GM foods are foods that contain components of GM crops—plants that have been genetically modified by the insertion of foreign genetic material. The foreign genetic material may have come not only from another plant but possibly from a species of another kingdom (e.g., animal, fungal, bacterial). The foreign genetic material is usually a gene that codes for a protein that gives the plant an advantage over similar crop plants. Examples of conferred traits include pest resistance, herbicide tolerance, delayed fruit ripening, improved fruit yield, increased nutrient content, etc. Currently, genetically modified (GM) foods do not have to be labeled as such in the US andfoods with less then 5% genetically modified content can be labeled "GMO-free". In Europe and Asia, genetically modified foods do require labeling if they contain >1% GM content.

**The first step** in the genetic modification process is to identify a protein that has the potential to improve a crop. 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. **The second step** is to isolate (clone) the gene that codes for the protein. **The third step** is to engineer the gene so that the crop plant's cells will read it correctly and manufacture the protein of interest. This is done by streamlining the gene to remove unnecessary introns, and adding or changing sequences that will enable the gene to be expressed within the crop's cells, including a promoter and a terminator (see Figure 1). The promoter serves as a docking site for RNA polymerase and a signal for where it should start transcribing a gene. The terminator is the signal to stop transcription. The native promoters and terminators of unmodified genes interact with other components of a host cell to turn genes on or off depending on cell type and situation, but scientists can engineer the constructs for GMOs so that the foreign gene is continually transcribed and the foreign protein is produced throughout the entire plant. The most common promoter used in GM crops is the 35S promoter from the cauliflower mosaic virus (CaMV 35S). This promoter is chosen because it is already designed by nature to activate transcription in all plant cell types. The most common terminator used in GM crops is the nopaline synthase (NOS) terminator from Agrobacterium tumefaciens. **In the fourth step** the engineered gene with the appropriate promoter and terminator, is introduced into the plant. The engineered gene is transferred into the cells of the callus by a variety of methods, each of which must get the DNA past the plant's cell wall, plasma membrane, and nuclear membranes. One method is to use a GM version of the soil bacterium Agrobacterium tumefaciens. This bacterium causes crown gall disease by inserting some of its DNA to a host plant's genome; this unusual natural property is exploited to transfer the engineered gene into the plant genome. A second method is electroporation, in which an electric current creates pores in cell membranes and allows the entry of the engineered DNA. A third method uses a device called a "gene gun" that physically shoots gold particles coated with the engineered DNA into the plant cells.. To assist this process, selectable markers are inserted into the cells along with the engineered gene. These may be antibiotic resistance markers, or visual markers like the gene for Green Fluorescent Protein. Once thetransformed cells have been isolated, they are induced with plant hormones to differentiate and grow into complete plants. **The fifth and final** step in making a GM crop is to back-cross the genetically engineered crop into the most current high-yielding crop strains that are being used in the field.

Since the release of the first genetically modified (GM) crop in the US in 1996, scientists have debated the use of these crops because of potential health and environmental risks. They argue that there is a potential for "superweeds" to arise through cross-pollination of natural weed species with herbicideresistant crops, or that "superbugs" will evolve that are no longer susceptible to the toxins in pest-resistant crops. Many are concerned about potential allergic reactions to novel proteins, antibiotic resistance arising from the selectable markers used to develop the crops, or other unforeseen effects on public health. Others voice concerns that not enough research has been done to fully understand the implications of altering plant diversity. People also voice concerns on the lack of government requirements for labeling of foods in the US. Proponents of GM foods argue that these crops are beneficial for the environment, because they reduce the use of herbicides and pesticides, chemicals that are potentially toxic to the environment and human health. In addition, these crops may preserve arable land by reducing stresses on the land, improve the nutritional value of food in developing.

How does one test foods and crops to identify which contain GM genomes (see Figure 3)? Two methods are currently used. **One**, the enzyme-linked immunosorbent assay (ELISA), identifies proteins. It is an antibody-based test, and it identifies the specific proteins produced by GM plants. ELISA can only test fresh produce, due to protein degradation during food processing. In addition, since ELISA identifies the proteins produced in GMO crops, the tests must be individualized according to the type of crop. For example, a Bt ELISA test can only detect Bt corn, and not herbicide-tolerant GM corn. However, ELISA is inexpensive and accurate, and can be performed in the field with little expertise. **The second test**, using the polymerase chain reaction (PCR), identifies sequences of DNA that have been inserted into the GM plant. In contrast to proteins, DNA is a relatively stable molecule, thus DNA fragments can be isolated from highly processed foods and are sufficiently intact to be amplified by PCR. A modified version of PCR, real-time PCR, can also quantitate the percentage of GM material in the food sample. In contrast to an ELISA test that is specific to a single crop, a single PCR test like this one can detect 85% of all GM crops. This is because genetic engineers use only a small number of regulatory sequences (promoter and terminator sequences) to control the expression of the inserted genes, and so these sequences are common to the majority of GM crops. Two of the most common regulatory sequences are the 35S promoter from cauliflower mosaic virus and the nopaline synthase (NOS) terminator from Agrobacterium tumefaciens, which are the sequences that can be easily detected by PCR.

**Extraction of DNA From Food Samples**

1. Find your screwcap tubes and label one “non-GMO” and one “test”.

2. Weigh out 0.5–2 g of certified non-GMO food and put it into the mortar.

3. Add 5 ml of distilled water for every gram of food. To calculate the volumes of water you

need, multiply the mass in grams of the food weighed out by 5 and add that many milliliters.

Mass of food = \_\_\_\_ g x 5 = \_\_\_\_\_ ml

4. Grind with pestle for at least 2 min to form a slurry.

5. Add 5 volumes of water again and mix or grind further with pestle until smooth enough

to pipet.

6. Pipet 50 μl of ground slurry to the screwcap tube containing 500 μl of InstaGene labeled

“non-GMO” using the 50 μl mark on a graduated pipet. Recap tube.

7. Repeat steps 2–5 to prepare the test food sample.

8. Pipet 50 μl of ground test food slurry to the screwcap tube labeled “test”. Recap tube.

9. Shake or flick the non-GMO food and test food InstaGene tubes and place tubes in

95°C water bath for 5 min.

10. Place tubes in a centrifuge in a balanced conformation and centrifuge for 5 min at max

speed.

11. Store tubes in a refrigerator

**Set Up PCR Reactions**

1. Number PCR tubes 1–6 and initial them. The numbers should correspond to the following

tube contents:

2. Place each tube in a capless microtube adaptor and place in the foam float on ice.

3. Referring to the table and using a fresh tip for each addition, add 20 μl of the indicated

master mix to each PCR tube, cap tubes.

4. Referring to the table and using a fresh tip for each tube, add 20 μl of the indicated DNA to

each PCR tube, being sure to avoid the InstaGene pellet at the bottom of the tubes. Mix by pipetting gently up and down; recap tubes.

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

**Electrophoresis of PCR products**

1. Set up your gel electrophoresis apparatus as instructed.

2. Obtain your PCR tube from the thermal cycler and place in the capless microtube adaptor.

Pulse-spin the tube for ~3 seconds.

3. Using a fresh tip each time, add 10 μl of Orange G loading dye (LD) to each sample

and mix well

4. Load 20 μl of the molecular weight ruler and 20 μl each sample into 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

5. The run time and voltage will depend on the type of gel you are running. Run an agarose

gel for 30 min at 100 V and run a polyacrylamide gel at 200 V for 20 min.

6. Stain in Fast Blast DNA stain. Refer to specific instructions depending on gel type.

Fig. 2. 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 . A molecular weight ruler (lane 5) is electrophoresed with the samples to allow the sizes of the DNA bands to be determined.