**Using the Polymerase Chain Reaction (PCR) to Identify Genetically Modified Organisms (GMOs) in Food Products**

**Introduction to the Polymerase Chain Reaction (PCR):**

The PCR is a laboratory technique that was created in the 1980s and has since revolutionized biological research. The purpose of PCR is to make billions of copies of a specific sequence of DNA. It’s much like the real DNA replication that occurs in a cell, but instead of the whole genome being copied, the PCR only copies a specific target sequence. PCR is used routinely in laboratories and medicine to verify whether a certain sequence is present in a sample genome. For example, PCR is used when diagnosing genetic diseases to determine if there is a defective gene present. PCR can also be used as a starting tool for many other biotechnology and research applications. It is a very powerful AND very simple technique. PCR will be used in this laboratory exercise to identify the presence of the Bt gene in food products containing corn.

Remember that **DNA replication** occurs during the interphase of mitosis. The DNA in the cell is used as a template to form identical copies of the DNA in preparation for one cell splitting into two. Below is a *partial* list of the molecules cells must have when they perform DNA replication:

|  |  |  |
| --- | --- | --- |
| **Some major “ingredients” required for actual cell DNA replication** | **Function of ingredient** | **Ingredient equivalents required for same function in the PCR** |
| Template DNA | The original from which the copy will be made | Template DNA |
| DNA helicase | To separate the two strands in the template DNA helix by breaking the hydrogen bonds between the bases | Heat |
| Free nucleotides | These will be assembled in the correct sequence to make the copy | Free nucleotides |
| DNA primase | Places a short piece of complementary DNA called a **primer** on the template DNA, which shows the DNA polymerase where to bind in order to begin DNA replication | Specially designed, pre-made primers |
| DNA polymerase | Binds and reads the original DNA sequence, then assembles the free nucleotides in the correct sequence to make a complementary copy | DNA polymerase |

PCR happens in a test tube, not a cell. Still, to make a copy of DNA, we still need some of the same ingredients required for actual DNA replication:
1. template DNA 2. free nucleotides 3. DNA polymerase 4. Primers
must all be added to the test tube.
In a PCR, heat is used to separate the two strands in the template DNA, not the enzyme DNA helicase. And specially designed, pre-made primers are added to the PCR tube. These primers are the key to how PCR can copy a specific target sequence. In the above table, the PCR equivalents to the cells required “ingredients” are listed.

**How the PCR works:**

Let’s say that in the following portion of a sample DNA, we want to do a PCR to make copies of just the target sequence included in boxes:

5' C A A T C G G C C A A G T T C C C A G G A G C A A G G T C T 3'

3' G T T A G C C G G T T C A A G G G T C C T C G T T C C A G A 5'

 **PCR Step 1: Denaturing**

For replication to occur, first the double stranded molecule must be separated into two strands. In a PCR, the double-stranded DNA is **denatured** (converted to single-stranded DNA) by heating it to 94oC. This heating breaks the **hydrogen bonds** between the two strands.

5' C A A T C G G C C A A G T T C C C A G G A G C A A G G T C T 3'

3' G T T A G C C G G T T C A A G G G T C C T C G T T C C A G A 5'

**PCR Step 2: Annealing**

This is where the primers come in. For a PCR, there must be two primers:
1. One primer will bind to the top strand (see below) because it has a sequence that is complementary to the DNA just after the sequence we want to copy.
2. One will bind to the bottom strand, because it has a sequence that is complementary to the DNA just before the sequence we want to copy.
 **if the primers do not match, the PCR will fail and no DNA copies will be made.**

The reaction tube is then cooled to 59oC. At this temperature, it is cool enough for the primers (in bold type) to bind or **anneal** to their complementary DNA sequences. However, it is still too hot for the two strands of DNA to come back together again.

5' C A A T C G G C C A A G T T C C C A G G A G C A A G G T C T 3'

**primer**

 **3' C G T T C C** **5'**

**primer**

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

3' G T T A G C C G G T T C A A G G G T C C T C G T T C C A G A 5'

 **PCR Step 3: Extension**

Next, the reaction tube is warmed to 72oC. At this temperature the DNApolymerase

attaches free nucleotides to the 3' end of the primers, thus making a copy of the sequence from both directions.

5' C A A T C G G C C A A G T T C C C A G G A G C A A G G T C T 3'

 **3' C G T T C C** **5'**

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

3' G T T A G C C G G T T C A A G G G T C C T C G T T C C A G A 5'

At the conclusion of the extension step, two complete double-stranded copies of the sequence have been produced which include the target sequence (see below).

5' C A A T C G G C C A A G T T C C C A G G A G C A A G G T C T 3'

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

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

3' G T T A G C C G G T T C A A G G G T C C T C G T T C C A G A 5'

 **These three steps (denaturing, annealing, extension) counts as one round of PCR.**
By starting the cycle over at step one, these now two molecules of double-stranded DNA can now serve as template for the polymerase. Each time this cycle is repeated, copies are made from copies and the number of template strands doubles (from 2 to 4 to 8 to 16 and so on). The reaction produces copies of the DNA sequence according to the following equation:

**# of copies = 2n**

**Where n = number of repeated cycles**

Most PCR reactions are repeated for 35 to 40 cycles. After 40 cycles, there are
1,099,511,627,776 copies of the DNA target sequence! That’s 1.099 x 10\*12 copies! Whew!
Each cycle takes about 5 minutes. An entire 40 cycle reaction requires just about 3 ½ hours.
 This rapid production of billions of copies of a sequence by PCR is called **amplification**.

The PCS is performed in a test tube in a machine called a **thermal cycler.** This machine

can rapidly heat and cool the samples according to how it has been programmed. It looks like a very expensive bread machine…

Once the target DNA sequences of interest have been amplified, they can be visualized using **gel electrophoresis.**

 **Using PCR to test for genetically modified foods**

With 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.
Genetically modified organisms (GMOs), particularly genetically modified crop plants, may potentially be able to solve both problems. The use of GMOs, however, has met with some opposition. Sometimes called “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 already “genetically modify” crops through selective crop breeding to encourage specific traits, such as high yield. 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.** Delta endotoxin 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 genes that delay fruit ripening, confer resistance to parasitic fungi or drought, increase crop yield, or result in improved fruits.

Objections to the use of GM crop plants include:
1. concerns that super-weeds will be created through cross-pollination with herbicide-resistant crops
2. concerns that super-bugs will evolve that are no longer susceptible to the toxins in pest-resistant crops
3. concerns that allergic reactions or other unforeseen effects on public health will arise
while proponents of GM foods argue these crops are actually better for the environment and public health because fewer toxic pesticides will be put into the environment.

Because there is no labeling requirement for products that contain GM foods in the US, there is no way for consumers to know whether the products they purchase contain GM ingredients. One way to test for the presence of GM foods is by using PCR.

That’s today’s lab…

In this laboratory exercise, we will complete an experiment to test the question
**“Do some food products contain corn that has been genetically modified with the Bt gene?”
 To answer this question, you will complete the following steps:**

**1) Extract corn DNA from food products.**

**2) Perform a PCR on the extracted DNA.** For the PCR, you will use **primers** specifically designed to target a sequence within the Bt gene.
 **If the Bt gene is present, amplification will be successful.
 If the Bt gene is not present, amplification will not be successful.**

**3) Perform a gel electrophoresis to visualize the presence or absence of amplified sequences.** Amplified sequences will appear as a specific band on the gel.
The presence or absence of this band and comparisons to control samples will confirm whether the food product contains the Bt gene.

**Exercise 1: Extracting corn DNA from food products**

Intact DNA fragments can withstand the kind of processing that occurs to make the corn products you will test. You will choose an unknown food sample from the supply bench. One group will unknowingly chose the negative control sample. The negative control sample is a known, certified, **non-Bt food**. After recording your unknown food sample number, obtain the following materials from the supply bench:

1 screwcap tube with 500 µL InstaGene matrix clean mortar and pestle

1 5ml transfer pipette permanent marking pen

5–50 µL or 20–200 µL micropipette sterile distilled water

pipette tips unknown food sample

**Procedure:**
1. Label the screwcap tube (containing the InstaGene matrix), with the sample number for the food product you chose. Use a permanent marker and write on the cap.

2. Weigh out 2 g of the test food sample and place in the mortar.

3. Using the transfer pipette, add 10 mL of distilled water to the mortar.

4. Grind with the pestle for at least 2 minutes until a slurry is formed. Add 10 mL of water again and mix or grind further with the pestle until the slurry is smooth enough to pipette.

5. Add 50 µL of the ground slurry to the screwcap tube using a micropipette. Recap the tube and shake well.

6. Place the screwcap tube in a boiling water bath for 5 minutes.

7. Remove the tube from the water bath, place it in the centrifuge in a balanced conformation, and spin for 5 minutes at maximum speed.

8. Carefully remove the tube from the centrifuge, being careful not to disturb the contents. The tube will contain pelleted debris at the bottom, and a clear liquid at the top. **The clear liquid on top contains the DNA** if you have obtained a successful extraction. Carry the tube to your bench and proceed to setting up the PCR.

Pelleted debris

Clear liquid containing the DNA

**Exercise 2: Setting up the PCR reactions**

You will set up two PCR reactions using the DNA you extracted from the food sample:
1. **a positive control tube (P)** this positive control tube will contain the sample DNA and specific primers **designed to amplify a target sequence present in all plants**. This is to make sure the extraction was successful. If your DNA extraction was successful, the PCR for the control tube will also be successful.
2. **your experimental tube**
 your experimental tube will contain your unknown sample’s DNA and specific primers **designed to amplify a portion of the Bt gene found in GM corn.** If your DNA extraction was successful AND it contains genetically modified Bt corn, the PCR for the experimental tube will be successful.

**Procedure:**
Obtain two PCR tubes for your sample.
Label one tube with your sample number and the letter **“P” for your positive control.**Label the other tube with your sample number and the letter **“B” for your experimental tube.**

1. Add 20 µL of your sample DNA to each tube. Remember, your sample DNA is in the clear liquid portion of the screwcap tube. Be very careful NOT to add any of the pelleted debris to the PCR tube.
2. I will then add the primers, DNA polymerase and free nucleotides to your PCR tubes.
 The PCR mix that includes the positive control plant primers is colored green.
 The PCR mix that includes the experimental Bt primers is colored red.
3. Tightly cap the tubes and tap each gently on the lab bench to make sure all of the liquid is at the bottom of the tubes.
4. Place the PCR tubes in the thermal cycler.
5. When your PCR is finished (this will be after class is over), I’ll place the tubes in the freezer until our next lab period.

Instrutor adds 20 µL **plant** primers, DNA polymerase, free nucleotides, and other PCR ingredients to this tube

Instructor adds20 µL

 **Bt** primers, DNA polymerase, free nucleotides, and other PCR ingredients to this tube

You add 20 µL of your extracted DNA to each tube

**Exercise 3: Electrophoresis of PCR products**

Next, we’ll visualize the DNA we have amplified using electrophoresis.
First, we’ll cast a 1% agarose gel using Tris-acetate-EDTA (TAE) buffer. While the gel is solidifying, we’ll prepare the PCR samples for electrophoresis.

1. Remove your PCR tubes from the freezer and allow them to thaw.
2. Using a fresh tip each time, add 10 µL of Orange G loading dye to each of your PCR samples. Tap the tubes on the bench to mix and to make sure all of the contents are collected at the bottom of the tube.
3. After the gel has solidified, place it in the gel rig and pour in TAE buffer to cover the gel 5 mm deep. Gently remove the comb, taking care not to rip the wells. Make certain that the sample wells left by the comb are completely submerged. If dimples are noticed around the wells, slowly add buffer until they disappear.
4. Use the image of the gel at the bottom of the next page to write down which tubes will be loaded into each sample well.
5. Draw 20 μL (the entire contents) of the first reaction tube into the micropipette tip.
6. Steady the micropipette over the proper well using two hands. Be careful to **slowly expel any air** in the micropipette tip end before loading the sample.
7. Dip the pipette tip through surface of buffer, position it over the well, and slowly expel the sample. Sucrose in the loading dye weighs down the sample, causing it to sink to the bottom of the well. Be careful not to punch the tip of the pipette through the bottom of the gel.
8. It is very important **not to move or bump the electrophoresis apparatus** or loaded gel after you have put in the samples!
9. Place the top on the electrophoresis chamber, and connect the electrical leads to the power supply – red to red (positive) and black to black (negative). Make sure both electrodes are connected to the same channel of power supply.
10. Turn the power supply on, set the voltage to 130 volts, and press run. Check to make sure that bubbles appear on the electrodes. As electrophoresis continues, the orange loading dye can be seen moving through the gel toward the positive pole of the electrophoresis apparatus.
11. When the orange loading dye has neared the end of the gel (about 1 cm away), the electrophoresis is finished. This will take approximately 1 hour.
12. Turn off the power supply, disconnect the leads from the inputs, and remove the top of the electrophoresis chamber.
13. Carefully remove the casting tray, and slide the gel into a staining tray labeled with your group name.
14. Stain the gel with “Fast Blast” blue dye for 3-5 minutes. Destain by rinsing with warm deionized water and agitation by placing the gel (in a tray with warm water) on the shaker table. Change the warm water approximately every 5 minutes until distinct blue bands appear on the gel and the blue color fades in the rest of the gel.
15. After the gel has been destained, place it on the light box to see whether bands were produced in the reaction tubes. Draw the results of the PCR on the gel image below.

Results of gel electrophoresis for PCR of food samples

Sample name:

Expected results from PCR of control and experimental samples

The “P” tube (green) contains the DNA sample and primers that amplify a target sequence present in all plants. Thus, if you have been able to obtain an adequate DNA extraction, this lane on the gel should show a band.
The “B” tube contains the DNA sample and primers that amplify a target sequence present in the Bt gene. Thus, if you have been able to obtain an adequate DNA extraction **AND** the DNA contains the Bt gene, this lane on the gel should also show a band.

One group in the class prepared the negative control food sample, which was certified non-GMO. Thus, we expect to see a band on the gel for the “P” tube if there was an adequate DNA extraction but NOT a band on the gel for the “B” tube, since we know there is no Bt gene present. If we see a band for this tube, uh oh. That indicates contamination of at least one of the reagents used.

 **Questions:**

1. What are the differences between actual DNA replication that takes place inside a cell and PCR?
2. Why is it necessary to have a primer on each side of the DNA segment to be amplified?
3. How many copies of the gene of interest should be present after 10 complete cycles of PCR?
4. Why did we perform PCR on the food samples in this laboratory exercise?
5. Why do you have to set up a PCR reaction with DNA from certified non-Bt food?
6. What would happen if some of the Bt-positive DNA got into the plant primer mix because of using a dirty pipette tip?
7. What are the purposes of the PCR reactions set up for the Bt-positive control DNA?
8. How would you explain the results of the following PCR experiment?

+P

+B

CP

CB

1P

1B