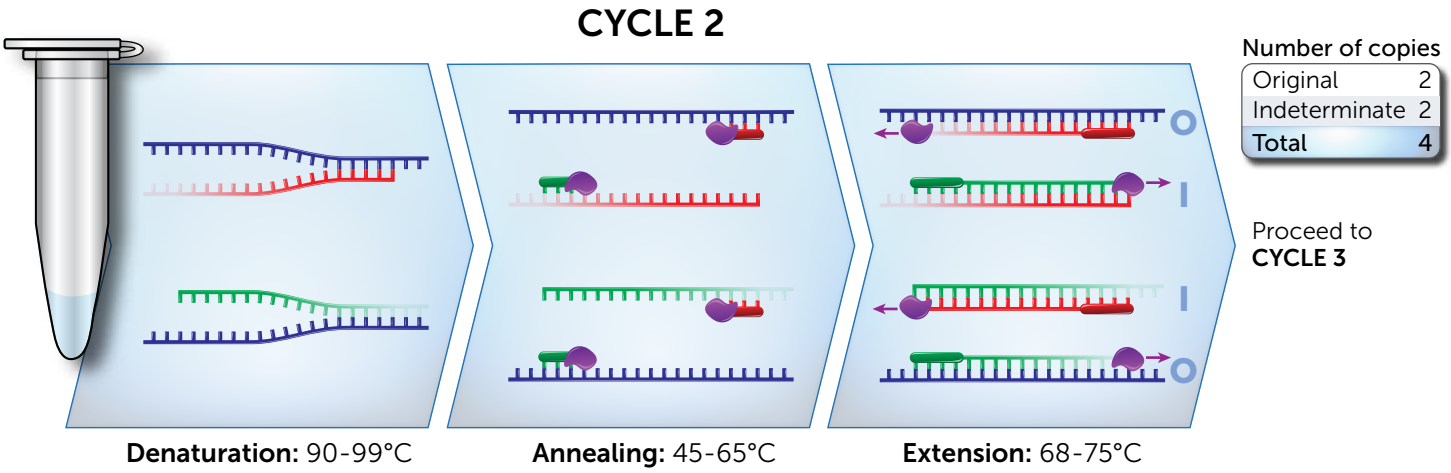
How do scientists determine the identity of a suspect from a single drop of blood at a crime scene? How can clinicians find a single base-pair mutation among the three billion base pairs of the human genome? Polymerase Chain Reaction (PCR), one of the most essential and ubiquitous techniques in modern molecular biology allows scientists to target and copy specific regions of the genome. PCR has revolutionized fields such as forensics, medical diagnostics, genetics, and biotechnology. Techniques based on PCR are at the heart of many advanced sequencing technologies.

In your previous lessons you have learned the mechanism of how PCR is used to copy DNA in a test tube using a few simple reagents and primers specifically designed to target a gene of interest. For a review of the history, biochemical mechanism, and applications of PCR, see **Appendix A**. In this lab, we will look in detail at the exponential nature of PCR amplification by analyzing the products of a PCR reaction as a function of cycle number. Exponential amplification is the key to understanding why PCR is so effective at producing billions of copies of a target in a short period of time.

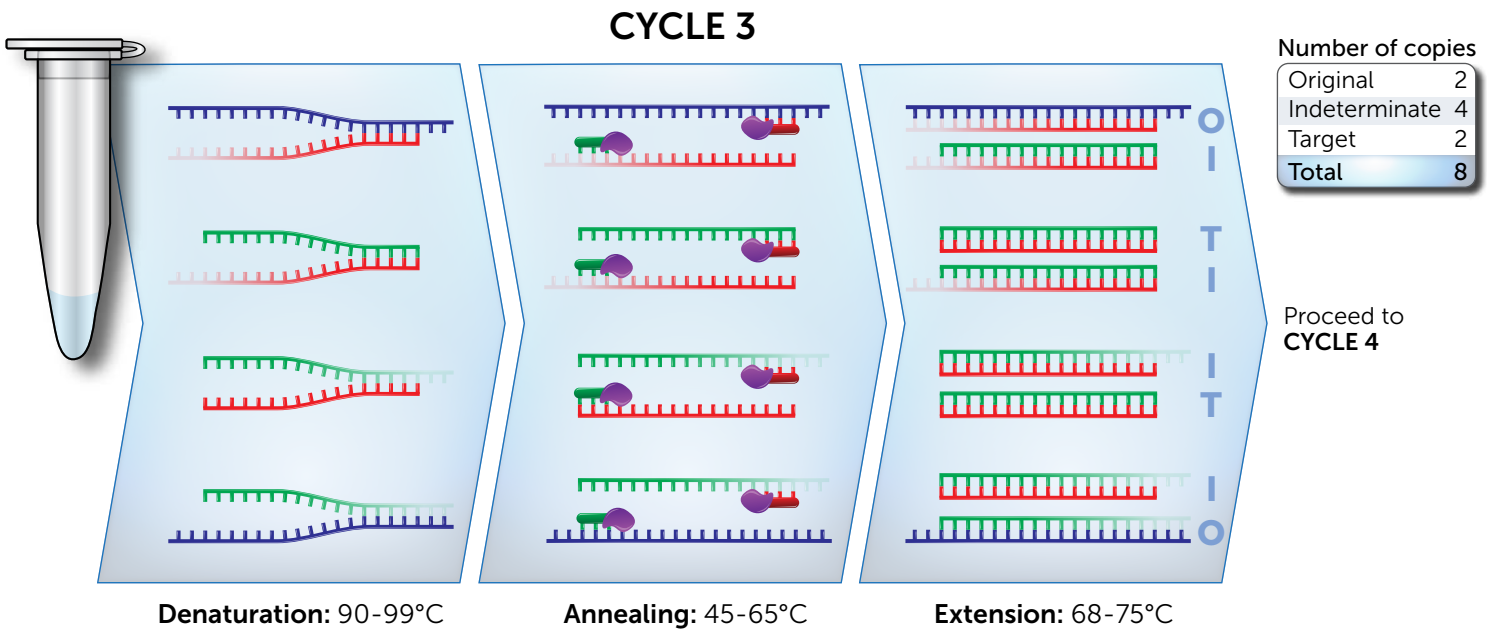
Let's start with the first cycle. The details of complementary base pairing and strand directionality are left out here for simplicity. Following the cycle through denaturation, annealing, and extension you can see that starting with one double-stranded piece of DNA, you will have two copies by the end of the first cycle. We will call these "copies of the original" because the original strands are still part of their structure. Note that we do not know how long the newly copied strand will be. The polymerase will extend the strand until the temperature is raised for the next cycle's denaturation. There is nothing to tell it where to stop.



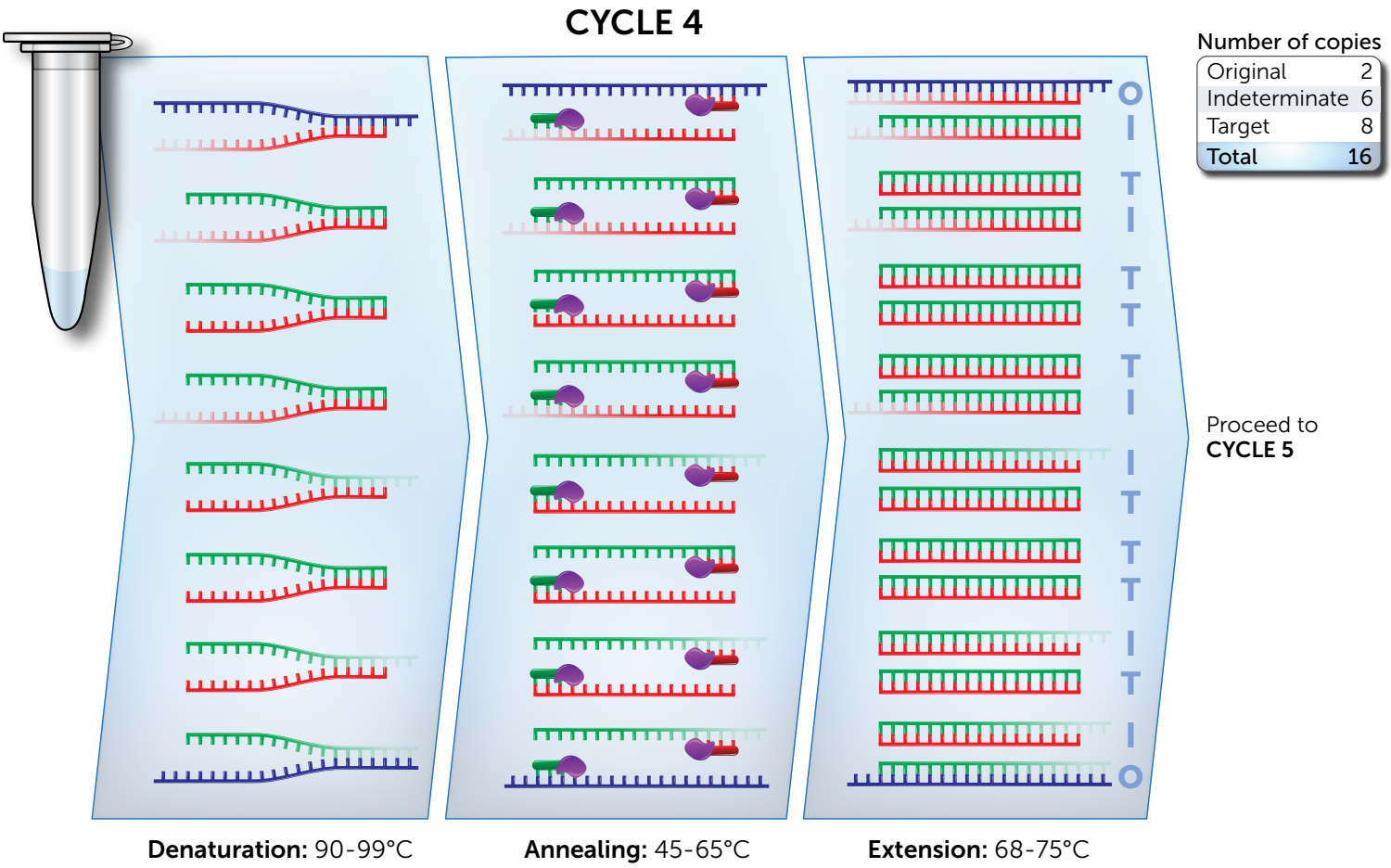
The second cycle begins when the temperature is again raised to 94°C. During denaturation, the two double-stranded fragments are separated into four single-stranded fragments. Each of these fragments is a template for copying during the extension step. Since two of these single strands are the original template they will be copied in the same way as in the first cycle. The copies produced in the first cycle will also serve as templates, but since we don't know how long they are to begin with we will call them "indeterminate length fragments".



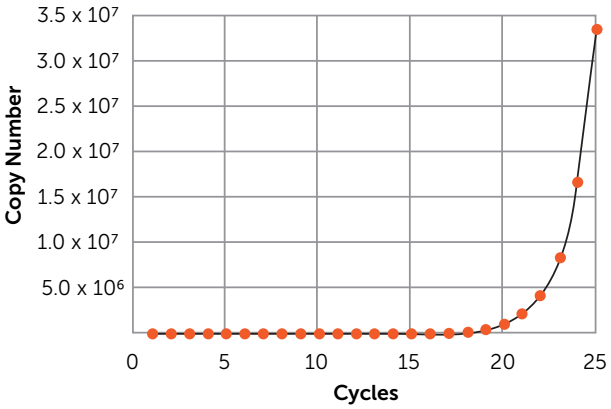

In the third cycle something new happens. Again, the original sequences are copied, the fragments of indeterminate length are copied, but now the sequences that were copied from the fragments of indeterminate length are themselves copied producing two fragments that are bracketed on both ends by the primers. We know exactly how long these fragments are- this is the target sequence that we designed the primers for. After the third cycle we have eight fragments in total, but only two of them are the target fragment.



Watch what happens after cycle four. Now we have two copies of the original, six fragments of indeterminate length and eight of target fragments! Even though the target didn't appear until the third cycle, it's increasing faster than the other two.




The PCR protocol will continue for another 20-30 cycles, but we are not going to draw all the products because it would quickly fill up multiple pages. Instead, we can try to spot a pattern in the relationship between cycle number and number of copies and use this relationship to determine the number of copies after any cycle number. We have plotted the total number of copies and the number of target length copies on the graph below:



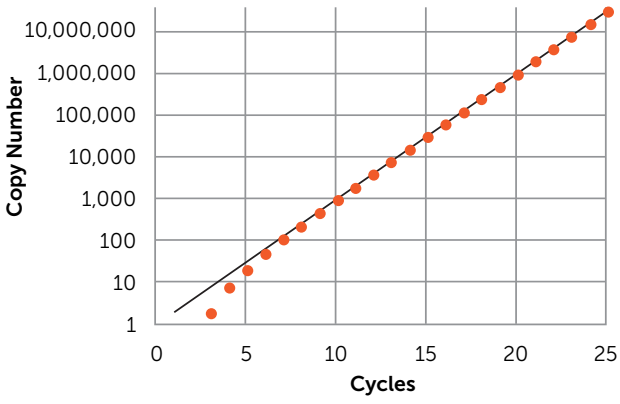



Table 1. DNA amplification over 25 PCR cycles

| Cycles | Total copies | Target copies |
|---|---|---|
| 1 | 2 | 0 |
| 2 | 4 | 0 |
| 3 | 8 | 2 |
| 4 | 16 | 8 |
| 5 | 32 | 22 |
| 6 | 64 | 52 |
| 7 | 128 | 114 |
| 8 | 256 | 240 |
| 9 | 512 | 494 |
| 10 | 1,024 | 1,004 |
| 11 | 2,048 | 2,026 |
| 12 | 4,096 | 4,072 |
| 13 | 8,192 | 8,166 |
| 14 | 16,384 | 16,356 |
| 15 | 32,768 | 32,738 |
| 16 | 65,536 | 65,504 |
| 17 | 131,072 | 131,038 |
| 18 | 262,144 | 262,108 |
| 19 | 524,288 | 524,250 |
| 20 | 1,048,576 | 1,048,536 |
| 21 | 2,097,152 | 2,097,110 |
| 22 | 4,194,304 | 4,194,260 |
| 23 | 8,388,608 | 8,388,562 |
| 24 | 16,777,216 | 16,777,168 |
| 25 | 33,554,432 | 33,554,382 |

In the left panel the number of copies over 25 cycles is plotted on a linear scale, and in the right panel the number of copies is plotted on a semi-log scale. The solid black line represents the total number of copies and the orange dots represents the number of target-length copies. Numbers used to generate these graphs are shown in **Table 1**.

Note that in the scenario we have just described we are starting with a single copy of template DNA. In most experiments you will start with hundreds or thousands of copies.

**CONSIDER THIS:** How will the number of fragments after a specific cycle change when you start with 4 times as much template DNA? What about 10 times as much DNA?

Understanding the mechanism of PCR amplification and the number of copies produced by a cycle is interesting on its own, but it also has practical implications for how we design PCR protocols. For many molecular assays the result is a band visualized on an agarose gel. The brightness of a band is proportional to the quantity of DNA in the band. There is a minimum brightness needed to detect the band with your eye or with a camera, and therefore a minimum number of DNA fragments needed to visualize a band. This is called the **detection limit**. The detection limit is a property of the dye and illuminator we were using to visualize the DNA. Note that you cannot see DNA directly. We are using fluorescence of GelGreen™, a dye that binds to DNA, as a reporter for DNA quantity. In a PCR reaction, the minimum number of fragments corresponds to a minimum number of cycles, a critical parameter to know when you are designing a PCR assay.

In this lab you will estimate the minimum number of cycles needed to see a band for a particular PCR reaction. The PCR reaction amplifies a section of the genome of bacteriophage lambda, a classic model system for molecular biology. **You will run the reaction for 10, 15, 20, 25, and 30 cycles, then visualize the results on an agarose gel.**

## Pre-lab questions

1. A certain species of bacteria divides every minute. Starting with one cell, after one minute it will have divided into two, and after two minutes each of those cells will have divided again for a total of four. If I have a petri dish that is half covered with bacteria, how long will I have to wait for the dish to be completely covered with bacteria?

2. Exponential phenomena are extremely common in subjects you will study in school and encounter in your daily life. Use the chart below to write down an example of exponential growth for each of the subjects below. Share your list with your lab partner or lab group.

| Subject | Example of exponential growth |
|---|---|
| Ecology/Environmental Science | |
| Business/Economics | |
| Medicine | |
| Physics | |

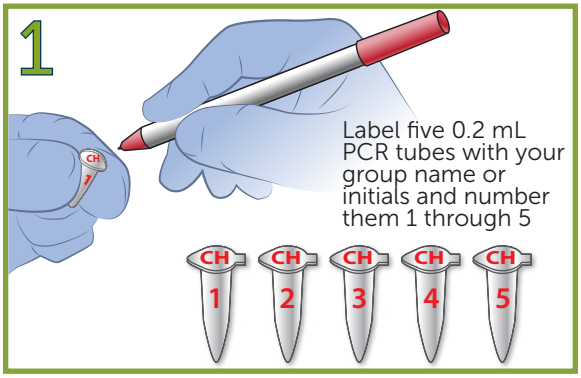
3. You have run two identical PCR reactions both with the same template, primers, and polymerase, and each reaction produced a single DNA band. One reaction was run for twenty PCR cycles and the other is run for thirty PCR cycles. Which DNA will band will travel farther on an agarose gel?

4. You and your lab partner set up two identical PCR reactions to run for 20 and 30 cycles. However, your lab partner forgot to record which tube was removed after 20 cycles and which after 30. Using gel electrophoresis, how can you tell the difference?

# Experimental Procedures

## Day 1: Set up and run your PCR amplification

### 1




Label five 0.2 mL PCR tubes with your group name or initials and number them 1 through 5

### 2

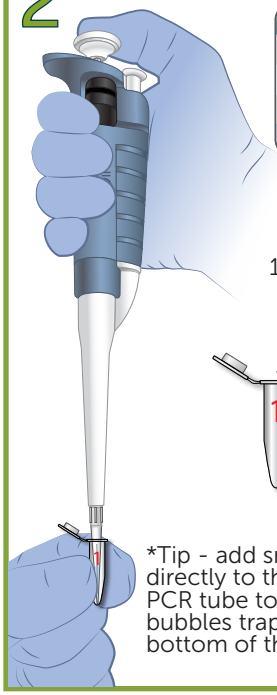
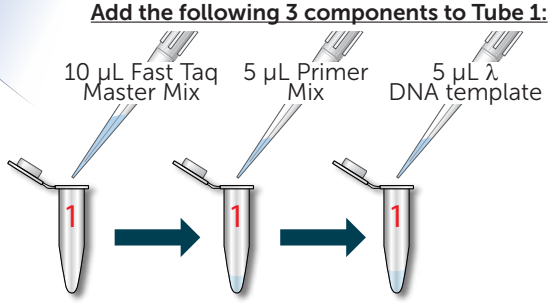


**Table 2. PCR Setup**

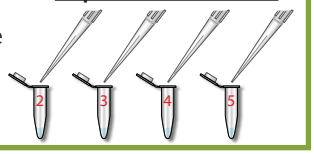
| Reagent | Volume |
|---|---|
| Fast Taq Master Mix | 10 µL |
| Primer mix (forward and reverse) | 5 µL |
| λ DNA template | 5 µL |
| **Total** | **20 µL** |

**Add the following 3 components to Tube 1:**

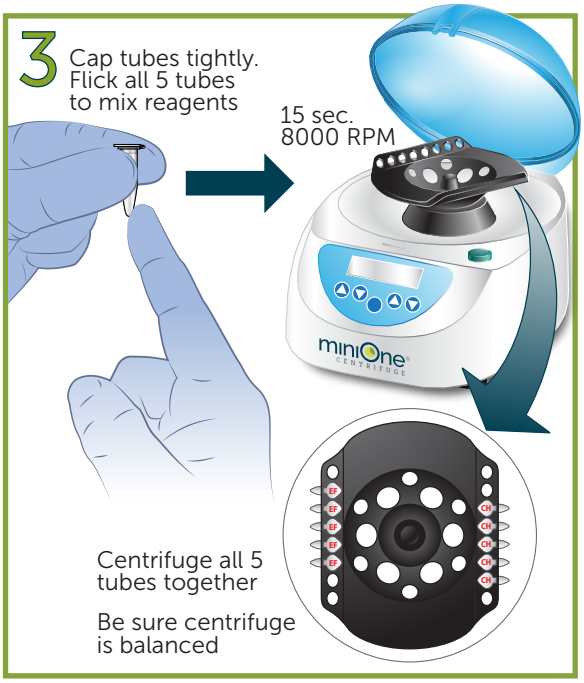



*Tip - add small volumes directly to the bottom of the PCR tube to avoid having bubbles trapped at the bottom of the tube.

**Repeat for Tubes 2-5**




### 3



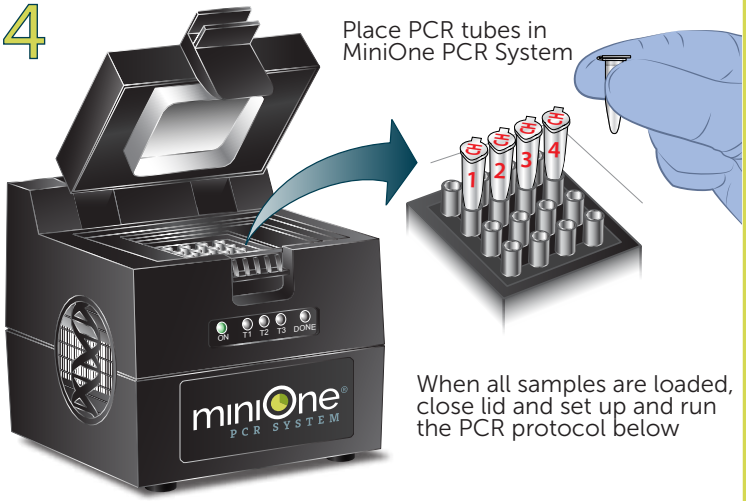

Cap tubes tightly. Flick all 5 tubes to mix reagents

15 sec.
8000 RPM

Centrifuge all 5 tubes together

Be sure centrifuge is balanced

### 4




Place PCR tubes in MiniOne PCR System

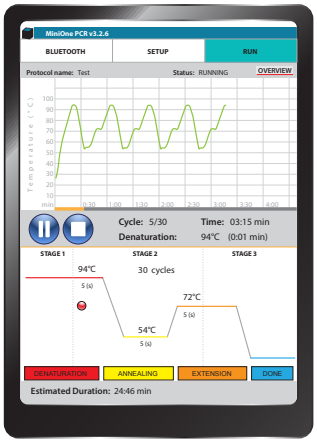
When all samples are loaded, close lid and set up and run the PCR protocol below

**Table 3. Cycling protocol for fragment amplification**

| Step | Duration | Temperature | Cycles |
|---|---|---|---|
| Denaturation | 5 sec | 94°C | |
| Annealing | 5 sec | 54°C | 30 |
| Extension | 5 sec | 72°C | |
| Final incubation | ∞ | 4°C | |

### 5

**Set a timer to remind you to take your tubes out at cycles 10, 15, 20, 25 and 30.**




Use the Mobile App to monitor the progress of the PCR reaction

Tap the **"Pause"** button when you want to remove a sample

**Wait until "Status: Paused" appears** above the graph before opening the lid and removing your sample.(See Appendix D)

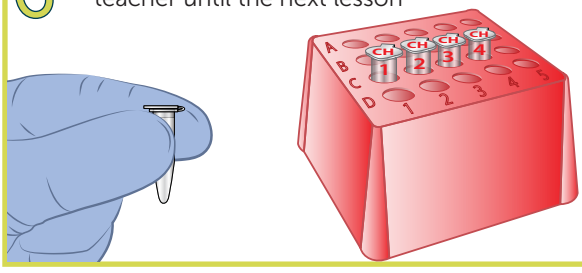
Record which tube was removed after which cycle

**Table 4. Cycle number record**

| Tube label | Number of Cycles |
|---|---|
| | 10 |
| | 15 |
| | 20 |
| | 25 |
| | 30 |

### 6

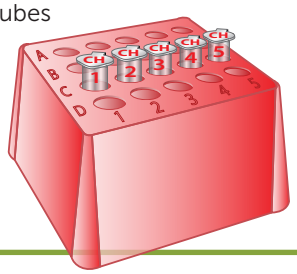
Give your samples to your teacher until the next lesson



# Experimental Procedures

Day 2: Visualize your results with gel electrophoresis

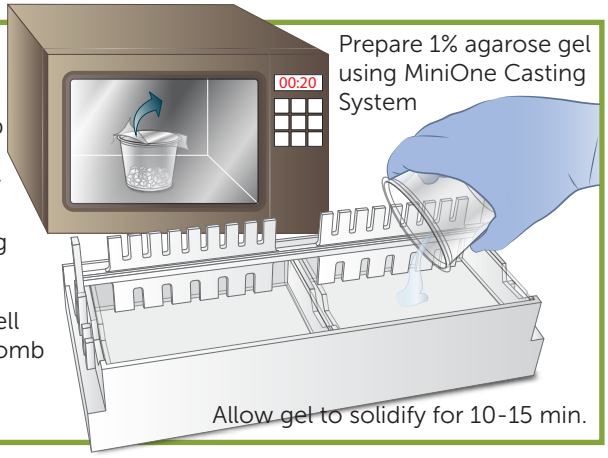
**1** Retrieve your PCR tubes



**2** Vent gel cup by peeling back slightly prior to microwaving

Prepare 1% agarose gel using MiniOne Casting System

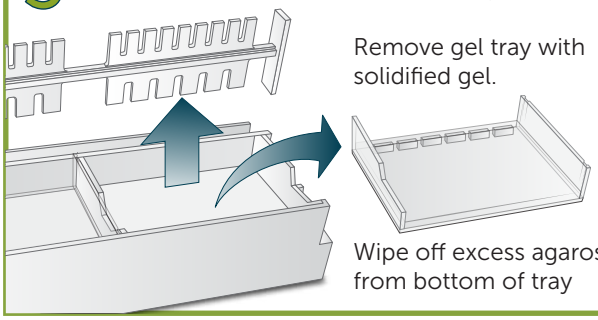
Use 6-well side of comb




Allow gel to solidify for 10-15 min.

**3** Carefully remove comb from gel.

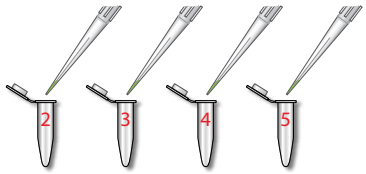
Remove gel tray with solidified gel.



Wipe off excess agarose from bottom of tray

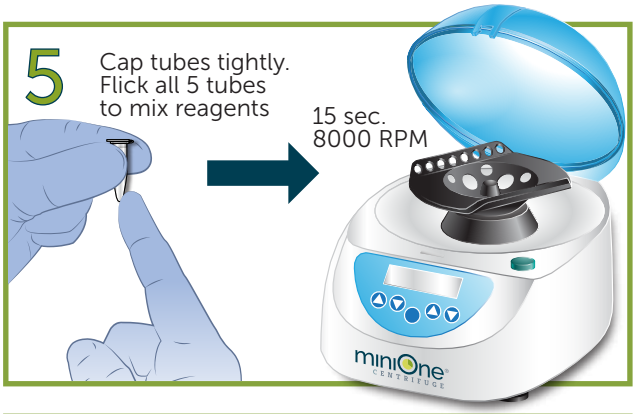
**4** 5 µL 5X Loading Dye


**Repeat for Tubes 2-5**




**5** Cap tubes tightly. Flick all 5 tubes to mix reagents

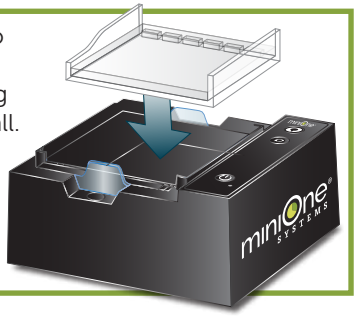
15 sec. 8000 RPM




**6** Connect the power supply to the back of the MiniOne Electrophoresis carriage. Plug the power supply into the wall.

Place gel and gel tray into tank

Make sure wells are aligned with marks on black viewing platform on negative end



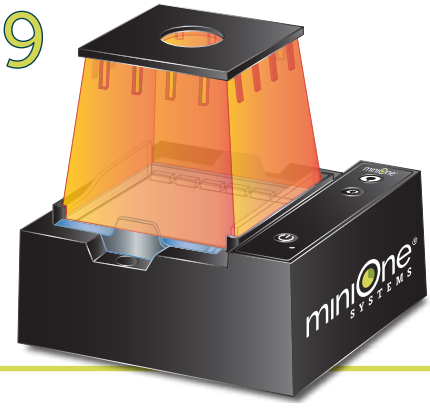

**7** Add 135 mL TBE running buffer


**8** Load 10 µL of each sample according to Table 5


**Table 5. Record the sample loaded into each lane**

| Well | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Sample | Marker | 10 cycles | 15 cycles | 20 cycles | 25 cycles | 30 cycles |

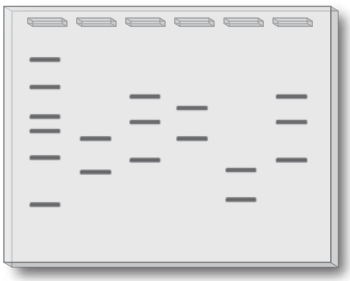
**9**




Place orange photo hood on carriage and press power button to start run

Run gel for 20 min.

**10** Document gels and discuss results with your class



## Gel Analysis Worksheet

**Directions:** After completing the gel electrophoresis portion this MiniLab, record an image of your gel and draw the results or tape the image on the template below:

Lane 1: _____

Lane 2: _____

Lane 3: _____

Lane 4: _____

Lane 5: _____

Lane 6: _____

## Post-lab Analysis Questions

1. Label the bands in the marker lane. The sizes of the bands in the marker lane are 100, 300, 500, 1000, and 2000 base pairs (bp). Compare the DNA fragment from each of your reactions to the bands in the marker lane to estimate its size.

2. In which of your samples can you see a band? How many PCR cycles was this sample run? From this number, estimate the minimum number of cycles needed to see a band.

3. Judging the relative brightness of bands with your eye can be tricky, but it's good enough for some rough observations. Comparing two bands with a difference of five cycles, how would you describe the relative intensity of the fluorescence? Is the brighter one five times brighter? Or more than five times brighter?

4. Based on your knowledge of how copy number increases with cycle number, how much brighter would you expect a 20-cycle band to be relative to a 15-cycle band? Do you think this difference will be observed in reality?

5. If you kept running the experiment through several more cycles, removing tubes after 30, 35, 45 cycles and so on, do you think that the bands would continue to get brighter indefinitely? Why or why not?

6. The team that developed the MiniOne Electrophoresis System determined that the minimum quantity of DNA needed to see a band is 10 nanograms. If you wanted to calculate the minimum cycle number needed to produce enough DNA to see a band (instead of testing it with an experiment), what additional information would you need?

7. In the introduction we followed a scenario where a single starting copy of DNA is used as the PCR template. In this reaction (and in most PCR reactions you will encounter), you will be starting with hundreds or thousands of copies. How would your gel look different if you had been starting with only one copy of the lambda genome?

8. Challenge Question: After you pressed the pause button on the MiniOne PCR mobile app, the pause did not begin until the end of the extension step. What would happen if you removed the tubes during the denaturation step?

Appendix A: Glossary

| | |
|---|---|
| Anealing | As the temperature of a PCR reaction is lowered, short pieces of DNA, called primers, bind to specific sequences within the genome targeting this region to be copied. Annealing temperature is specific to the primers used in your reaction- typical settings are 45-65°C for 5-30 seconds. |
| Buffer | An ionic compound added to aqueous solution that helps maintain a consistent pH. Buffers are essential in PCR because the DNA polymerase's function is sensitive to pH changes. A running buffer is also used in electrophoresis to conduct electricity between the electrodes. |
| Cycle | A cycle refers to one round of denaturation, annealing, and extension steps of the PCR reaction. The number of cycles needed for a particular reaction will depend on how much DNA you are starting out with and how much DNA you are trying to produce. With high starting concentration, 20-25 cycles are sufficient to produce enough DNA to visualize on a gel. Where the starting concentration is low or large quantities of product are needed, 35-40 cycles can be used. |
| Denaturation | Denaturation uses high temperature to break the hydrogen bonds between bases on opposing DNA strands. Double-stranded DNA is split into single-stranded DNA exposing the bases so they can be copied. Typical settings are 90-98°C for 5-30 seconds. |
| Dependent variable | The dependent variable is a measure of the effect that is being tested or experimented on in a scientific study. |
| Detection limit | The lowest quantity of a substance that will produce a measurement on a given instrument, i.e. the smallest quantity of the substance that can be distinguished from the background. |
| dNTPs | Nucleotides, the molecular building blocks of DNA. |
| Enzyme | An enzyme is a biological catalyst that speeds up a chemical reaction without changing the products or being consumed by the reaction. Most enzymes are proteins and they control a wide range of reactions in cells, from copying DNA to extracting energy from food. |
| Extension | At around 70°C the polymerase gets to work and starts adding nucleotides (dNTPs) to the 3' end of the annealed primers, copying the complementary strand. Typical settings are 72°C for 5 seconds – 5 minutes. |
| Final Extension | In some protocols an additional extension step is used. This ensures that the polymerase can add the final base pairs onto the end of the strands, which is necessary in some applications. The typical duration is 2-10 minutes. |
| Independent variable | The variable that is changed or controlled by the experimenter in a scientific study. |
| Initial denaturation | When copying a piece of genomic DNA, an initial denaturation step is often used to make sure the long strands of DNA are fully separated and freed from bound proteins before thermal cycling begins. Typical settings are 90-96°C for 30 seconds-10 minutes. |
| Lambda phage (λ) | A bacterial virus, or bacteriophage, that infects *E. coli*. Lambda phage is a classic laboratory model system for molecular genetics. |
| Monomer | A molecule that can be bonded with other similar molecules to form a polymer. |
| Polymer | A molecule that consists of many similar units bonded together. |
| Template | DNA containing the sequence that will be copied in a PCR reaction. Can be a short DNA fragment or a whole genome. |
| Thermal Cycler | Also called a PCR machine, a thermal cycler is an instrument that automatically changes the temperature of the PCR reaction according to a program set up by the user. It heats and cools the reaction between denaturation, annealing, and extension temperatures over a specified number of cycles. |

## Appendix B: Polymerase Chain Reaction

Polymerase chain reaction (PCR) addresses two major challenges in molecular biology- producing usable quantities of DNA for analysis and isolating a specific region of the genome. First, the DNA that scientists want to analyze is often collected in extremely small quantities (for example, a single drop of blood from a crime scene). To have usable quantities of DNA we must make many copies using the original DNA as a template. Second, among the whole genome we must find only the part we want to analyze, whether it is a gene that causes a disease or a sequence that can help identify a species. This is no small matter since the human genome is over 3 billion base pairs (bp) long and we are often interested in regions less than 300 bp long.

### The History of PCR

As with many ideas in biotechnology, nature provides us with most of the tools we need. Every time a cell divides it makes two replicates of its entire genome with the help of specialized enzymes. The phenomenon of complementary base pairing should give you a hint as to how a specific region can be targeted. In the late 1970s Frederick Sanger developed a method for copying DNA in vitro that used short pieces of DNA, called primers to initiate replication by a DNA polymerase, similar to the RNA primers in your model of cellular DNA replication. An artificial primer used in the Sanger method has a sequence that allows it to bind at only one location in the target DNA sequence.

Using only one DNA primer, the Sanger method can only produce one copy of the target at a time. In 1983 Kary Mullis proposed a modification to this method where a second primer is used to initiate replication using the first copy as a template. Recall that DNA has an antiparallel structure where one strand runs in the opposite direction relative to the second strand. To use the first copy as a target for replication, the second primer that Mullis added needed to initiate replication in the opposite direction, thus one primer is called the forward primer and the other is called the reverse. With the original sequence and its copy serving as templates, two copies are produced instead of one. Further, these two copies can each be used as templates in a second round of copying, which produces four copies. After each round, or cycle, the number of copies has doubled. Over multiple cycles, billions of copies of the DNA sequence between the two primers are generated. This method is called Polymerase Chain Reaction (PCR) – polymerase because of the enzyme that is used to copy DNA and chain reaction because the products of one cycle serve as templates for the next round of copying. This rapid and efficient technology for generating usable quantities of DNA won Mullis the Nobel Prize in Chemistry. The first application of PCR was a test for sickle cell anemia.

### How PCR Works

Instead of trying to recreate the cell's intricate biochemical machinery in a test tube, scientists rely on heat to control the various steps of the PCR reaction. For DNA to be copied the nucleotide bases must be exposed, which means double-stranded DNA must be separated into single strands. Just as heat applied to an ice cube weakens the hydrogen bonds between water molecules and causes a phase transition to liquid water, heat applied to double-stranded DNA weakens hydrogen bonds between bases causing separation of the strands into single-stranded DNA. As with ice, this is sometimes called melting, but is commonly referred to as denaturation. In a PCR cycle, denaturation is performed at 90-98°C for 5-30 seconds. This temperature is just below the boiling point of water.

Once the template DNA has been separated into single strands, the temperature is lowered to allow primers to bind to their target sequences. Just as in the water analogy, lower temperature favors the formation of hydrogen bonds between molecules, in this case the primer and the template DNA. This step, called annealing, is performed at 45-65°C for 5-30 seconds. The ideal temperature and duration of the annealing step depends on the sequences of the primers being used. It must be low enough that hydrogen bonds are able to form between the primer and specific complementary sequence, but not so low that non-specific, or random, binding between primer and template occurs.

DNA polymerase binds to the primer-template complex and begins to add nucleotides (dNTPs) onto the 3' end of the primer. This step, called extension, results in a new DNA copy attached to the template as double-strand-

ed DNA. The duration of the extension step depends on the length of the DNA segment being copied and can be anywhere from 5 seconds to 5 minutes.

At this point you may have noticed a problem – DNA polymerase, which the entire PCR process relies on, is a protein enzyme that needs a very specific three-dimensional structure to copy DNA. Before getting to the extension step, the entire PCR mixture, including the polymerase, has already been through the denaturation step where the reaction was heated almost to boiling. Anyone who has cracked an egg into a frying pan will know what high heat does to proteins!

In the early days of PCR, fresh polymerase was added to the reaction tube every cycle to replace the polymerase that had been destroyed by the denaturation step. Having to open the tube and add new enzyme for up to 30 cycles was expensive, inefficient, and increased the chances of contaminating the reaction. The innovation that would remove these obstacles came from an unusual source – Yellowstone National Park.

In the 1970s, scientists had isolated a species of bacteria called *Thermus aquaticus* from a hot spring in Yellowstone. *T. aquaticus* thrives at 75-80°C and can survive much higher temperatures. Its enzymes are similarly heat-tolerant. *E. coli*, the original source for the polymerase used in PCR, thrives at 37°C, the same temperature as the gut of mammals. Since the biochemistry of DNA is similar across the tree of life, polymerase from *T. aquaticus* (called Taq Polymerase) can replace *E. coli* polymerase in PCR, with the modification that the extension step is performed at 70-75°C.

This innovation led to PCR becoming the ubiquitous, inexpensive, and efficient tool that it is today. Reactions can be set up once, sealed inside a tube, and placed in an automated thermal cycler. The thermal cycler (or PCR machine) is a specialized instrument that accurately and rapidly changes the temperature of the tube between denaturation, annealing, and extension. After 20-40 cycles of these three temperatures, billions of copies of the desired DNA product can be produced, often in less than 1-2 hours. The amplified DNA can be analyzed with gel electrophoresis at the end of the experiment or detected as they are being formed using more advanced equipment.

## Appendix C: Gel Electrophoresis

Gel electrophoresis is a technique used in many areas of science to separate and analyze the components of complex chemical mixtures. Mixtures of DNA, RNA, proteins, or dyes can be separated into their individual components based on molecular size and electrical charge using a separation matrix within an electric field.

The gel used in gel electrophoresis is a tangle of polymers forming a three-dimensional matrix with water-filled pores through which molecules migrate. A higher density of polymers creates smaller pores. Like the holes in a sieve or colander, the size of the pores has to be the appropriate size for the molecules being separated. Gels can be made from different substances depending on the application. One of the most commonly used and effective materials is agarose, a polymer extracted from seaweed. Agarose gels are formed (or cast) by pouring molten (melted) agarose into a tray where it solidifies into the desired shape as it cools. A comb is placed while the agarose is molten and then removed after the agarose gel is solidified. This creates wells into which the samples are loaded.

After the gel solidifies it is placed in an electrically conductive buffer between parallel positive (+ anode) and negative (- cathode) electrodes. A voltage is applied between the electrodes, creating a uniform electric field within the gel. Molecules in the wells begin to move under the influence of the electric field: positively charged molecules migrate toward the cathode and negatively charged molecules migrate toward the anode.

The speed of a molecule's movement in an electric field is determined by the strength of its electric charge relative to its molecular weight. This is quantified as the charge to mass ratio. Speed of movement within a gel is also influenced by the size of the molecule relative to the pores in the gel. The polymers in the gel are like an

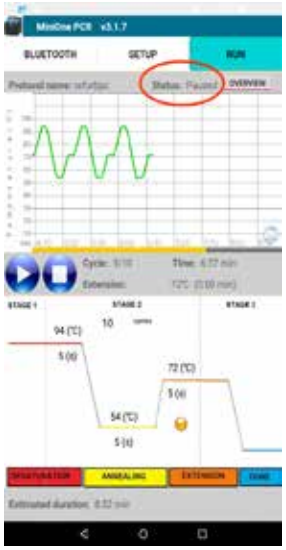
obstacle course: smaller molecules maneuver easily through the pores, traveling faster and farther than large, bulky molecules. However, a large molecule can move faster through a gel than a smaller molecule when the strength of its charge relative to its mass is significantly higher. Shape can also affect how a molecule moves through the gel. Long spaghetti-like molecules will move slower than compact molecules, which slip easily through the pores. Molecules of the same size, shape, and charge will move together and form a distinct band. If multiple types or sizes of molecules are present in the sample, they will separate from each other and each will form a distinct band.

## Appendix D: PCR App Pause Screenshots

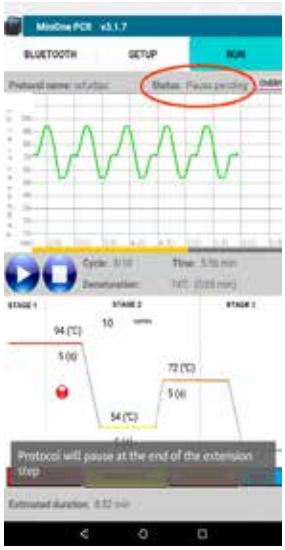
**NOTE:** If you accidentally hit the STOP button instead of PAUSE, a warning asking if you really want to abort the run will be displayed. Press "NO" and you will return to your protocol, and from there you can select the PAUSE button.

### Amazon Fire

Paused

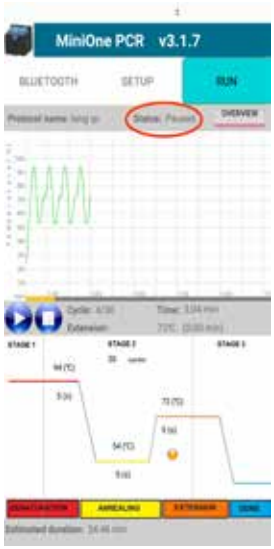


Pause Pending




### Android Phone

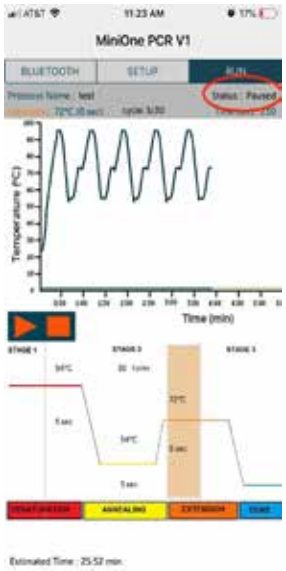
Paused




Pause Pending


### iPhone

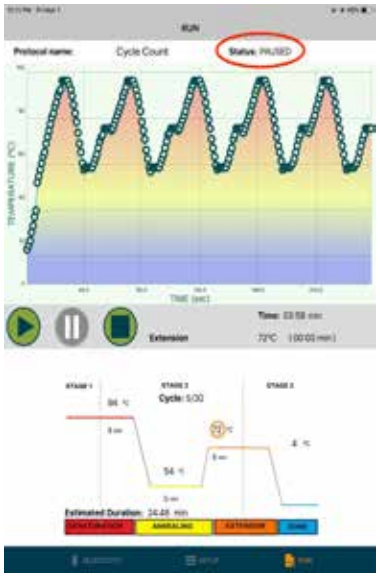
Paused




Pause Pending


### iPad

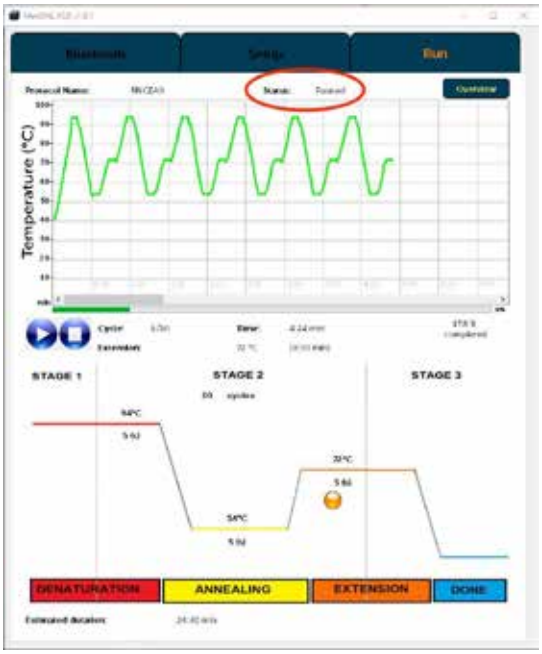
Paused



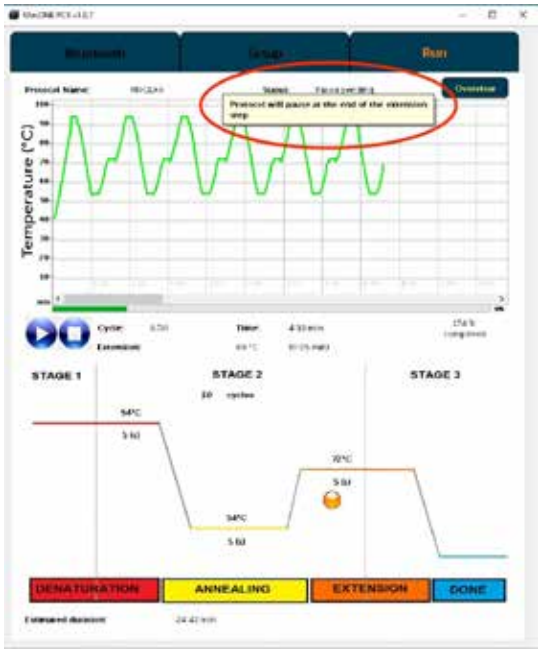
Pause Pending

PC

Paused




Pause Pending




## Appendix E: Recommended Reading

Scitable by Nature Education: Useful resource for a variety of topics in molecular biology.
https://www.nature.com/scitable/content/gel-electrophoresis-is-a-laboratory-technique-used-116897192
https://www.nature.com/scitable/content/replication-7689643

DNA electrophoresis sample loading: Quick video from Kirkwood Community College shows how to load a DNA horizontal electrophoresis gel. Shows proper technique and some common mistakes.
https://www.youtube.com/watch?v=tTj8p05jAFM

Play the DNA – the Double Helix Game: In this game your job is to first make exact copies of a double-stranded DNA molecule by correctly matching base pairs to each strand, and to then determine which organism the DNA belongs to.
http://www.nobelprize.org/educational/medicine/dna_double_helix/index.html

DNA Learning Center: an animation illustrating exponential amplification and the mechanism of PCR:
https://www.dnalc.org/resources/animations/pcr.html

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