DETECTION OF GENETICALLY MODIFIED ORGANISMS BY PCR

Ref. PCROMG (4 practices)

1. EXPERIMENT OBJETIVE

The objective of this experiment is to introduce students to the principles and practice of **Polymerase Chain Reaction (PCR)** as a tool for the detection of genetically modified organisms.

Students will acquire basic knowledge about the molecular biology of the process of obtaining a GMO.

2. BACKGROUND INFORMATION

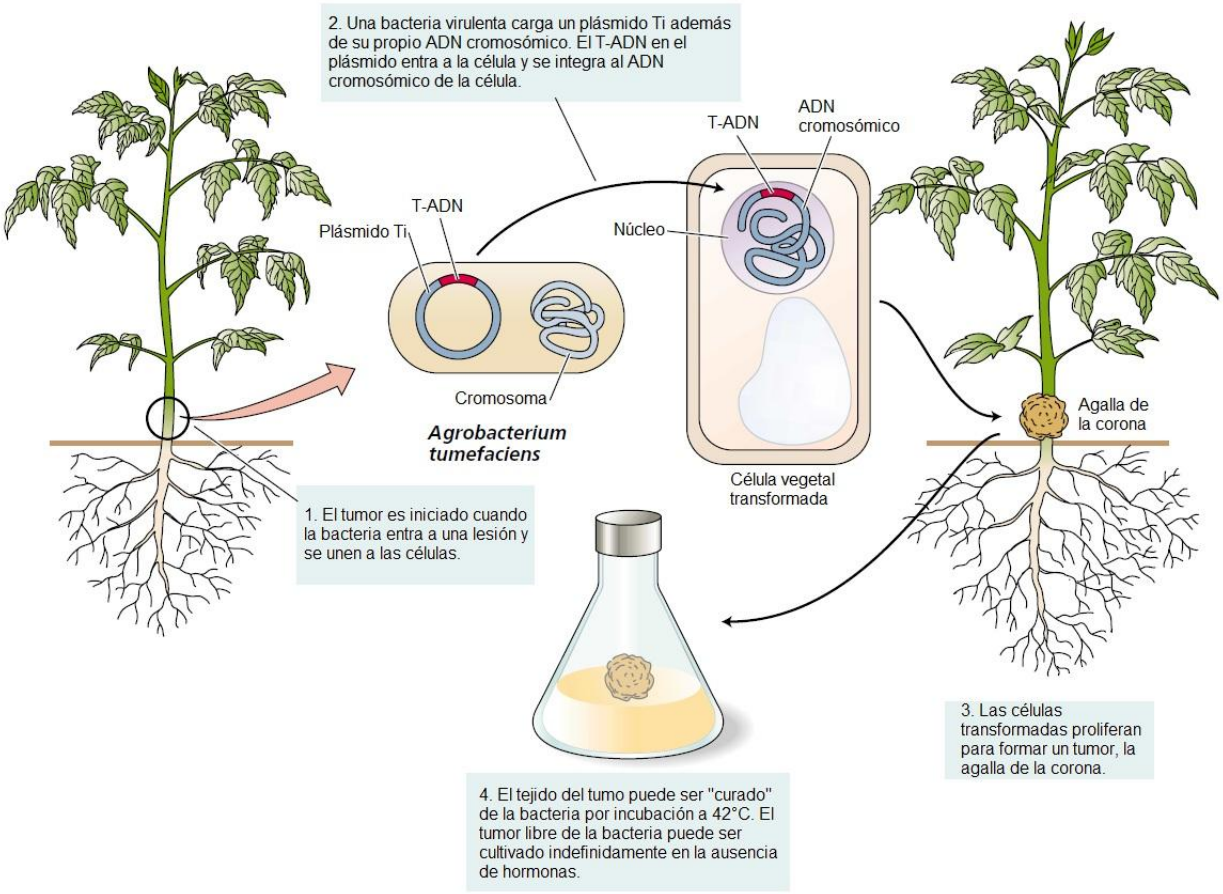
2.1 Genetically modified or transgenic organisms.

Genetic engineering has produced pest-resistant crop plants. The one that gains is the environment, because the use of pesticides is diminished; but the most paradoxical thing is that the organizations that have been dedicated to protect the environment have been those that have been noisier opposed to the introduction of these plants, which are called **genetically modified (GM)**.

The first difficulty in working with this technique is the introduction of the desired DNA fragment (useful gene) into the plant cell, and then into the plant genome.

Gill disease causes the formation of an irregular "tumour" in the stem of the plants, known as gill. The cause is a common soil bacterium called ***Agrobacterium tumefaciens***, which opportunely infects plants where it has been damaged by, say, the biting of herbivorous insects. The bacterial parasite carries out the attack by building a tunnel through which it deposits a packet of its own genetic material into the plant cell. The packet consists of a DNA fragment that is carefully extracted from a special plasmid and then wrapped in a protective cover before being sent through the tunnel. Once delivered the DNA packet, it integrates, as would the viral DNA, into the DNA of the host cell. However, unlike a virus and once it has been lodged, this DNA fragment does not make more copies of itself. Instead, it produces plant growth hormones and specialized proteins that serve as nutrients for the bacteria, simultaneously favouring the division of plant cells and bacterial growth and creating a closed circuit of positive intercommunication: growth hormones cause cells to multiply and in each cell division the invading bacterial DNA is copied together with that of the host cell, so that more and more bacterial nutrients and plant growth hormones are produced.

The consequence of this frenzy of uncontrolled growth is the appearance in the plant of an irregular mass, the gill, very useful for the bacteria because it constitutes a kind of factory in which the plant is forced to produce precisely what the bacterium needs, and even in larger quantities.



Agrobacterium is a prefabricated transfer system for introducing foreign DNA into plants, a natural genetic engineer. So that a gene of choice could be inserted into the ***Agrobacterium*** plasmid and then transferred to the plant cell, so that when the genetically modified bacterium infected the host, it would insert the chosen gene into the chromosome of the plant cell.

As the debate about GM foods revolves around us, it is important to understand that our practice of taking foods that have been genetically modified is actually thousands of years old. In fact, both our domestic animals, the origin of the meat we eat, and the crop plants that supply us grains, fruits and vegetables, are genetically far removed from their wild ancestors.

The effect of centuries of artificial selection: the corn and its wild ancestor teocinte to the left.

Many of the wild ancestors of crop plants offered relatively little to the first farmers: they were difficult to grow and their production was scarce. For agriculture to work, it was necessary to change it. Early farmers understood that the desirable characteristics were maintained from generation to generation involved an inborn modification (we would say genetics). In this way began the huge agricultural program of our ancestors. The activity depended on an artificial selection, according to which the farmers only raised those individuals who had the desired traits, for example, the cows that produced more milk. In effect, farmers did what nature does in the course of natural selection: choosing from the range of genetic variations available, in order to ensure that the next generation is enriched with those that are better adapted to the consumption, in the case of the farmers, and the survival, in the case of the nature. Biotechnology has offered us a way to generate desired variations, so we do not have to wait for them to appear naturally; is not, of itself; more than the last of a series of methods that have been used to *genetically modify* our food.

2.2 Genetically modified corn (Bt corn).

Weeds are difficult to eliminate, there are also herbivorous insects that take advantage of our agriculture, etc. for all these attacks on our agriculture have been and continue to use **pesticides, the full scope of the risks of its use is not very clear**. Farmers who grow organic crops have always had their tricks to avoid pesticides. An ingenious method has a toxin obtained from a bacterium to protect plants from insects. *Bacillus thuringiensis* (Bt) naturally attacks the intestinal cells of insects, resulting in the death of the insect. This has inspired genetic engineers, what if, instead of indiscriminately applying bacteria to crops, the Bt toxin gene could be introduced into the genome of the crop plants? The farmer would not need to sprinkle his crops anymore because every bite of the plant would be deadly to the insect that would ingest it and harmless to us.

Today we have a full range of Bt design crops, including Bt maize, Bt potato, Bt cotton, and Bt soybeans, and the net effect has been that pesticide use has been greatly reduced. It is estimated that since 1996 the result of using Bt crops has been an annual reduction of 9 million liters of pesticides in the United States.

In the European Union are allowed the cultivation of a Bt corn, called **MON810** of the multinational Monsanto. Transgenic maize has been cultivated in Spain since 1998. Since then, varieties of the *Syngenta Bt 176* event (withdrawn from the market since January 2005) and a large number of Monsanto's **MON810** varieties have been grown currently growing. In 2011, 97,346.31 hectares of Monsanto's Bt maize were grown in Spain.

2.3 The MG food debate.

This debate has combined two problems. First, purely scientific questions of whether GM foods pose a threat to our health or the environment. Second, there are economic and political issues focused on the aggressive practices of multinational corporations and the effects of globalization. Much of the rhetoric has focused on companies engaged in agricultural issues, especially Monsanto.

A meaningful assessment of GM food should be based on scientific, non-political and economic considerations. However, let's look at some of the more common statements:

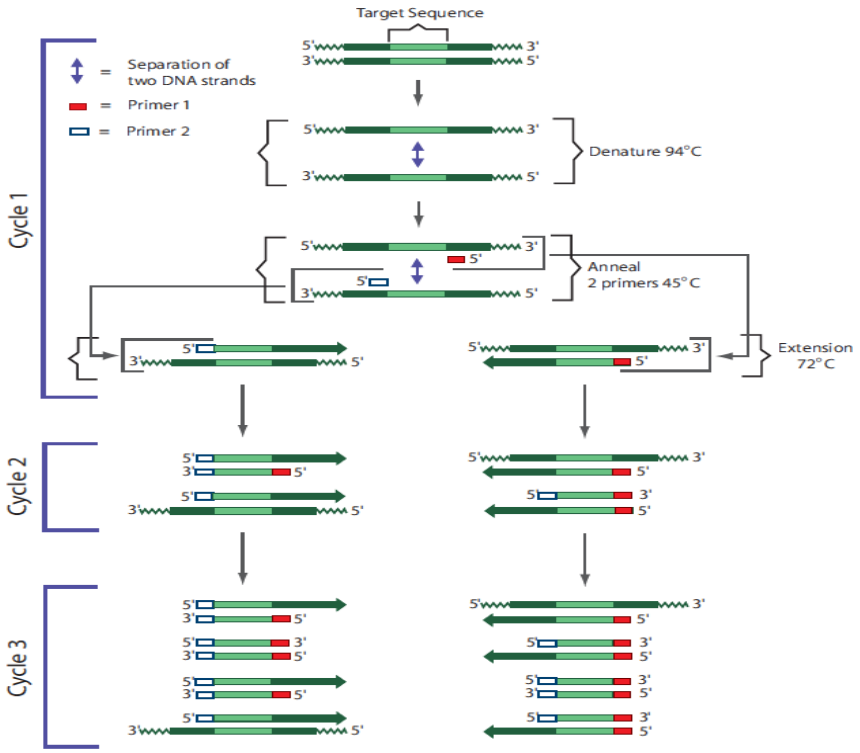
- It is not natural. Currently no one can take a strictly natural diet. Ancient farmers often crossed different species, creating entirely new ones with no direct equivalents in nature. Wheat, for example, is the result of a series of crosses. In this way, our wheat is a combination of the characteristics of several ancestors that perhaps nature would never have invented.
- Produce allergens and toxins in our food.
- It is indiscriminate and will harm the species to which it is not directed. While the toxin incorporated into Bt plants only affects insects that feed on plant tissue, pesticides unequivocally affect all harmful and non-harmful insects that are exposed to them.

- It will carry an environmental disgrace with the appearance of "supercizañas". At this point, the concern is that genes that confer resistance to herbicides migrate from the genome of the crop to that of weeds through interspecies hybridization.

2.4 PCR analysis.

In a PCR reaction, the first step is the preparation of the DNA sample that is extracted from various biological sources or tissues. In PCR, the DNA or gene to be amplified is defined as "target" and the synthetic oligonucleotides used are defined as "primers". A set of 2 primers of between 20-45 nucleotides are chemically synthesized that correspond to the ends of the gene to be amplified. Each primer binds to one end of each DNA strand and is the starting point of the amplification.

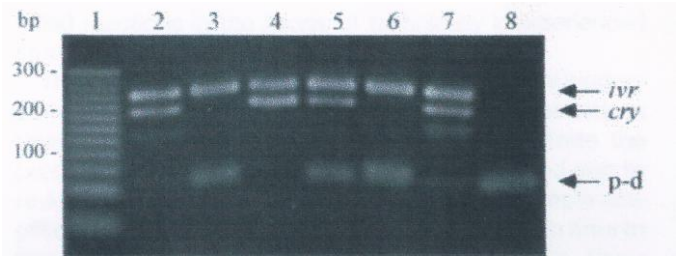
A typical PCR reaction contains template DNA, Taq polymerase and the 4 dNTPS in an appropriate reaction buffer. The total reaction volume is 25-50 µl. In the first step of the PCR reaction, the complementary strands of DNA are separated (denatured) from each other at 94°C, while the Taq polymerase remains stable. In the second step, known as annealing, the sample is cooled to a temperature between 40-65°C allowing hybridization of the 2 primers, each to a strand of the template DNA. In the third step, known as extension, the temperature is raised to 72°C and the Taq polymerase adds nucleotides to the primers to complete the synthesis of a new complementary strand.



These three steps, denatured-annealing-extension, constitute a PCR cycle. This process is repeated for 20-40 cycles by amplifying the object sequence exponentially. The PCR is performed on a thermocycler, an instrument that is programmed for rapid heating, cooling and maintenance of the samples for several times. The amplified product is then detected by removal of the reaction mixture by agarose gel electrophoresis.

PCR detection BT maize

The primers used, on the one hand, amplify endogenous maize regions, in this case the **invertase gene (Ivr1) which will give rise to a fragment of 226 bp**, and, on the other hand, will also amplify foreign genes introduced in the transgenic formation process, in this case **the Bacillus thurigiensis Delta (CryI Ab) endotoxin gene which will give rise to a 184 bp fragment**.



Agarose gel analysis of PCR products from different maize-containing foods. It can be observed that food with normal maize only presents a band of 226 bp (3 and 6), while foods with transgenic maize will present the 2 bands 226 and 184 bp (2, 4, 5 and 7).

In this practice a simulated PCR will be performed, since the instrument to carry out the PCR has a very high cost, for it will be used NON-TOXIC dyes that will migrate in the agarose gel as if they were the amplified DNA fragments.

3. STATEMENT OF FACTS

We will go to the market to buy different food products containing corn to see if it belongs to a normal variety or transgenic.

For this the first thing we will do will be the DNA extraction of the different selected foods: Corn grains brand A; Corn grains brand B; cereal bran brand A; cereal bran brand B; Corn pancakes.

Next, we will use the PCR MIX for the detection of Bt transgenic maize.

4. EXPERIMENT COMPONENTS

COMPONENT		STORE
10x Concentrated electrophoresis buffer	2 x 50 ml	
Agarose	1.75 gr	
Micropipette 20 µl	1	
Tips rack	1	
Samples microtubes	6	at 4ºC

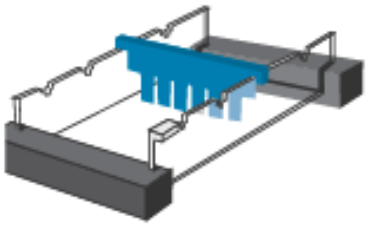
Add 450 ml of distilled water to each 10x Electrophoresis Buffer container to make 2 x 500 ml of 1x Electrophoresis Buffer which is the Working Buffer.

5. EXPERIMENT PROCEDURES

5.1 Agarose gel preparation

A) Mold preparation

Take the mold to make the gels and close the ends with the stops so that the agarose does not go out. Then place the comb to form the wells.



B) Agarose gel preparation

1.b) Use a 100 ml beaker or erlenmeyer to prepare the gel solution.

2.b) **For 7 x 7 cm gels**: Add **32 ml of 1x electrophoresis buffer** plus **0.30 g of agarose**, stir the mixture to dissolve the agarose clumps.

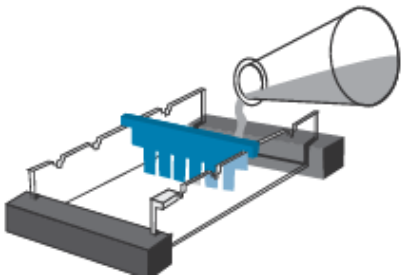
For 7 x 10 cm gels: Add **42 ml of 1x electrophoresis buffer** plus **0.40 g of agarose**, stir the mixture to dissolve the agarose clumps.

Make sure the 450 ml of distilled water has been added to the 10x Electrophoresis Buffer

3.b) Heat the mixture to dissolve the agarose. The fastest method is the use of a microwave, a heating plate can also be used, in both cases, in order for the agarose to dissolve **the solution must be brought to boiling point**. The final solution should appear clear without apparent particles.

4.b) **Cool** the agarose solution to about 55°C (to accelerate the process can be cooled by placing the container under a water tap and shaking). If there is excessive evaporation of the liquid, add electrophoresis buffer.

5.b) Add the agarose solution to the mold.

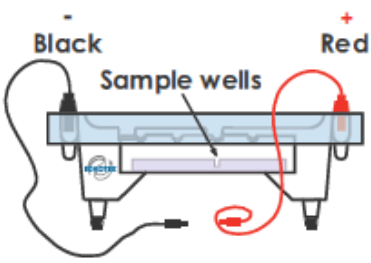


6.b) Allow the gel to solidify. To accelerate the process, the gel can be planted and then put it in a refrigerator (if the electrophoresis is performed the next day, keep the gel at 4ºC).

C) Gel preparation for electrophoresis

1.c) After the gel has solidified carefully remove the stops.

2.c) Place the gel in the electrophoresis chamber correctly oriented with the wells closest to the negative pole (black color).



3.c) Fill the electrophoresis chamber with **300 ml of 1x electrophoresis buffer**. ***The electrophoresis buffer can be used for 2 electrophoresis practice. Once the electrophoresis is finished, store this used buffer in a different container; don't mix a electrophoresis buffer new with one used buffer.***

4.c) Ensure that the gel is completely covered with tampon.

5.c) Remove the comb that has formed the wells very carefully to do not break any well.

6.c) Proceed to the load of the gel and carry out the electrophoresis.

5.2 Gel load and electrophoresis

Note: If you are unfamiliar with loading agarose gels, it is advisable to practice load before performing the experiment, or carry out the complete experiment before doing it with the students.

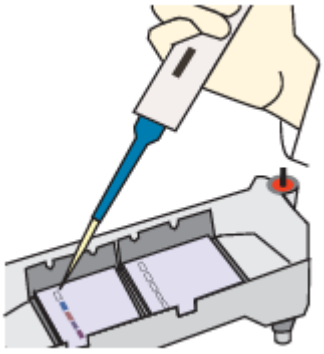
A) Electrophoresis samples

Check the volume of the all samples. Sometimes small drops of the sample may be on the walls of the microtubes. Make sure that the entire amount of sample is uniform before loading the gel. Centrifuge briefly the sample microtubes, or tap microtubes over a table to get the entire sample in the bottom of the microtube.

1.a) Six different samples presented in 6 tubes of a different color each one are supplied, loading the samples in the following order:

WELL	SAMPLE	DESCRIPTION
1	**GREEN**	Molecular weight marker
2	**BLACK**	Corn grains brand A
3	**RED**	Corn grains brand B
4	**LILAC**	Cereal bran brand A
5	**BLUE**	Cereal bran brand B
6	**YELLOW**	Corn pankakes

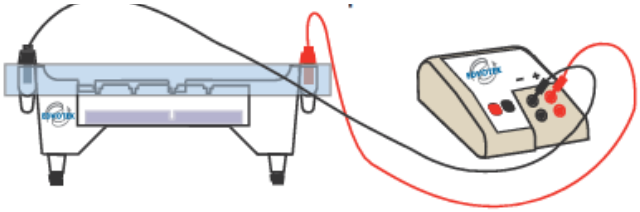
2.b) Load 20 microliters of each sample, using the fixed volume micropipette with a pipette tip supplied.



B) Carry out electrophoresis

1.b) After the samples have been loaded, place the electrophoresis apparatus cover on the electrode terminals carefully.

2.b) Insert the plug of the black cable into the black input of the power supply (negative input). Insert the red cable plug into the red input of the power supply (positive input).



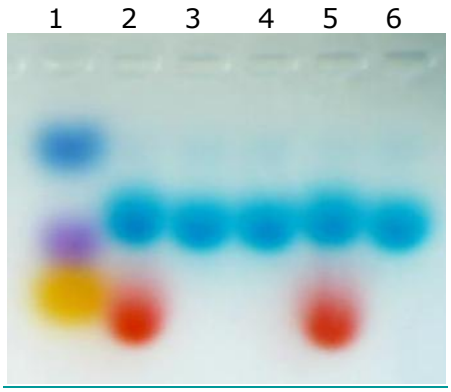
3.b) Set the power supply **at 75 volts (30 minutes) or 150 volts (20 minutes)**. **Watch that the dyes do not come out of the gel.**

4.b) After 10 minutes the separation of the dyes will begin to be observed.

5.b) After the electrophoresis is finished, **turn off the power supply**, disconnect the cables and remove the cover.

6.b) Place the gel in a white light transilluminator (if not available, a sheet of white paper may also be used).

6. PRACTICE RESULTS



1: Molecular weight marker.
2: Corn grains brand A.
3: Corn grains brand B.
4: Cereal bran brand A.
5: Cereal bran brand B.
6: Corn pankakes.

7. QUESTIONS AND ANSWERS ABOUT THE PRACTICE

A series of questions can be asked of students about the practice:

1. **What is the function of the 4 nucleotides (dATP, dCTP, dGTP, dTTP) in a PCR reaction?**

The 4 dNTPs are the components of DNA. For DNA synthesis a template DNA and 2 primers are required, the opposite strand of the template is synthesized following the Watson-Crick base pairing rule.

2. **Why are there 2 different primers?**

They present a different sequence that coincides with the beginning and end of the gene or sequence to be amplified (template DNA).

3. **Which products we have purchased are normal and which GMOs?**

The corn flour of brand B is transgenic while the rest of products containing corn, this is not modified.

Foods that have normal corn are the grams of brand B corn, brand A bran cereal and corn pancakes, while transgenic foods are brand A corn and brand B cereal bran.

4. **Explain how a normal variety of a transgenic variety of Bt maize differs in an agarose gel?**

The PCR reaction contains 2 sets of different primers, one amplifying a fragment of a gene from the maize invertee that will be present in all samples, and the other set will amplify a fragment of the exogenous gene of the *Bacillus thuririgensis* toxin which will also be present present in transgenic varieties.

5. **What is your opinion on genetically modified or transgenic foods?**

For any further questions or queries, please contact us info@bioted.es