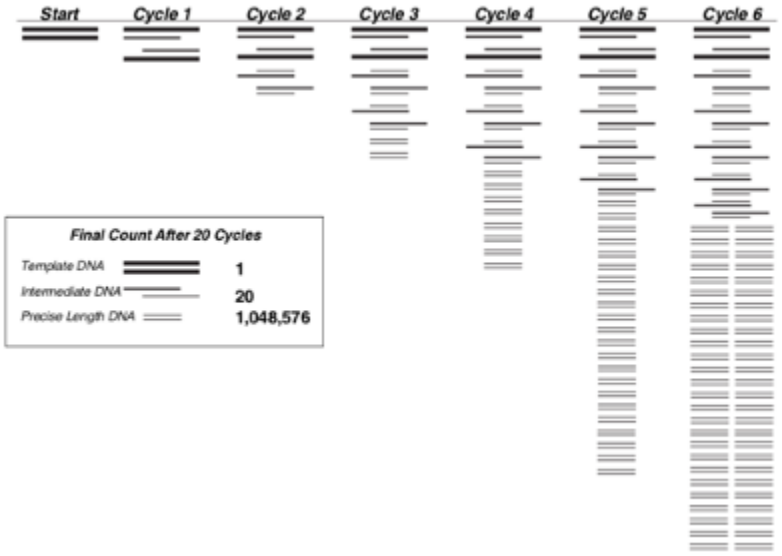
LAB: Identification and detection of GMO's using PCR

INTRODUCTION:

This can be done in several ways. One 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 part of this experiment genomic DNA is extracted from food samples. In the second part PCR reactions will be used to amplify GMO and natural plant sequences from the DNA. In the third part electrophorese will be used to visualize amplified samples of the DNA.

A Review of PCR:



Results

The DNA fragments amplified from the 35S promoter and NOS terminator are 203 and 225 base pairs (bp) respectively.

The PCR product generated from the photosystem II gene is 455 bp.

Agarose gels should be 3% agarose gel
PAGE will require: 5% polyacrylamide gel.

Part 1: Extraction of DNA From Food Samples

Protocol:

https://www.youtube.com/watch?v=RIPsjrXMMmw

Setting up the PCR Reactions

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

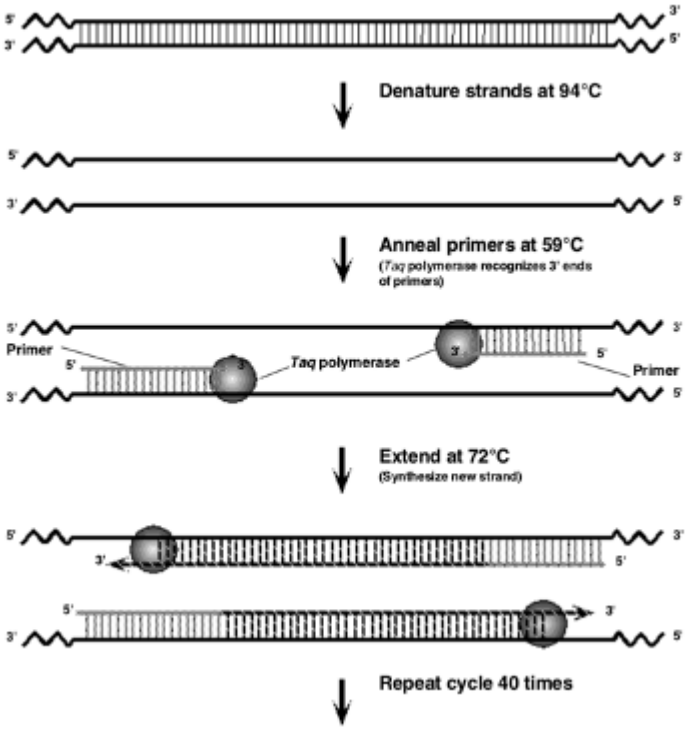
Here is a PCR Review if you need it:

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.

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. 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.



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 240, 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 Part:

PCR to test for the presence of

- Ø the 35S promoter of the cauliflower mosaic virus (CaMV 35S)
- Ø terminator of the nopaline synthase (NOS) gene of Agrobacterium tumefaciens.
- Ø The integrity of the plant DNA extracted from food is tested using PCR to identify a third sequence of DNA, the photosystem II chloroplast gene, which is common to most plants.

- Ø Controls: The integrity of the polymerase chain reaction is tested by amplifying the 35S promoter and the photosystem II gene sequences directly from template DNA provided.

Ø Controls: Potential contamination of the test samples is also identified by extracting DNA from a 'certified non-GMO food' control provided and performing PCR to test for the presence of GMO sequences.

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**) 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.

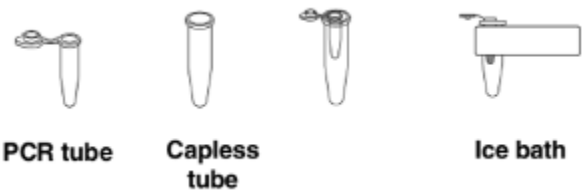
The second PCR reaction you carry out will determine whether or not your DNA sample contains GM DNA sequences. This is done by identifying DNA sequences that are common to most (~85%) of all GM plants using primers specific to these sequences. These primers are **colored red** and are in the GMO master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

Protocol – Part Two: Set Up PCR Reactions

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

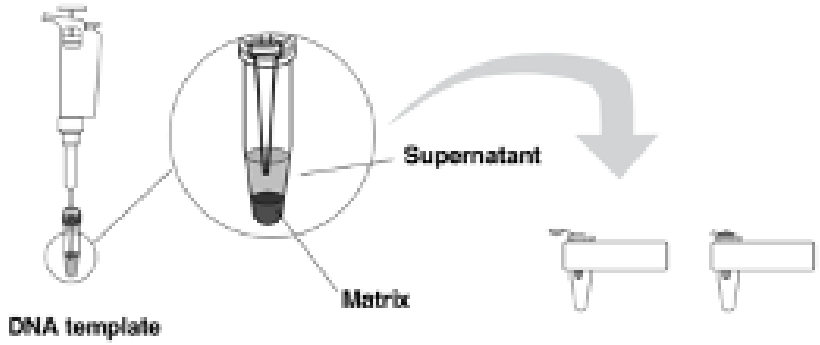


2. Keep the tubes on ice for the remaining steps.

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

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.

4. Using a fresh pipet tip for each tube, **add 20 µl of the DNA to each tube** as indicated in the table. Take care not to transfer any of the InstaGene beads to your PCR reaction. If the beads are disrupted, re-centrifuge your DNA samples to pellet the beads.



5. Place the PCR tubes in the thermal cycler.