Student Manual

Background

With the world population exploding and farmable land disappearing, agricultural specialists are concerned about the world's ability to produce enough food to feed the growing population. Environmentalists are concerned about the overuse of pesticides and herbicides and the long-term effects of these chemicals on the environment and human health. Might there be a solution to both of these problems? The biotechnology industry thinks so. Its proponents believe genetically modified organisms (GMOs), particularly genetically modified (GM) crop plants, can solve both problems. This proposed solution, however, has met with great opposition throughout the world. Dubbed "frankenfoods" by opponents and restricted in most European countries, GMOs are widely produced and sold in the United States. Currently in the US, foods that contain GMOs do not have to be labeled as such.

Genetic manipulation of crop plants is not new. Farmers have been genetically modifying crops for centuries and crop breeding to encourage specific traits, such as high yield, is still an important part of agriculture today. However, there is now the option to place genes for selected traits directly into crop plants. These genes do not have to originate from the same plant species—in fact, they do not have to come from plants at all. One popular class of GM crops has a gene from the soil bacterium *Bacillus thuringiensis* (Bt) inserted into their genomes. Bt crops produce a protein called delta-endotoxin that is lethal to European corn borers, a common pest on corn plants. Farmers who plant Bt crops do not have to apply pesticide because the plants produce the toxic protein inside their cells. When the corn borers feed on the genetically modified plant, they die. Other GMOs include those that are herbicide-resistant delayed for fruit ripening, are resistant to fungi or drought, have increased crop yield, or bear improved fruits.

Many people object to the use of GM crop plants. They argue that there is a potential to create super-weeds through cross-pollination with herbicide-resistant crops or that super-bugs will evolve that are no longer resistant to the toxins in pest-resistant crops. Many are concerned with potential allergic reactions to the novel proteins or antibiotic resistance arising from the selectable markers used to develop the crops or other unforeseen effects on public health. Proponents of GM foods argue these crops are actually better for the environment. Fewer toxic chemicals are put into the environment and thus fewer toxic chemicals can harm the environment and human health. In addition, these crops can preserve arable land by reducing stresses on the land, improve the nutritional value of food in developing countries, and allow crops to be grown on previously unfarmable land.

Whatever position one takes in the GMO debate, it would be beneficial to be able to test foods found in the grocery store for the presence of GMO-derived products. This can be done in several ways. One would be to use an antibody-based test such as the enzyme-linked immunosorbent assay (ELISA), which can detect the proteins that are produced specifically by GM crops. However, the ELISA is not useful for testing foods that have been highly processed, because the proteins have most likely been destroyed and different GM foods produce different proteins. Another method is to use the polymerase chain reaction (PCR) to look for a DNA sequence common to GM foods. DNA is more resistant than proteins to processing and can be extracted from even highly processed foods. It is these GMO DNA sequences that we will be testing for in this laboratory.

In the first lesson you will extract genomic DNA from food samples, in the second lab you will run PCR reactions to amplify GMO and natural plant sequences from the DNA, and in the third lab you will electrophorese the amplified samples to visualize the DNA.

Let's see if your favorite food contains GMOs!

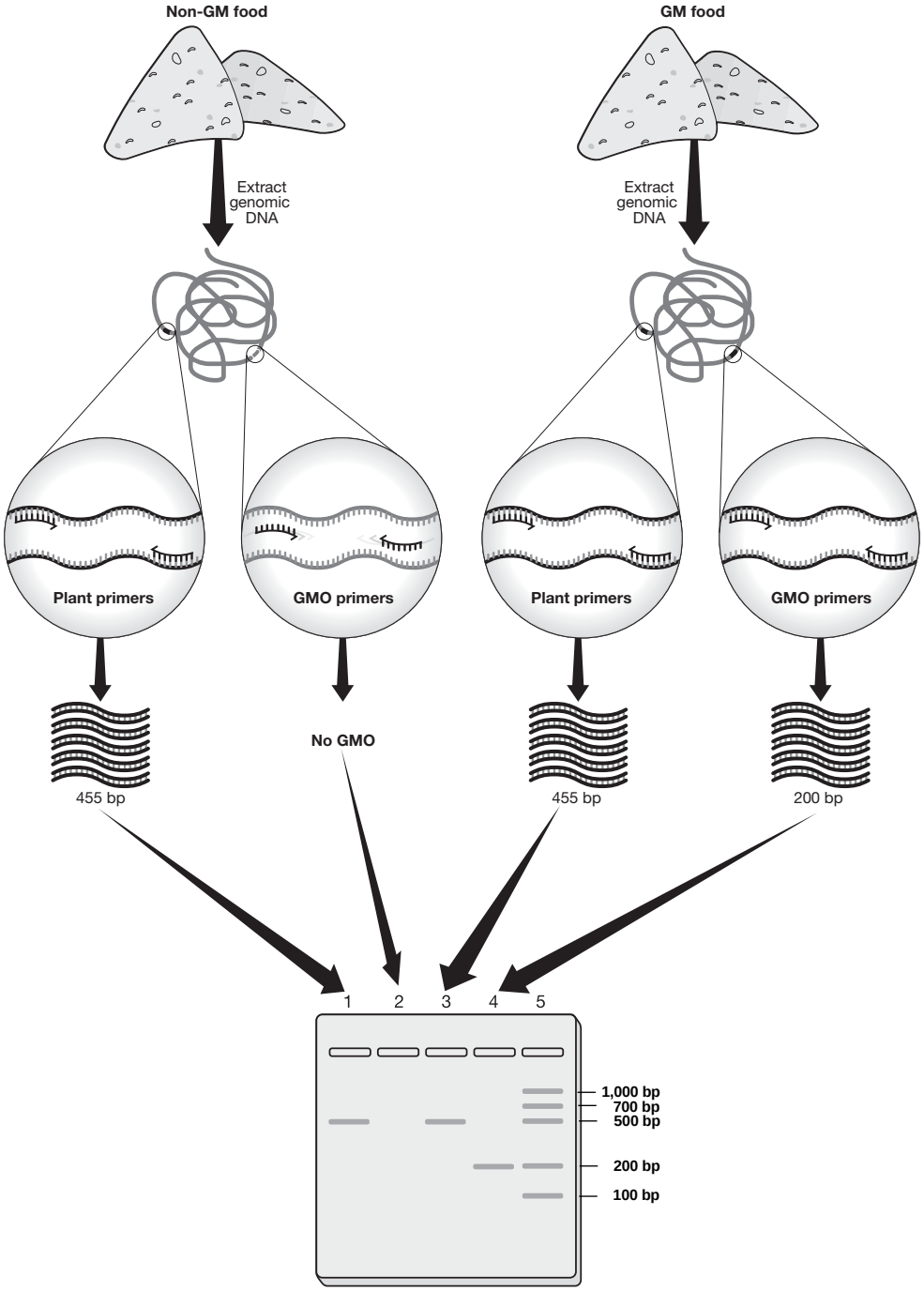


Fig. 1. Detecting GM foods by PCR. Genomic DNA is extracted from test foods (Lesson 1) and then two PCR reactions are performed on each test food genomic DNA sample (Lesson 2). One PCR reaction uses primers specific to a common plant gene (plant primers) to verify that viable DNA was successfully extracted from the food. No matter whether the food is GM or not, this PCR reaction should always amplify DNA (See lanes 1 and 3 of the gel above). The other PCR reaction uses primers specific to sequences commonly found in GM crops (GMO primers). This PCR reaction will only amplify DNA if the test food is GM (See lane 4). If the test food is non-GM, then the GMO primers will not be complementary to any sequence within the test food genomic DNA and will not anneal, so no DNA will be amplified (see lane 2). To find out whether DNA has been amplified or not, the PCR products are electrophoresed on a gel and stained to visualize DNA as bands (Lesson 3). A molecular weight ruler (lane 5) is electrophoresed with the samples to allow the sizes of the DNA bands to be determined.

Lesson 1 Extraction of DNA From Food Samples

In this lesson you will extract DNA from a control non-GMO food and a grocery store food item that you will test for the presence of GMOs. The most commonly modified foods are corn and soy-based, and so the test food could be fresh corn or soybeans, or a prepared or processed food such as cornmeal, cheese puffs, veggie sausage, etc. You will process the non-GMO control first.

You will first weigh your food sample, then grind it with water to make a slurry. You will then add a tiny amount of the slurry to a screwcap tube containing InstaGene matrix and boil it for 5 minutes.

The cellular contents you are releasing from the ground-up sample contain enzymes (DNases) that can degrade the DNA you are attempting to extract. The InstaGene matrix is made of negatively charged microscopic beads that “chelate” or grab metal ions out of solution. It chelates metal ions such as Mg^{2+}, which is required as a cofactor in enzymatic reactions. When DNA is released from your sample in the presence of the InstaGene matrix, the charged beads grab the Mg^{2+} and make it unavailable to the enzymes that would degrade the DNA you are trying to extract. This allows you to extract DNA without degradation. Boiling the samples destroys these enzymes.

After you centrifuge the samples to remove the InstaGene matrix and debris, the supernatant will contain intact extracted DNA. This extracted DNA will be used in the next laboratory as your target DNA.

Lesson 1 Extraction of DNA From Food Samples

Focus Questions

1. How can you test a food to find out if it contains material derived from a genetically modified organism (GMO)?

2. In what organelles is plant DNA located?

3. Many foods containing GM crops are highly processed. Can you suggest how DNA from whole plants may differ from that extracted from processed foods, e.g., corn chips, cornmeal, etc.?

4. What molecules are present in the cell that might interfere with DNA extraction?

5. Why do you also perform analysis on food that is known to be a non-GMO food control?

6. Why was the non-GMO food control prepared prior to your test food sample?

Student Protocol – Lesson One

Materials and supplies required at the workstation prior to beginning this exercise are listed below.

Student Workstation

Material	Quantity
Screwcap tube with 500 µl InstaGene matrix	2
Beaker of distilled water	1
Food samples	1 or 2
Disposable plastic transfer pipets (DPTP)	2
2–20 µl micropipet (if preparing non-GMO food control)	1
2–20 µl pipet tips, aerosol barrier	1 rack
Mortar and pestle	1
Marking pen	1

Common Workstation

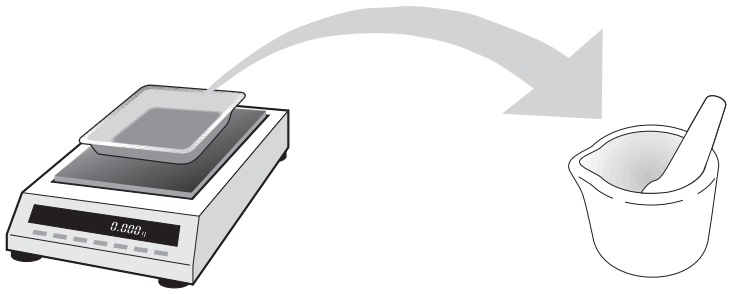
Material	Quantity
Water bath set to 95-100ºC	1
Microcentrifuge or mini centrifuges	3–4
Balance and weigh boats	1

Protocol

Note: ALWAYS process the non-GMO control before the test sample to reduce the risk of contamination.

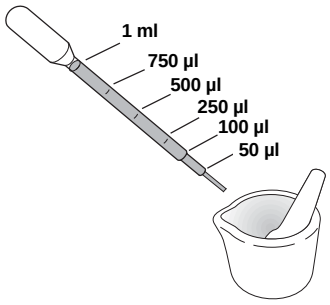
Grind non-GMO food control (your instructor may perform this step for you)

1. Find your screwcap tubes containing 500 µl of InstaGene matrix and label one “non-GMO” and one “test”.
2. Weigh out 0.5–2 g of the certified non-GMO food control and place in mortar.

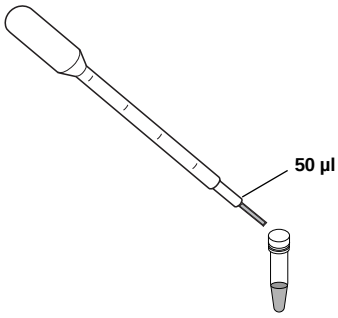


2. Using the transfer pipet, add 5 ml of distilled water for every gram of food using the graduations on the transfer pipet. To calculate the volume of water you need, mulitply the mass in grams of the food weighed out by 5 and add that many millimeters.

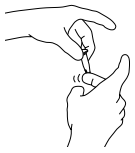
   Mass of Food = _____ g x 5 = _____ ml



3. Grind with pestle for at least 2 min until a slurry is formed.
4. Add 5 volumes of water again and mix or grind further with pestle until the slurry is smooth enough to pipet.
5. Add **50 µl** of ground slurry to the screwcap tube containing 500 µl of InstaGene matrix labeled “non-GMO” using a transfer pipet.



6. Recap tube and shake well.



7. Wash mortar with detergent and dry.

Grind Test Food

1. **Weigh out 0.5–2 g of test food and place in mortar.**
2. Using the transfer pipet, add 5 ml of distilled water for every gram of food using the graduations on the transfer pipet. To calculate the volume of water you need, mulitply the mass in grams of the food weighed out by 5 and add that many millimeters.

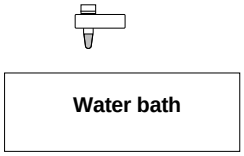
   Mass of food = _____ g x 5 = _____ ml

3. Grind with pestle for at least 2 min until a slurry is formed.

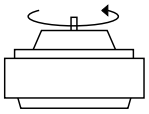
4. Add 5 more volumes of water and mix or grind further with pestle until the slurry is smooth enough to pipet.
5. Add **50 µl** of ground slurry to the screwcap tube labeled “Test” using the 50 µl mark on a transfer pipet.
6. Recap tube and shake well.

Process Samples to Extract DNA

1. Place non-GMO food control and test food sample tubes in 95°C water bath for 5 min.



2. Place tubes in a centrifuge in a balanced conformation and spin for 5 min at max speed.



3. Store tubes in refrigerator until the next lesson.

Lesson 2 Set Up PCR Reactions

In the last laboratory, you extracted DNA from a certified non-GMO food sample and a test food sample that you are analyzing for the presence of GMO DNA sequences. In this lab you will prepare those two samples and a positive control (GMO-positive template DNA) for the polymerase chain reaction (PCR).

PCR is DNA replication in a test tube. PCR allows you to amplify specific sections of DNA and make millions of copies of the target sequence. Your experiment is to determine whether or not the DNA you extracted from food in Lesson 1 contains or does not contain the target sequences of interest typically found in genetically modified (GM) foods.

PCR Review

PCR is such a powerful tool because of its simplicity and specificity. All that is required are minute quantities of the DNA template you want to amplify, DNA polymerase, two DNA primers, four DNA base pair subunits (deoxyribonucleotide triphosphates of adenine, guanine, thymine, and cytosine) and buffers.

Because PCR identifies a specific sequence of DNA and makes millions of copies of (or amplifies) that sequence, it is one of the most useful tools of molecular biology. Scientists use PCR to obtain the large amounts of a specific sequence of DNA that are necessary for such techniques as gene cloning, where DNA is physically moved from one genome to another. You are using the property of PCR that allows identification of a specific sequence, namely, the ability of PCR to search out a single sequence of a few hundred base pairs in a background of billions of base pairs. For example, the corn genome has 2.5 billion base pairs of DNA. This sequence is then amplified so that there are millions of copies of it so that it stands out from the few copies of the original template DNA.

PCR locates specific DNA sequences using primers that are complementary to the DNA template. Primers are short strands of DNA (usually between 6 and 30 base pairs long) called oligonucleotides. Two primers are needed to amplify a sequence of DNA, a forward primer and a reverse primer. The two primers are designed and synthesized in the laboratory with a specific sequence of nucleotides such that they can anneal (bind) at opposite ends of the target DNA sequence on the complementary strands of the target DNA template. The target DNA sequence is copied by the DNA polymerase reading the complementary strand of template DNA and adding nucleotides to the 3' ends of the primers (see fig 2). Primers give the specificity to the PCR, but they are also necessary because DNA polymerase can only add nucleotides to double-stranded DNA.

During PCR, double-stranded DNA template is separated by heating it, then each primer binds (anneals) to its complementary sequence on each of the separated DNA strands, and DNA polymerase elongates each primer by adding nucleotides to make a new double-stranded DNA (see fig 2).

The DNA polymerase used in PCR must be a thermally stable enzyme because the PCR reaction is heated to 94°C, which would destroy the biological activity of most enzymes. The most commonly used thermostable DNA polymerase is *Taq* DNA polymerase. This was isolated from a thermophillic bacterium, *Thermus aquaticus*, which lives in high-temperature steam vents such as those in Yellowstone National Park.

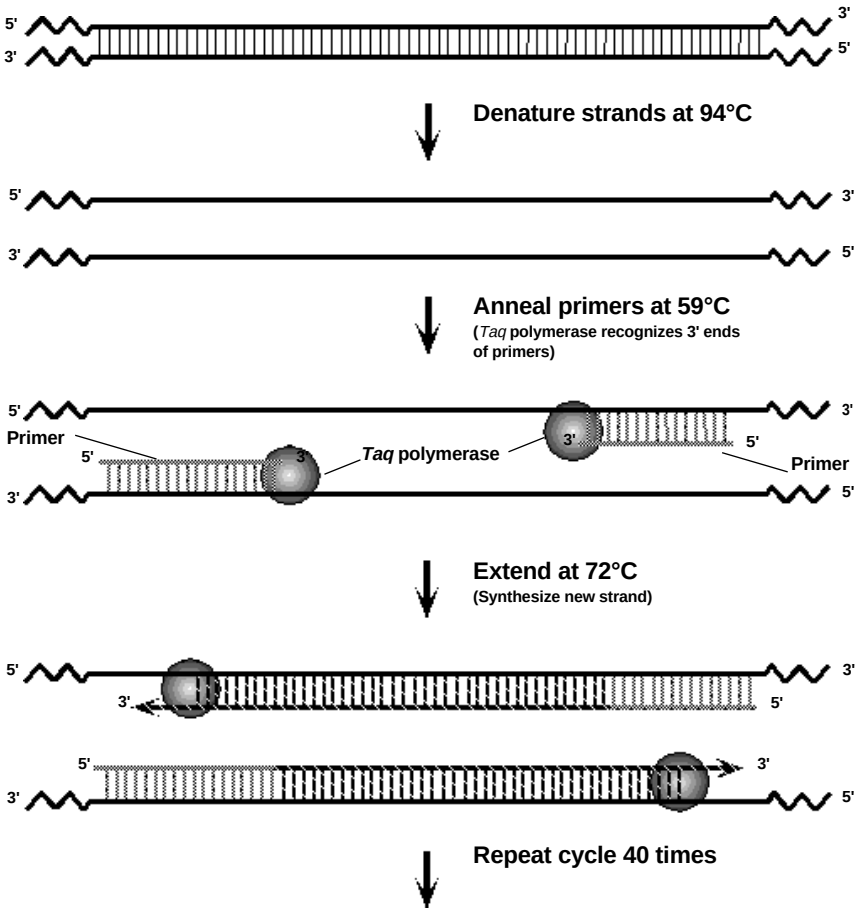


Fig. 2. A complete cycle of PCR.

PCR Step by Step

PCR has three steps, a denaturing step, an annealing step, and an elongation step. During the denaturing step, the DNA template is heated to 94°C to separate (or denature) the double-stranded DNA molecule into two single strands. The DNA is then cooled to 59°C to allow the primers to locate and anneal (bind) to the DNA. Because the primers are so much shorter than the template DNA, they will anneal much more quickly than the long template DNA strands at this temperature. The final step is to increase the temperature of the PCR reaction to 72°C, which is the optimal temperature for the DNA polymerase to function. In this step the DNA polymerase adds nucleotides (A, T, G, or a C) one at a time at the 3' end of the primer to create a complementary copy of the original DNA template. These three steps form one cycle of PCR. A complete PCR amplification undergoes multiple cycles of PCR, in this case 40 cycles.

The entire 40 cycle reaction is carried out in a test tube that has been placed in a thermal cycler or PCR machine. This is a machine that contains an aluminum block that can be rapidly heated and cooled. The rapid heating and cooling of this thermal block is known as thermal cycling.

Two new template strands are created from the original double-stranded template during each complete cycle of PCR. This causes exponential growth of the number of target DNA molecules, i.e., the number of target DNA molecules doubles at each cycle; this is why it is called a chain reaction. Therefore, after 40 cycles there will be 2^{40}, or over 1,100,000,000,000 times more copies than at the beginning. Once the target DNA sequences of interest have been sufficiently amplified, they can be visualized using gel electrophoresis. This allows researchers to determine the presence or absence of the PCR products of interest.

Your Task for This Lesson

For this experiment you will set up two PCR reactions for each DNA sample, which makes 6 PCR reactions in total. One PCR reaction, using the plant master mix (PMM), is a control to determine whether or not you have successfully extracted plant DNA from your test food. This is done by identifying a DNA sequence that is common to all plants by using primers (colored green in the kit) that specifically amplify a section of a chloroplast gene used in the light reaction (photosystem II). Why is this necessary? What if you do not amplify DNA using the GMO primers? Can you conclude that your test food is not GM or might it just be that your DNA extraction was unsuccessful? If you amplify DNA using the plant primers, you can conclude that you successfully amplified DNA, therefore whether or not you amplify DNA with your GMO primers, you will have more confidence in the validity of your result.

The second PCR reaction you carry out will determine whether or not your DNA sample contains GM DNA sequences. This is done by identifying DNA sequences that are common to most (~85%) of all GM plants using primers specific to these sequences. These primers are colored red and are in the GMO master mix (GMM).

Why do you have to set up a PCR reaction with DNA from certified non-GMO food? What if some GMO-positive DNA got into the InstaGene or master mix from a dirty pipet tip or a previous class? This DNA could be amplified in your test food PCR reaction and give you a false result. By having a known non-GMO control that you know should not amplify the GMO target sequences, you can tell if your PCR reactions have been contaminated by GMO-positive DNA.

Lesson 2

Focus Questions

1. What chemicals and molecules are needed for PCR, and what is the function of each component?

2. Examine the 150 base promoter sequence below.

5'TAGAAAAGGA AGGTGGCTCC TACAAATGCC ATCATTGCGA TAAAGGAAAG

GTATCATTC AAGATGCCTC TGCCGACAGT GGTCCCAAAG ATGGACCCCC

ACCCACGAGG AGC ATCGTGG AAAAAGAAGA CGTTCCAACC ACGTCTTCAA3'

Write in the sequence of the complementary strand and mark the 3' and 5' ends of the complementary strand.

Remembering that DNA polymerases can only add nucleotides to the 3' end of DNA, design a forward primer and a reverse primer, each 10 bases long, to amplify a target sequence of the DNA that is at least 100 bp long. Write the sequence of the primers below, with their 3' and 5' ends indicated. Also indicate on the sequence above which strand they are complementary to (will anneal to).

Forward primer sequence:

Reverse primer sequence:

4. Why are you performing two PCR reactions on each DNA sample?

5. What is the purpose of the GMO-positive control DNA?

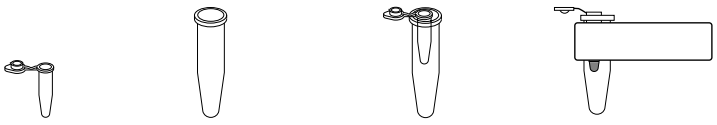
Student Protocol – Lesson Two Set Up PCR Reactions

Student Workstations

Material	Quantity
Ice bath containing 3 tubes	1
GMO master mix (red) (on ice)	1
Plant master mix (green) (on ice)	1
GMO positive control DNA (on ice)	1
Test food DNA (from previous lab)	1
Non-GMO food control DNA (from previous lab)	1
PCR tubes	6
PCR adaptors	6
Foam microtube holder	1
Marking pen	1
2–20 µl adjustable-volume micropipet or fixed-volume 20 µl micropipet	1
2–20 µl pipet tips, aerosol barrier	1 rack

1. Number six PCR tubes 1–6 and label them with your initials. The numbers correspond to the following tube contents:

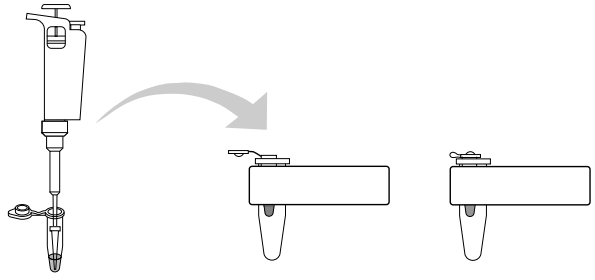
Tube Number	DNA	Master Mix
1	20 µl Non-GMO food control DNA	20 µl Plant master mix (green)
2	20 µl Non-GMO food control DNA	20 µl GMO master mix (red)
3	20 µl Test food DNA	20 µl Plant master mix (green)
4	20 ul Test food DNA	20 µl GMO master mix (red)
5	20 µl GMO positive control DNA	20 µl Plant master mix (green)
6	20 µl GMO positive control DNA	20 µl GMO master mix (red)



PCR tube

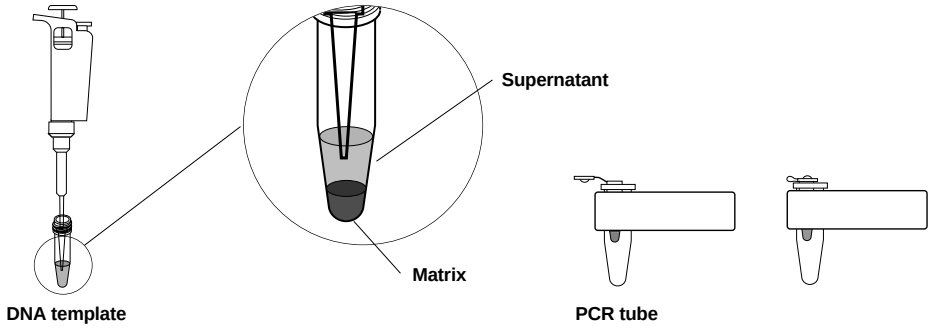
Capless tube

2. Keep the tubes on ice for the remaining steps.
3. Using a fresh tip each time, add 20 µl of the indicated master mix to each tube. I.E. add 20 µl of green plant master mix (PMM) to tubes 1, 3, and 5. Then add 20 µl of red GMO master mix (GMM) to tubes 2, 4, and 6. Cap each tube.



Master mix

4. Using a fresh pipet tip for each tube, add 20 µl of the DNA to each tube as indicated in the table above. Take care not to transfer any of the InstaGene beads to your PCR reaction. If the beads are disrupted, recentrifuge your DNA samples to pellet the beads. Add 20 µl of non-GMO food control DNA to tube 1 and pipet up and down to mix. Discard your tip. Use a fresh tip to add 20 µl of non-GMO food control DNA to tube 2 and mix. Discard your tip. Similarly add 20 µl of test food DNA to tubes 3 & 4, and add 20 µl of GMO positive control DNA to tubes 5 & 6, changing your tip for every tube. Recap tubes.



5. When instructed to, place the PCR tubes in the thermal cycler.

Lesson 3 Electrophoresis of PCR Products

You have completed your PCR amplification. You cannot, however, at this point determine whether or not you have PCR products. To do this, you must visualize your products. You will do this using gel electrophoresis.

Your PCR product bands are very small compared to those in other DNA experiments you may have done. For example, fragments produced from a *Hin*dIII digest of lambda DNA are 23,130, 9,416, 6,557, 4,361, 2,322, 2,027, and 500 base pairs (bp). The product band sizes in this lab are 455 bp for the plant primers and 200 bp for the GMO primers, and a 1% gel would not separate these bands. Instead, a tighter gel matrix is needed to impede the movement of these bands so that they are separated more on the gel and can be seen. Therefore, if you are using agarose electrophoresis, you will use a 3% agarose gel. Alternatively, your teacher may elect to use a polyacrylamide gel, which has smaller pores, to separate your products. Polyacrylamide gel electrophoresis (PAGE) is used to separate smaller molecules for visualization.

Regardless of the gel type, you will load a molecular weight ruler (DNA standard) so that you have a reference to determine your product bands' sizes. The gel will then be stained with Fast Blast stain to make the bands visible.

Lesson 3

Focus Questions

1. Why did you resolve your PCR products by electrophoresis?

2. Explain why DNA fragments separate according to size in an electrophoresis gel.

3. Why do you need a molecular weight ruler alongside your samples?

4. What results do you expect in each lane? Fill in the chart below.

Lane	Sample	Expect band (Yes, No, Don't know)?
1	Sample 1: Non-GMO food control with plant primers	
2	Sample 2: Non-GMO food control with GMO primers	
3	Sample 3: Test food with plant primers	
4	Sample 4: Test food with GMO primers	
5	Sample 5: GMO positive control DNA with plant primers	
6	Sample 6: GMO positive control DNA with GMO primers	

Lesson 3

Student Workstation

Material	Quantity
Gel (3% agarose or 10% polyacrylamide)	1
Samples from previous lab period	6
Running buffer (1x TAE for agarose gels or 1x TBE for polyacrylamide gels)	300–350 ml
Orange G loading dye	1 vial
PCR molecular weight ruler	1 vial
2–20 µl adjustable-volume pipet or fixed-volume 20 µl micropipet	1
1–20 µl pipet tips, aerosol barrier	1 rack
Gel electrophoresis chamber (may be shared by 2 workstations)	1
Power supply (may be shared by multiple workstations)	1
Fast Blast DNA stain, 1x or 100x depending on protocol (at common workstation)	1
Gel staining tray	1

Protocol

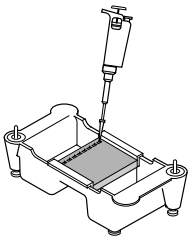
1. Set up your gel electrophoresis apparatus as instructed.

   Details on setting up electrophoresis equipment can be found in the Instructor's guide.

2. Using a fresh tip each time, add 10 µl of Orange G loading dye to each sample and mix well.

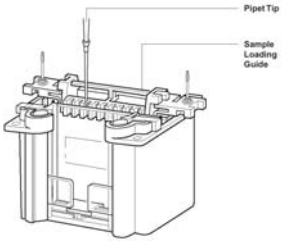
3. Load 20 µl of the PCR molecular mass ruler and 20 µl of each sample onto your gel in the order indicated below.

Lane	Sample	Load volume
1	Sample 1: Non-GMO food control with plant primers	20 µl
2	Sample 2: Non-GMO food control with GMO primers	20 µl
3	Sample 3: Test food with plant primers	20 µl
4	Sample 4: Test food with GMO primers	20 µl
5	Sample 5: GMO positive DNA with plant primers	20 µl
6	Sample 6: GMO positive DNA with GMO primers	20 µl
7	PCR molecular weight ruler	20 µl
8	Leave empty	



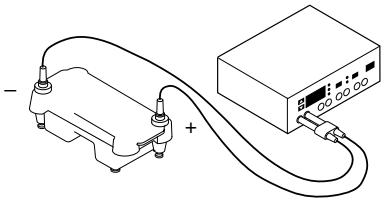
Agarose gel

or



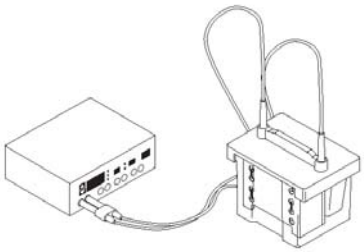
Polyacrylamide gel

4. The run time and voltage will depend on the type of gel you are running.
    - Run an agarose gel at 100 V for 30 minutes. Do not let the orange dye front migrate out of the agarose gel.
    - Run a polyacrylamide gel at 200 V for 30 minutes and do not let the red GMO primer dye front run out of the gel.



Agarose Gel Electrophoresis

or

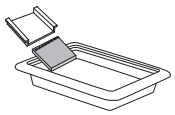


Polyacrylamide Gel Electrophoresis

5. Stain the gel in Fast Blast DNA stain. Refer to specific instructions below for your gel type.

Staining of Agarose Gels

1. When electrophoresis is complete, turn off the power and remove the lid from the gel box.
2. Carefully remove the gel tray and the gel from the gel box. Be careful, the gel is very slippery. Nudge the gel off the gel tray with your thumb and carefully slide it into your plastic staining tray.

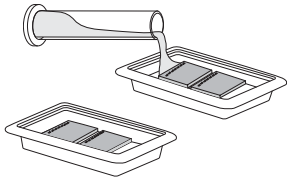


3. There are two protocols for staining your gel. Your instructor will inform you which one you will use.

    Protocol One: Overnight Staining

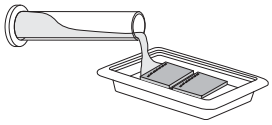
    a. Immerse your gel in 1x Fast Blast.

    b. Let the gels stain overnight, with gentle shaking for best results. No destaining is required.



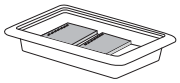
Protocol Two: Quick Staining (requires 20 minutes)–This method will allow you to see bands quickly (within 15 min) but may require extensive destaining to obtain optimal band-to-background intensity. Note: it is important to use **warm** water for destaining steps of this protocol.

a. Immerse your gel in 100x Fast Blast.



b. Stain the gel for 5 minutes with gentle agitation. Save the used stain for future use.

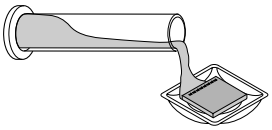
c. Transfer the gels into a large washing container and rinse with **warm** (40–55°C) tap water approximately 10 seconds.



d. Destain by washing three times in **warm** tap water for 5 minutes each with gentle shaking for best results. You should be able to visualize bands after 10 min if you view the gel with light coming through the bottom of the staining tray. If necessary continue destaining in **warm** water until the desired contrast is reached.

Staining of Polyacrylamide Gels

1. When gels are finished running, turn off the power supply and disconnect the leads. Remove the lid and lift out the electrode assembly and clamping frame.
2. Pour out the running buffer from the electrode assembly. Open the cams and remove the gel cassettes.
3. To keep the gel free of contamination from your fingertips, wear gloves to handle the gels from this point on. Lay a gel cassette flat on the bench with the short plate facing up. Cut the tape along the sides of the gel cassette. Carefully pry apart the gel plates, using a spatula or your fingertips. The gel will usually adhere to one of the plates. Transfer the plate with the gel adhering to it to a tray containing 1x Fast Blast stain (see below), allowing the liquid to detach the gel from the plate. The gel may also be lifted directly (and gently!) from the plate and placed into the stain.



4. Bands will start to appear after 10 minutes and staining will be complete in 1 hour. However, gels can be left in stain overnight. No destaining is required.

Lesson 4 Drying Gels and Analysis of Results

For a permanent record of the experiment, gels can be dried between cellophane sheets and incorporated into lab notebooks. To document the wet gels, they can be scanned, photocopied (a yellow backing provides optimal contrast), or traced onto acetate film. Your teacher will direct you on what method to use.

GelAir™ Drying Method

Materials Needed for Drying 8 Gels Using Gel Drying System	Quantity
GelAir cellophane	4 sheets
GelAir assembly table	1
GelAir drying frames	2
GelAir clamps	16
GelAir drying oven (optional)	1
Distilled water	500 ml

Procedure

1. Prewet 2 sheets of cellophane in a container of water for 15–20 seconds.
2. Place a plastic drying frame on the GelAir assembly table. Center one sheet of cellophane on the assembly table.
3. Carefully lay a gel on the cellophane, positioning it to accommodate other gels (up to six total). If there are bubbles between the gel and the cellophane, gently push them out with your gloved finger. Note polyacrylamide gels must have the ridge at the bottom of the gel removed by chopping them off (not slicing) using a plastic card, e.g., an I.D. card.
4. Flood the gels with water and lay the second sheet of cellophane on top of them. If you are drying polyacrylamide gels, try not to trap any bubbles in the sandwich since bubbles will cause cracks in the gel during drying. If there are any bubbles, gently push them out with a gloved finger. Because of their thickness, you cannot avoid bubbles at the edges of agarose gels, but avoid bubbles between the cellophane and the face of the gel.
5. Place the square metal frame on top of the cellophane sandwich. Secure the eight clamps onto the frame, two on each side. If you are not using a GelAir drying oven, place the frames in a well-ventilated area for 12–36 hours. If you have a GelAir drying oven, place up to four drying frames into the oven, turn the heater switch on, and set the dial to 3 hours. The dryer will shut off automatically.
6. When the gels are completely dry, they will be flat. Remove the clamps and take the gel/cellophane sandwich from the frame. Trim the excess cellophane surrounding the dried gels with scissors.

Cellophane Sandwich and Plastic Container Method

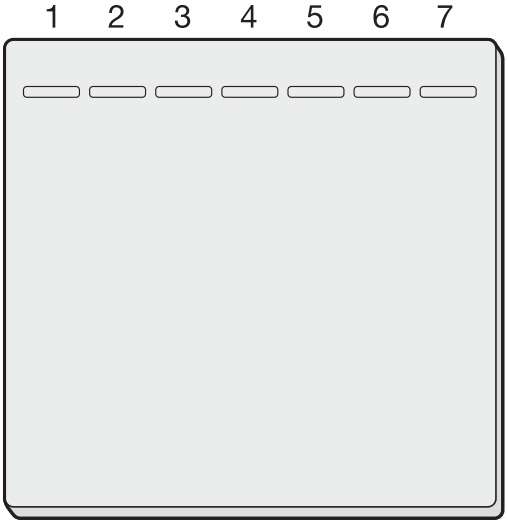
Materials Needed for Drying 8 Gels Using Plastic Containers	Quantity
GelAir cellophane	16 sheets
Plastic container	8
Rubber bands	16
Distilled water	500 ml
Materials needed for drying 8 gels using plastic containers	**Quantity**

Procedure

1. Prewet 2 sheets of cellophane in a container of water for 15–20 seconds.
2. Place one sheet of cellophane over a plastic container. Pull the cellophane taut so that it makes a flat surface over the top of the container, and use a rubber band to hold the sheet in place.
3. Place a gel onto the cellophane. Flooding the surface of the cellophane around the gel with water will aid in the removal of bubbles.
4. Place the second sheet of wetted cellophane over the gel. Because of their thickness, you cannot avoid bubbles at the edges of agarose gels, but avoid bubbles between the cellophane and the face of the gel. Secure the second sheet of cellophane to the box with a second rubber band.
5. Allow the gel to dry for several days in a well-ventilated area.

Analysis of Results

Lane	Sample	Bands?	Band Sizes (bp)
1	Sample 1: Non-GMO food control with plant primers		
2	Sample 2: Non-GMO food control with GMO primers		
3	Sample 3: Test food with plant primers		
4	Sample 4: Test food with GMO primers		
5	Sample 5: GMO positive control DNA with plant primers		
6	Sample 6: GMO positive control DNA with GMO primers		
7	PCR molecular weight ruler		



Lesson 4

Focus Questions

1. What was your test food?

2. Did your test food generate a 200 bp band with GMO primer (lane 4)?

3. What does this tell you about the GMO status of your food?

4. What other information do you need to confirm the GMO status of your sample?

5. How do the results of your other five PCR reactions help support or undermine your result for your test food?

6. If you were to repeat the procedure what laboratory practice might yield better results?

Appendix A

Introduction to PCR

In 1983, Kary Mullis at Cetus Corporation developed the molecular biology technique that has since revolutionized genetic research, earning him the Nobel Prize in 1993. This technique, termed the **polymerase chain reaction (PCR)**, transformed molecular biology into a multidisciplinary research tool. Many molecular biology techniques used before PCR were labor intensive, time consuming, and required a high level of technical expertise. Additionally, working with only trace amounts of DNA made it difficult for researchers in other biological fields (pathology, botany, zoology, pharmacy, etc.) to incorporate molecular biology into their research schemes.

PCR had an impact on four main areas of biotechnology: gene mapping, cloning, DNA sequencing, and gene detection. PCR is now used as a medical diagnostic tool to detect specific mutations that may cause genetic disease, in criminal investigations and courts of law to identify suspects on a molecular level, and in the sequencing of the human genome. Prior to PCR, the use of molecular biology techniques for therapeutic, forensic, pharmaceutical, or medical diagnostic purposes was not practical or cost-effective. The development of PCR technology changed these aspects of molecular biology from a difficult science to one of the most accessible and widely used tools in genetic and medical research.

PCR and Biotechnology - What Is It and Why Did It Revolutionize an Entire Research Community?

PCR produces exponentially large amounts of a specific piece of DNA from trace amounts of starting material (template). The template can be any form of double-stranded DNA, such as genomic DNA. A researcher can take trace amounts of DNA from a drop of blood, a single hair follicle, or a corn chip and use PCR to generate millions of copies of a desired DNA fragment. In theory, only a single molecule of double-stranded template DNA is needed to generate millions of copies. Prior to the development of the PCR technique, it would have been impossible to do forensic or genetic studies with a minute sample containing only a few molecules of source DNA. The ability to amplify a precise sequence of DNA to a sufficient quantity that a researcher can analyze and manipulate is the true power of PCR.

PCR amplification requires the presence of at least one DNA template strand. In this kit, plant DNA isolated from grocery store foods provides the template strands. One of the main reasons PCR is such a powerful tool is its simplicity and specificity. All that is required are inexpensive buffers, four DNA subunits (deoxynucleotide triphosphates of adenine, guanine, thymine, and cytosine), a DNA polymerase, two DNA primers, and minute quantities of the template sequence that one wants to amplify. Specificity comes from the ability to target one specific segment of DNA (or gene) out of a complete genome through the use of sequence-specific primers.

PCR Makes Use of Two Basic Processes in Molecular Genetics

1. Complementary DNA strand hybridization
2. DNA strand synthesis via DNA polymerase

In the case of PCR, complementary strand hybridization takes place when two different oligonucleotide primers anneal to each of their respective complementary base pair sequences on the template. The two primers are designed and synthesized in the laboratory with a specific sequence of nucleotides such that they can anneal at the opposite ends and on the opposite strands of the stretch of double-stranded DNA (template) to be amplified.

Before a region of DNA can be amplified, one must identify and determine the sequence of an area of DNA upstream and downstream of the region of interest. These areas are then used to determine the sequences of oligonucleotide primers that will be synthesized and used as starting points for DNA replication. Primers are needed because DNA polymerases can only add nucleotides to the end of a preexisting chain. The DNA polymerase used in PCR must be a thermally stable polymerase because the polymerase chain reaction cycles between 59°C and 94°C. The thermostable DNA polymerase (*Taq*) used in PCR was isolated from a thermophilic bacterium, *Thermus aquaticus*, which lives in high-temperature steam vents such as those found in Yellowstone National Park.

Two new template strands are created from the original double-stranded template during each complete cycle of the strand synthesis reaction. This causes exponential growth of the number of template molecules, i.e., the number of DNA strands doubles at each cycle. Therefore, after 30 cycles there will be 2^{30}, or over 10^9, times more copies than at the beginning. Once the DNA of interest has been sufficiently amplified, it can be visualized using gel electrophoresis. This allows researchers to determine the presence or absence of the desired PCR products.

PCR Step by Step

PCR involves a repetitive series of cycles, each of which consists of template denaturation, primer annealing, and extension of the annealed primer by *Taq* DNA polymerase. Before beginning DNA amplification, genomic DNA is prepared from samples-in this lab, from plant-derived food items.

Following sample preparation, the template DNA, oligonucleotide primers, thermostable DNA polymerase (*Taq*), the four deoxynucleotides (A, T, G, C), and reaction buffer are mixed in a single micro test tube. The tube is placed into the MyCycler™ thermal cycler. These thermal cyclers contain an aluminum block that holds the samples and can be rapidly heated and cooled across wide temperature differences. The rapid heating and cooling of this thermal block is called **temperature cycling** or **thermal cycling**.

The first step of the PCR temperature cycling procedure involves heating the sample to 94°C. At this high temperature, the template strands separate (denature). This is called the **denaturation step**.

The thermal cycler then rapidly cools to 59°C to allow the primers to anneal to the separated template strands. This is called the **annealing step**. The two original template strands may reanneal to each other, or compete with the primers for the primers' complementary binding sites. However, the primers are added in excess such that the primers actually outcompete the original DNA strands for the primers' complementary binding sites.

Lastly, the thermal cycler heats the sample to 72°C for *Taq* DNA polymerase to extend the primers and make complete copies of each DNA strand. This is called the **extension step**. *Taq* polymerase works most efficiently at this temperature. Two new copies of each complementary strand are created. There are now two sets of double-stranded DNA (dsDNA). These two sets of dsDNA can now be used for another cycle and subsequent strand synthesis.

At this stage, a complete temperature cycle (thermal cycle) has been completed.

Temperature cycle = denaturation step + annealing step + extension step

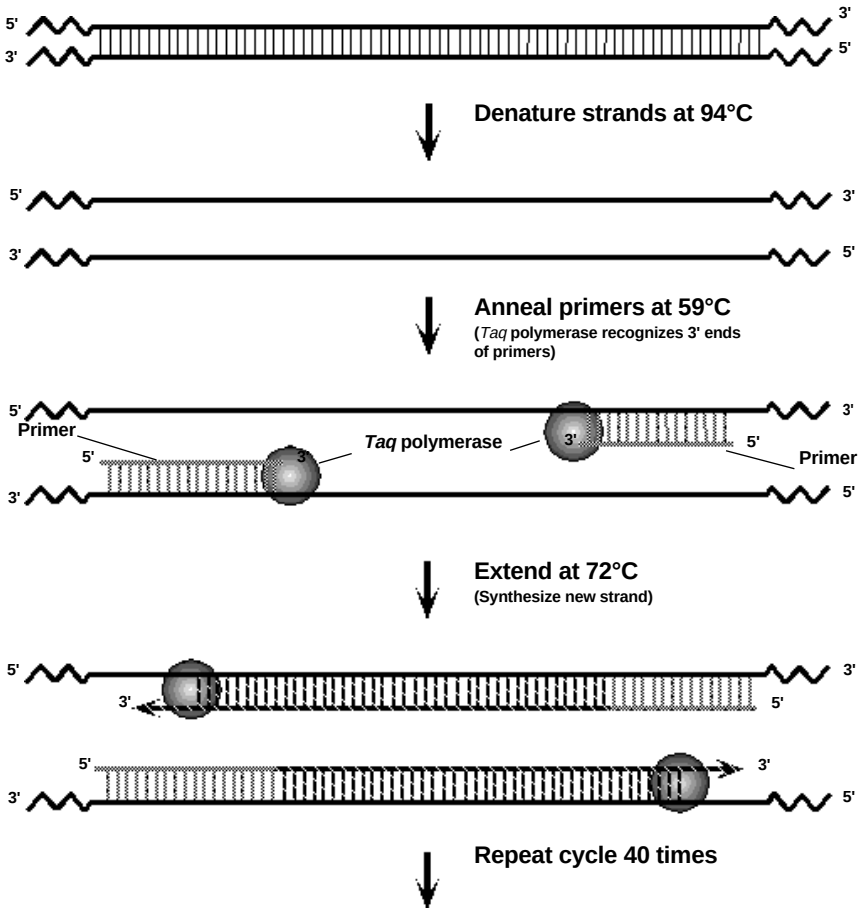


Figure A1. A complete cycle of PCR.

Usually, thermal cycling continues for about 40 cycles. After each thermal cycle, the number of template strands doubles, resulting in an exponential increase in the number of template DNA strands. After 40 cycles there will be 1.1×10^{12} more copies of the original number of template DNA molecules.

PCR generates DNA of a precise length and sequence. On the first cycle, the two primers anneal to the original genomic template DNA strands at opposite ends and on opposite strands. After the first complete temperature cycle, two new strands are generated that are shorter than the original template strands but still longer than the length of the DNA that the researcher wants to amplify. It isn't until the third thermal cycle that fragments of the precise length are generated.

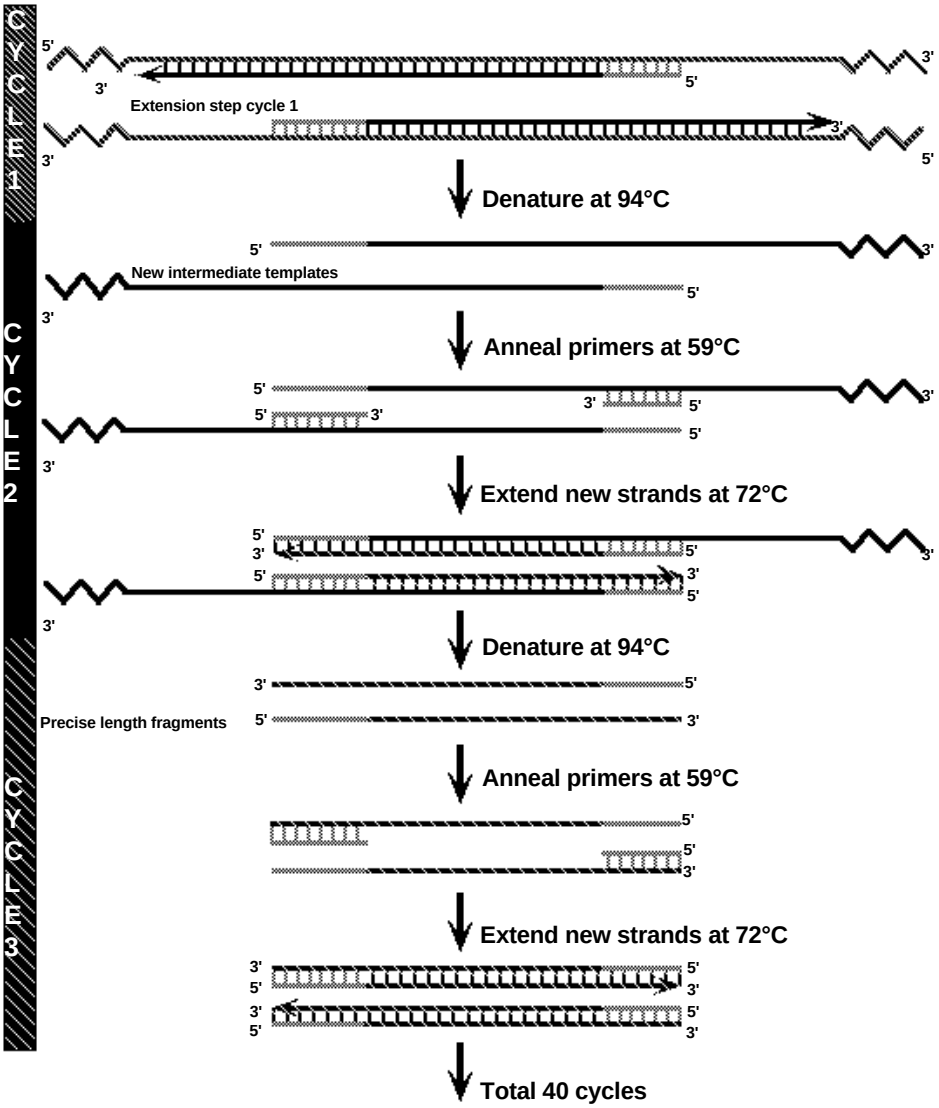


Figure A2. Generation of precise-length fragments.

It is the template strands of the precise length that are amplified exponentially (X^n, where X = the number of original template strands and n = the number of cycles). There is always one set of original long-template DNA molecules that are never fully duplicated. After each thermal cycle, two intermediate-length strands are produced, but because they can only be generated from the original template strands, the intermediate strands are not exponentially amplified. It is the precise-length strands generated from the intermediate strands that are amplified exponentially at each cycle. Therefore, if 20 thermal cycles were conducted from one double-stranded DNA molecule, there would be 1 set of original genomic template DNA strands, 20 sets of intermediate template strands, and 1,048,576 sets of precise-length template strands. After 40 cycles, there would be 1 set of original genomic template DNA strands, 40 sets of intermediate template strands, and 1.1×10^{12} sets of precise-length template strands.

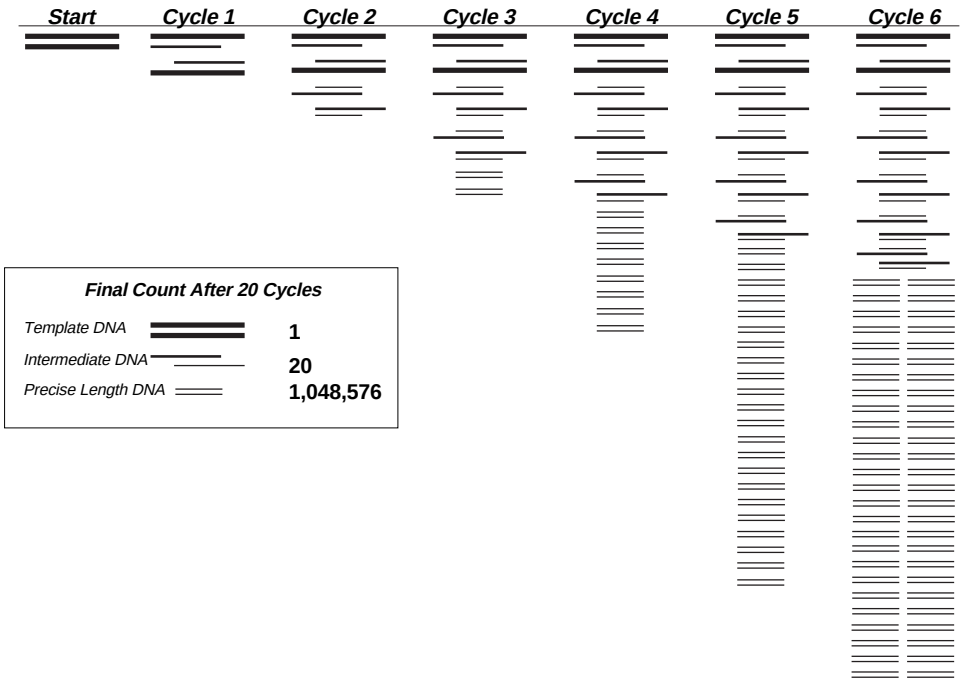


Figure A3. Schematic of PCR amplification of DNA fragments.

Appendix B

PCR Amplification and Sterile Technique

PCR is a powerful and sensitive technique that enables researchers to produce large quantities of specific DNA from very small amounts of starting material. Because of this sensitivity, contamination of PCR reactions with unwanted DNA is always a possible problem. Therefore, utmost care must be taken to prevent cross-contamination of samples. Steps to be taken to prevent contamination and failed experiments include:

1. Filter-type pipet tips. The end of the barrels of micropipets can easily become contaminated with aerosolized DNA molecules. Pipet tips that contain a filter at the end can prevent aerosol contamination from micropipets. DNA molecules that are found within the micropipet cannot pass through the filter and contaminate PCR reactions. Xcluda® aerosol barrier pipet tips (catalog #211- 2006EDU and 211-2016EDU) are ideal pipet tips to use in PCR reactions.
2. Aliquot reagents. Sharing of reagents and multiple pipetting into the same reagent tube can easily introduce contaminants into your PCR reactions. When at all possible, divide reagents into small aliquots for each team, or if possible, for each student. If only one aliquot of a reagent does become contaminated, then only a minimal number of PCR reactions will become contaminated and fail.
3. Change pipet tips. Always use a new pipet tip when entering a reagent tube for the first time. If a pipet tip is used repeatedly, contaminating DNA molecules on the outside of the tip will be transferred to other solutions, resulting in contaminated PCR reactions. If you are at all unsure if your pipet tip is clean, err on the safe side and discard the tip and get a new one. The price of a few extra tips is a lot smaller than the price of failed reactions.
4. Use good sterile technique. When opening tubes or pipetting reagents, leave the tubes open for as little time as possible. Tubes that are open and exposed to the air can easily become contaminated by aerosolized DNA molecules. Go into reagent tubes efficiently, and close them as soon as you are finished pipetting. Also, try not to pick tubes up by the rim or cap, as you can easily introduce contaminants from your fingertips.
5. Bleach at a concentration of 10% destroys DNA, so wiping down surfaces and rinsing plastic pipet barrels, mortars, and pestles with 10% bleach can get rid of any surface DNA contamination that may arise.

Appendix C

Glossary of Terms

Aliquot – The division of a quantity of material into smaller, equal parts.

Annealing – Binding of single-stranded DNA to complementary sequences. Oligonucleotide primers anneal to denatured (single-stranded) DNA strands.

Bt – *Bacillus thuringiensis* – In the context of GM crops Bt refers to a specific modification in which a gene for a member of the Cry family of proteins from the soil bacterium *Bacillus thuringiensis* is inserted into the crop. The gene confers resistance to the European corn borer.

Back-cross – In the context of GMO crops, the method by which a newly made genetically engineered crop is repeatedly bred into a commercially viable crop to transfer the genetic modification into a high yield or commercially viable background.

Callus – An undifferentiated mass of plant cells.

Chelate – To bind metal ions in solution. An example of a common chelating agent is EDTA (ethylene diamine tetraacetic acid).

Cofactor – Ion or other small molecule needed by an enzyme to function properly. For example, *Taq* DNA polymerase needs Mg^{2+} in order to function properly. Mg^{2+} is considered a cofactor.

Denaturation – The process of melting apart two complementary DNA strands. In vivo denaturation is accomplished by enzymes; in the (in vitro) PCR reaction, denaturation is accomplished by heat.

DNase – Enzyme that degrades DNA.

dNTPs – Commonly used abbreviation for all four deoxynucleotide triphosphates (dATP, dTTP, dGTP, dCTP) used in synthesizing DNA.

Ethidium bromide – A fluorescent dye that is used to detect DNA. It intercalates between DNA base pairs and fluoresces when exposed to ultraviolet light.

Exons – The coding regions of a transcribed messenger RNA that get spliced together and leave the nucleus for translation into protein sequence.

Extension – Elongation of a primer by addition of dNTPs (deoxynucleotide triphosphates — dATP, dTTP, dCTP, or dGTP) by a DNA polymerase. Extension follows the base pairing rule and proceeds in the 5' to 3' direction.

Genomic DNA – The sum total of the DNA that is found within a cell.

Genetic engineering – The process by which scientists change the genetic makeup of an organism.

GM – Genetically modified

GMO – Genetically modified organism

InstaGene™ matrix – Microscopic beads that bind divalent cations in solution. The binding of divalent cations to these beads prevents their availability to enzymes that can degrade DNA.

Intron – Region of a transcribed messenger RNA that is spliced out of and is not translated into protein sequence.

Lysis – The process of rupturing a cell to release its constituents. In this laboratory, plant cells are lysed to release genomic DNA for the PCR reactions.

Master mix – A premixed reagent solution designed for PCR reactions, containing all of the necessary components (dNTPs, primer, buffer, salts, polymerase, Mg^{2+}) of the reaction except the template DNA.

Nucleotide – A fundamental unit of DNA or RNA. Consists of a sugar (deoxyribose or ribose), phosphate, and nitrogenous base (adenine, cytosine, guanine, thymine, or uracil).

PCR – Polymerase chain reaction. A process used to amplify (synthesize large quantities from a small starting sample) DNA within a test tube.

Primer – A small chain of nucleotides (usually 16–24 bases in length) that provides a free end for DNA polymerase to extend from. Primers for PCR are designed (synthesized in a laboratory) to be complementary to specific sequences near the target DNA sequence, so that they will "anchor" to the template and provide a starting point for the DNA polymerase to copy the region of interest.

***Taq* DNA polymerase** – Heat-stable DNA polymerase that was isolated from the heat-stable bacterium *Thermus aquaticus*. This DNA polymerase is commonly used in PCR reactions.

Template – The DNA that contains the sequence to be copied (into a complementary sequence) in a DNA-synthesizing reaction. Double-stranded DNA serves as a template for replication of copies of itself, because each strand's sequence serves as a template for the replication of the other strand's sequence. A single-stranded DNA, on the other hand, can only serve as template for copies of its complementary sequence, and not for copies of itself.