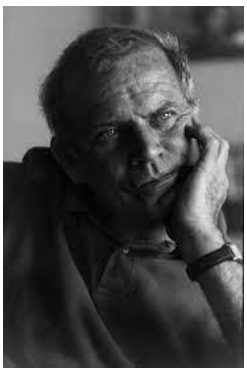
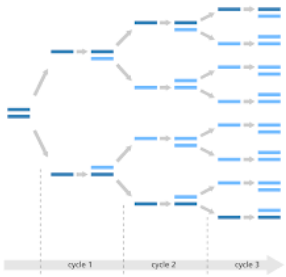
DNA Profiling

By: Sofia Carantón León

For this experiment, we needed to know two processes PCR (Polymerase chain replication) and how the gel electrophoresis is made.



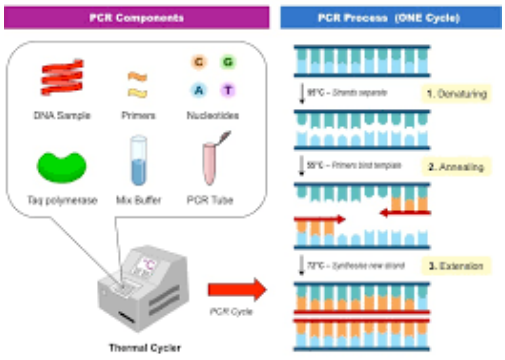
First of all, the **PCR** is a process developed in 1983 by an American biochemist called Kary Mullis. Is a common tool, easy to understand and use, in medicine and biology labs, used to generate copies of small sections of DNA or a gene from a tissue or organism, as skin, hair, saliva, microbes and blood. Same as detecting the presence or absence of a gene for pathogens, while infections or diseases such as tumors and microbes are present and when generating DNA forensic profiles. The process is quicker than others and is able, in a quantitative way, how much of a particular sequence is present. Which later, can be studied in greater detail, helping scientists search for patterns and information.



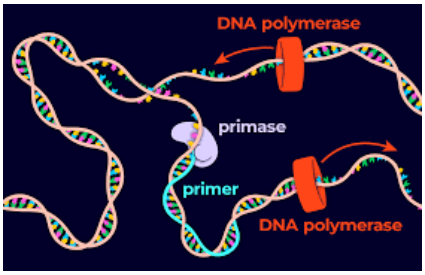
On the other hand, it's important to understand that, like other methods, it has its own limitations. It's a sensitive technique, which can lead to contamination in the samples creating wrong results. Also, this operation needs some prior sequence data. These primers can show non-specifically sequences, that may seem similar but not equal to target DNA. At the same time, some wrong nucleotides can be incorporated into the sequence by a polymerase, although are in a slow rhythm.

This procedure requires a *DNA template*, *primers*, which are short DNA fragments with a defined sequence that specifies the exact DNA product and is used as an extension for DNA polymerase, *nucleotides*, and *DNA polymerase*, the key enzyme that links nucleotides (A, T, C, G) to form a PCR product.

To start the method, the components need to be mixed in a test tube and placed in a machine that is a thermal cycler, whose function is to allow repeated cycled DNA amplification, which happens in three steps. The machine increases and decreases the temperature of the block where the tube is placed, with precise and programmed steps. First, the reaction solution is heated over the melting point of two of the complementary strands of the target DNA, allowing denaturation (two strands separate). Then, the temperature decreases, having the process of hybridization or annealing (accepting the primers to bind the target DNA). This only occurs if they are complementary base pairing. Finally, the temperature increases again, and with this DNA polymerase can extend the primers adding nucleotides to the DNA that is being created.

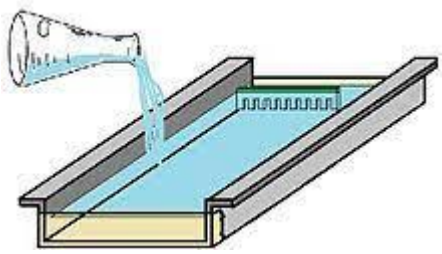


The PCR has been used in many ways since it was created. Now a days is used most commonly in genotyping, cloning, mutation detection, sequencing, microarrays, forensics, and paternity tests.



For reading the PCR results, there are two main methods. The first one is using a chemical dye (ethidium bromide), intercalating between both the strands of a duplex. The second one is using fluorescent dyes for labeling the PCR nucleotides. It allows it to be incorporated into the product. But, although the ones already mentioned, the most used is called gel electrophoresis. The sub-method of this other is called Agarose gel electrophoresis, which is used to visualize, analyze, and determine the PCR product since is the easiest to use.

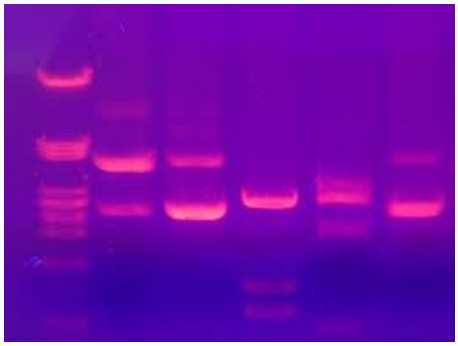
The second process is **gel electrophoresis**, created during the 1930's by Arne Tiselius. which is used to separate the mixtures of proteins, RNA, and DNA, according to their molecular size. This separation occurs by an electrical field through the gel with small pores, that has a speed inversely related to the molecule lengths. Migration is the movement of charged molecules. This means that a negative molecule can migrate and is attracted to a positive charge. (Opposite poles). This method helps to distinguish different DNA fragments. Although, it's important to know that this procedure has a high cost, since many societies can afford it, but it can be home-made.



For building the model with items that can be found at home. The gel can be made with agar found in most Asian food markets. Also, we need color dyes from food such as M&M's or Skittles. The method is made of 5 steps. The first one is the preparation of the gel. For this, in a flask, we add 100ml of water and 2g of baking soda and mix, later we add 1g of Agar and mix again. Heat in a microwave for about a minute until it is clear without grams. And let cool for 15 – 20 minutes. Now the mold is made with a gel comb. Pour the mixture into the mold and wait 1 – 2 hours. The second step is to prepare the dye, for this in a beaker place 3ml of water, and later in each reservoir place a little bit of the water, add 5ml of corn syrup for density in the mixture, and 2 drops of food dyes. And the third and final step, the comb is removed, and it creates six wells for each sample. With a knife cut the ends of the mold for the electrodes of the power supply. After this, pour 2g of baking soda into 100 ml of water submerging all the mold. With stainless steel facing upwards and five batteries of 9 volts. Finally, connect the positive ends to the steel with an alligator clip and draw drops of the dyes into each of the wells, wait for 1- 2 hours.

This procedure has some applications which include forensic applications, paternity tests, and measuring protein levels. Since it is connected with the PCR they share a common administration.

The results can be observed by the distinct color bands. This is because the dyes or the food contains different macromolecules. They separate depending on the speed. To see DNA, the gel is marked with fluorescent dye. When the banding pattern size is visible, it means that the gel has been done correctly. It's important to know that imagining a horizontal line running across the size of DNA can me judge. Also, the estimated size of DNA can be done by matching them with the closest band.

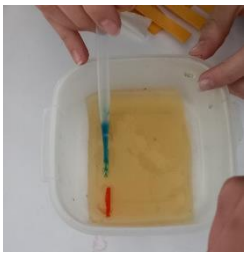


After knowing how these processes work and can be interpreted, a model was made. Which gives the next results:

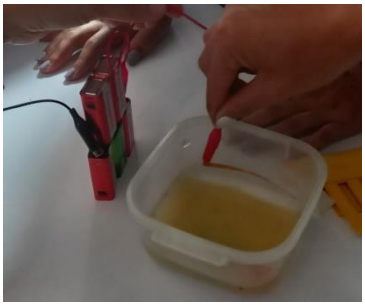
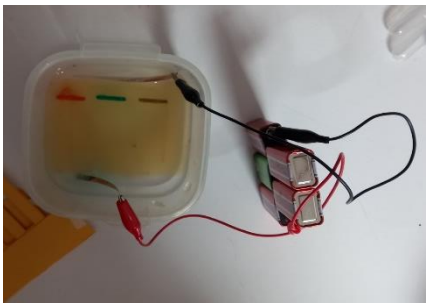
In these images, we see that we are preparing the mold and comb. We use all the ingredients and wait until its at the perfect consistency.

We are cutting and preparing the mold for the color dyes. Adding the mix and the water. With precision and determination for the results. Some of us cut, others prepare and others just observe and guide the procedure. Everyone participated in this stage.

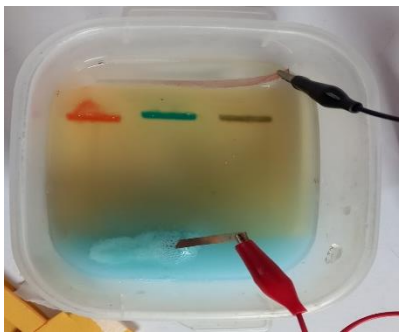
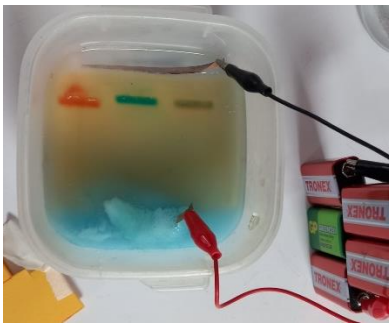





After having the final mixture, we add the dyes with calm, taking turns to do it. We use red, blue and purple. We had green but since this color was created through a combination of colors it would be more difficult to have results.


When the dyes were in position, the batteries and the steel were put in position and connected between them. To start the process of the positive and negative charge molecules.

When some time passed, we saw bubbles and a blue color in one of the charges. This indicated that the procedure was done in the correct way, giving results of the expected source. But at the end, the class wasn't enough for seeing the whole results, so that's why the results were until the half.

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