DETECTION OF TRANSGENIC CORN BY PCR

Ref. PCR6 (25 students)

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

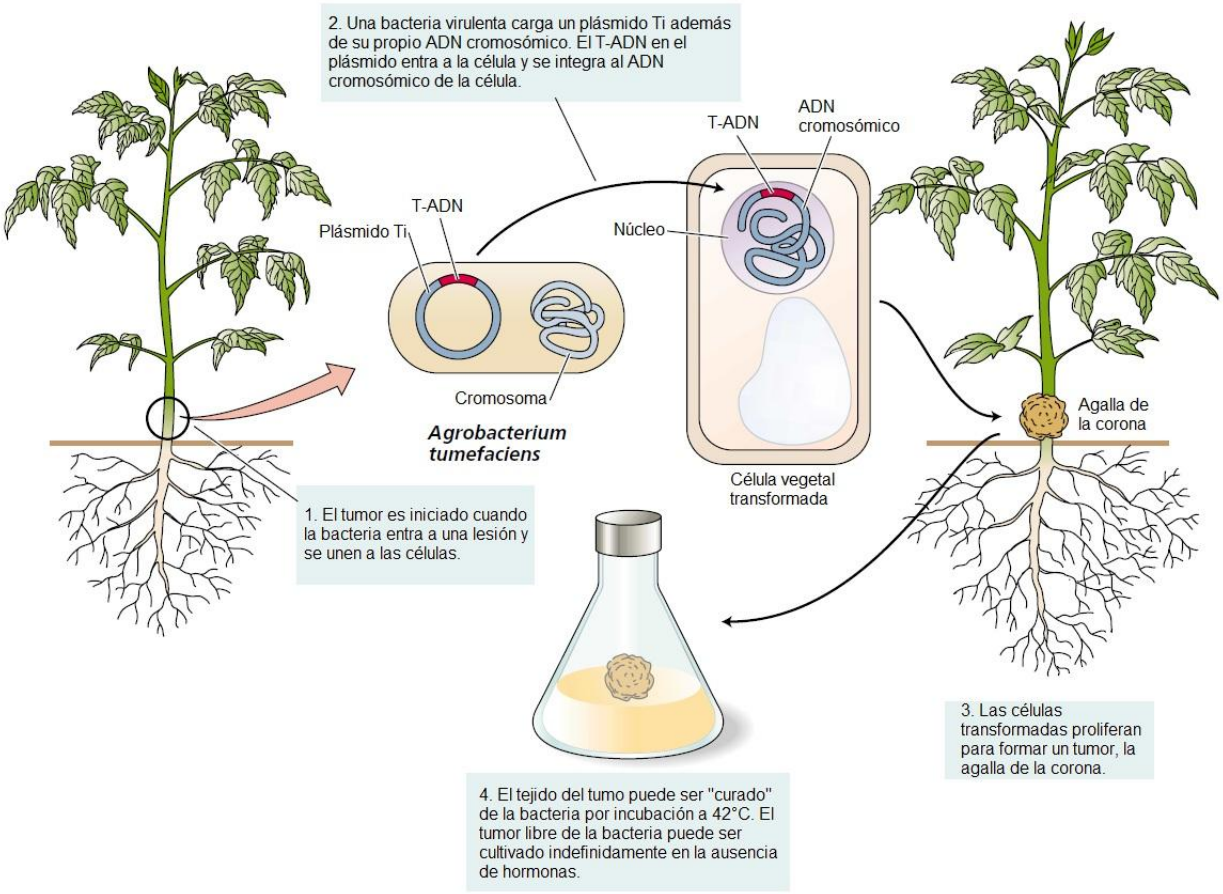
2.1 Genetically modified or transgenic organisms.

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:

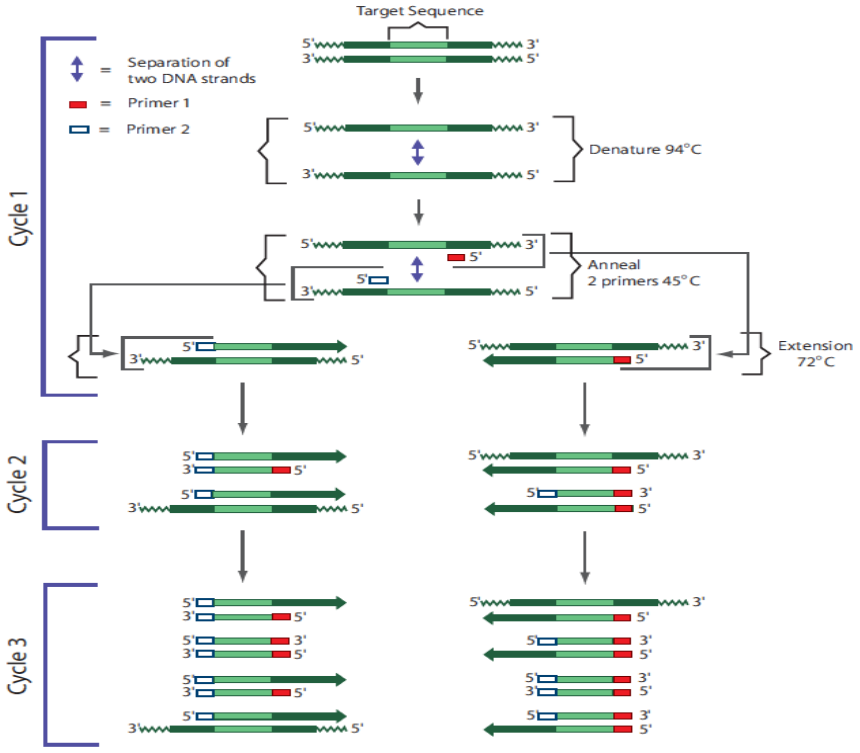
- It is not natural. Currently no one can take a strictly natural diet. Ancient farmers often crossed different species, creating entirely new ones with no direct equivalents in nature. Wheat, for example, is the result of a series of crosses. In this way, our wheat is a combination of the characteristics 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.

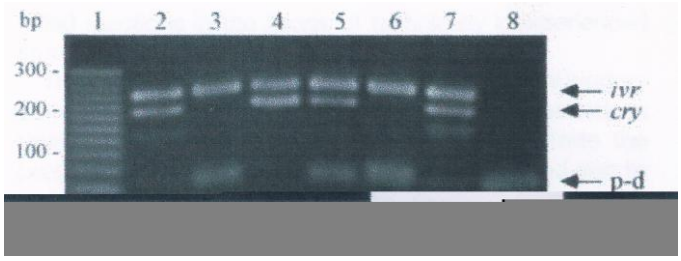
A typical PCR reaction contains template DNA, Taq polymerase and the 4 dNTPS in an appropriate reaction buffer. The total reaction volume is 25-50 µl. In the first step of the PCR reaction, the complementary strands of DNA are separated (denatured) from each other at 94°C, while the Taq polymerase remains 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).

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: 1. Maize flour brand A; 2. Maize flour brand B; 3. Corn flour BIO; 4. Corn grits. Other products with maize can also be used (it is important to work with pulverized samples, for example, using a grinder). **SEE EXTRACTION PROTOCOL (ANNEX 1)**.

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

4. EXPERIMENT COMPONENTS

COMPONENT		STORE
10x Concentrated electrophoresis buffer	100 ml	
Agarose	6.0 gr	
Corn samples for extraction	4 samples	
DNA food extraction Kit	For 25 samples	
Mix PCR detection of transgenic corn	2 x 235 µl	at -20ºC
Positive control normal DNA	10 µl	at -20ºC
Positive control transgenic DNA	10 µl	at -20ºC
Gelsafe DNA staining	25 µl	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 EXTRACTION OF DNA FROM FOOD CONTAINING MAIZE

There will be 4 working groups.

Four food samples containing maize are supplied and the presence of normal or transgenic maize DNA is to be detected: 1. Maize brand A; 2. Maize brand B; 3. BIO corn flour; 4. Corn seed. Other products with maize can also be used (it is important to work with pulverized samples, for example, using a grinder). **SEE EXTRACTION PROTOCOL (ANNEX 1).**

For the extraction of the samples supplied, use 100 mg and for the samples to be evaluated, start with 200 mg.

5.2 PCR REACTION

NOTE: Always use filter tips and change tips every time an action is taken to avoid contamination that can lead to false results.

1. Use 2.5 µl (100-250 ng) of the DNA from each DNA extraction.

IMPORTANT:

a) **Prepare a negative amplification control** by placing 2.5 µl of nuclease-free water instead of DNA, to see if the reagents or micropipettes and tips may be contaminated with DNA, do not amplify nothing.

b) **Prepare a positive amplification control** by placing 2.5 µl of the normal DNA positive control and transgenic DNA.

2. The typical concentrations of the primers and parameters used will depend on each system used. A typical final concentration of primers is 0.5 µM.

REAGENTS	VOLUME
MIX PCR	**22,50 µl**
DNA (100-250 ng)	2,5 µl
Total volume	**25 µl**

3. Mix well; the red dye included in the polymerase facilitates the process.

4. Perform the amplification process.

IMPORTANT:

For activation of the "HOT STAR" Polymerase it is necessary to program an initial denatured step of 10 minutes at 95ºC, then program the 30 or 40 specific cycles of each product to be amplified.

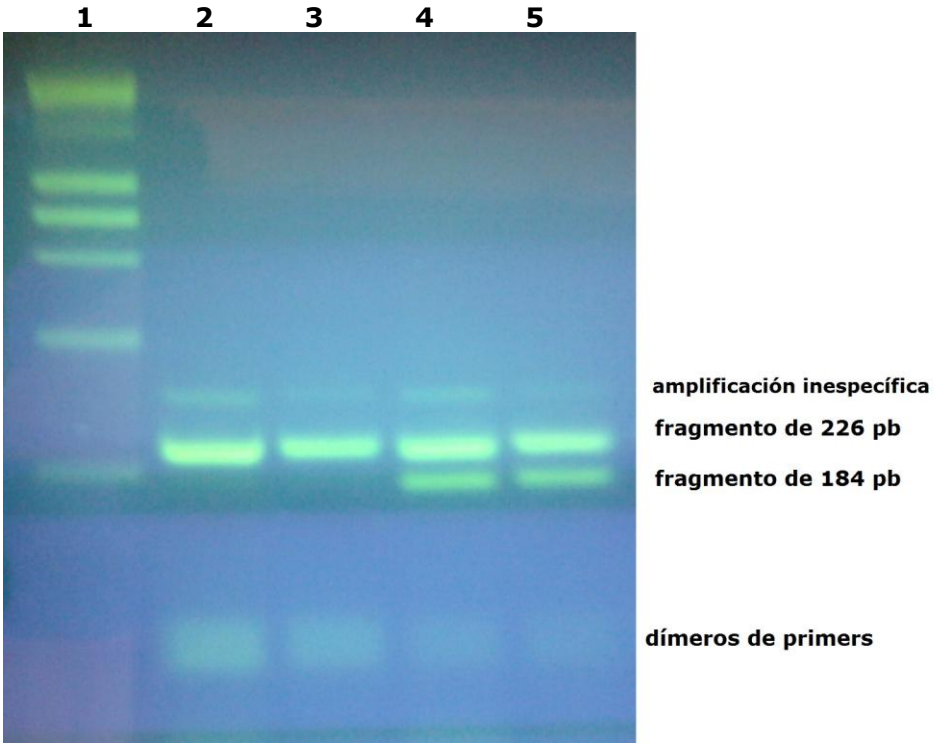
TRANSGENIC PCR PROGRAM

STEP	TEMPERATURE	TIME
Denaturetion HOT STAR	**95ºC**	**10 minutes**
PCR cycles **Carry out 35 cycles**	**95ºC** **66ºC** **72ºC**	**30 seconds** **30 seconds** **45 seconds**
Final extension	**72ºC**	**10 minutes**
Final	**4ºC**	

5. The PCR product can be load directly onto **a 3.0% agarose gel** after PCR, as the red dye acts as a loading buffer.

6. Use the method of DNA detection or staining used in the laboratory. We recommend using the GELSAFE supplied with the kit.

6. PRACTICE RESULTS



3% Agarose gel

1: Molecular weight marker.
2: Positive control of normal DNA.
3: Positive control of normal DNA.
4: Positive control transgenic DNA.
5: Positive control transgenic DNA.

An example of a result of the amplification of the normal positive DNA and transgenic DNA controls provided in the kit is presented. We can observe in the case of normal corn the only band of 226 bp and in the case of transgenic maize the presence of the 2 bands.

It is also observed a larger fragment that is a nonspecific amplification but that does not affect the result, and the formation of dimers of primers, very habitual thing and that is the union of the primers forming major fragments.

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.

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

ANNEX 1

Protocol for extracting DNA from 100-200 mg of foods containing corn

Given the wide variety of samples that comprise maize-containing foods, it is difficult to present a universal protocol for all samples.

The main and most important step to obtain good yields is a good break and homogenization of the sample that will be specific for each type of sample. In all cases and for greater effectiveness liquid nitrogen should be used to spray the sample.

In solid powder samples (flours, etc.) homogenize with a hand electric homogenizer; in large solid samples (corn flakes, chocolate, cookies, etc.) use a coffee grinder to spray a large sample and then weigh the required amount of powder.

1. Weigh 100-200 mg of the sample into a 2.0 ml microtube and add 1**.2 ml of CTAB-1 Buffer**. Vortex vigorously. **Incubate at 70°C for 30 minutes**. Repeat vortex several times during incubation. **In the samples supplied in the kit weigh only 100 mg**.

2. **Centrifuge at 14,000 rpm for 10 minutes**. A pellet will appear and a layer of grease on the surface, introduce the pipette tip through this surface layer, trying to collect only **600 µl of supernatant** which is the transparent liquid with color (avoid taking pellet and surface layer) and place in a microtube 1.5 ml.

3. Add **300 µl of the Lysis/Union Buffer + 25 µl Proteinase K** to the 600 µl supernatant. Mix well. **Incubate at 70 ° C for 10 minutes**.

4. Add **225 µl of Isopropanol**. Mix well.

5. Add **half of the liquid** into the Spin microcolumn reservoir with its collection tube. **Centrifuge at 10,000 rpm for 60 seconds**. Remove the collection tube.

6. Repeat point 5 **with the remaining liquid**.

7. Place the Spin microcolumn into a new collection tube and add **500 µl of Disinhibition Buffer** to the reservoir. **Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

8. Add **500 µl of Wash Buffer** to the Spin microcolumn reservoir. **Centrifuge at 12,000 rpm for 60 seconds**. Remove the liquid.

9. 2nd Washing. Add **500 µl of Wash Buffer** to the Spin microcolumn reservoir. **Centrifuge at 14,000 rpm for 60 seconds**. Remove the liquid.

10. **Centrifuge at maximum speed for 3 minutes to remove residual ethanol**.

11. Remove the collection tube and insert the Spin microcolumn into a 1.5 ml microtube. Add **150 µl of Elution buffer** (preheated to 70°C) in the center of the white membrane. Incubate for 2 minutes.

12. **Centrifuge at full speed for 60 seconds**. The microtube now contains the DNA.