SIMULATED PCR

Ref. PCR Simulada (4 practices)

1. EXPERIMENT OBJETIVE

The aim of this experiment is to introduce students to the principles and practice of Polymerase Chain Reaction (PCR).

Students will learn the relationship between the number of PCR cycles and the amount of amplified DNA.

2. BACKGROUND INFORMATION

PCR has had an extraordinary impact on several aspects of biotechnology.

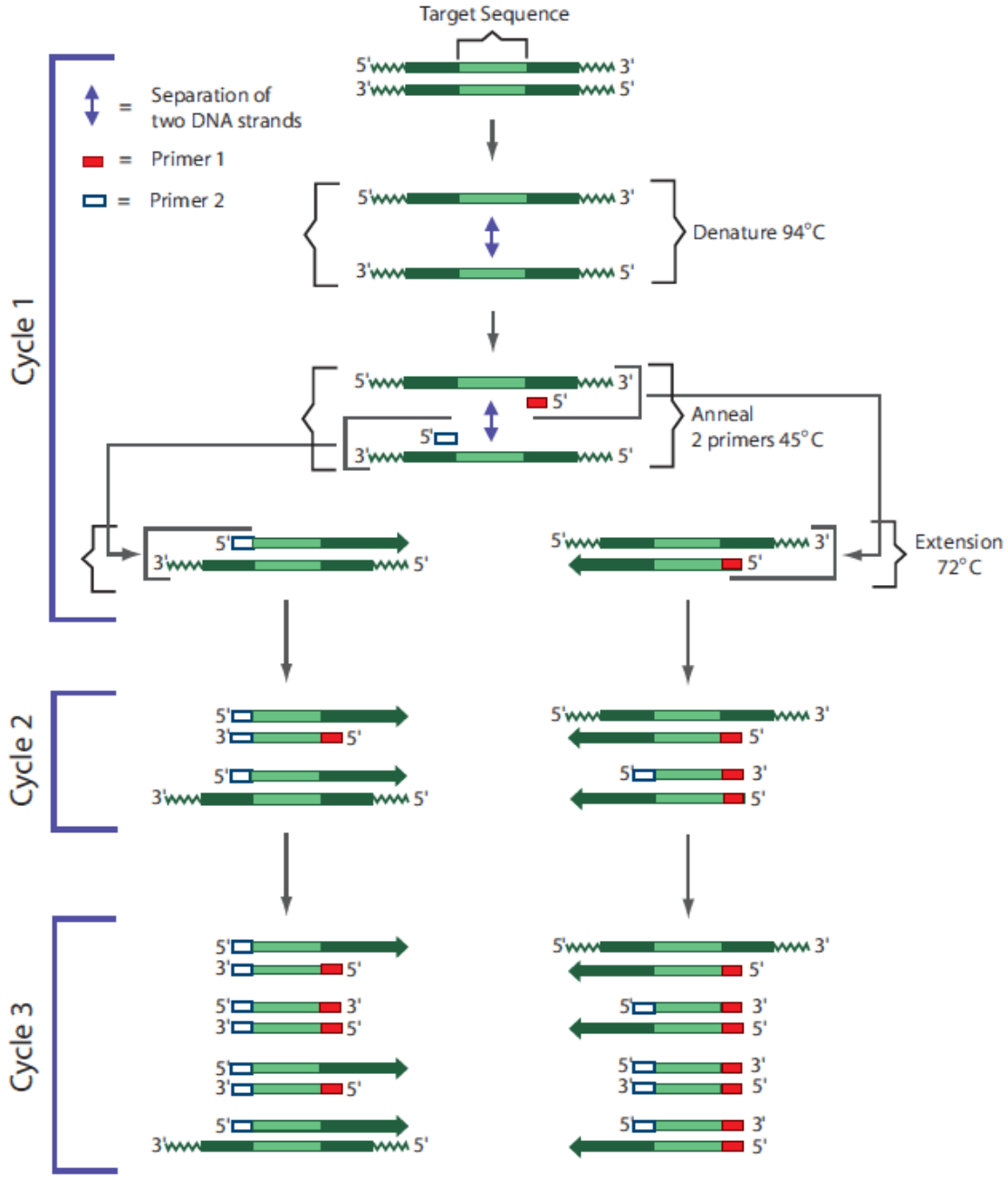
PCR has revolutionized research and diagnosis based on Molecular Biology. PCR is a simple, accurate and highly reproducible process that provides the advantage of starting with a small amount of DNA and being able to amplify it so that it has enough to perform experiments.

A large number of diagnostic tests have been developed, it has also been used in DNA mapping and sequencing in genome projects and is being used in forensic determinations, paternities, clinical diagnosis, etc.

In all cases DNA segments are amplified and subsequently subjected to various analyzes and studies.

In a PCR reaction, the first step is the preparation of the DNA sample that is extracted from several 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 to 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, denaturation-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.

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, in addition the relationship between the number of cycles and the amount of amplified DNA can be observed.

3. 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	5	at 4ºC

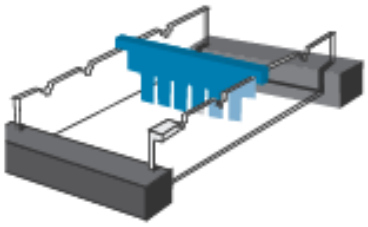
Add 450 ml of distilled water to each 10x Electrophoresis Buffer container to make 2 x 500 ml of 1x Electrophoresis Buffer which is the Working Buffer.

4. EXPERIMENT PROCEDURES

4.1 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.

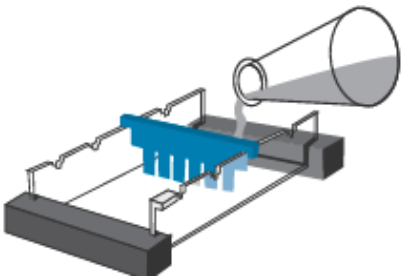
For 7 x 10 cm gels: Add **42 ml of 1x electrophoresis buffer** plus **0.40 g of agarose**, stir the mixture to dissolve the agarose clumps

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.

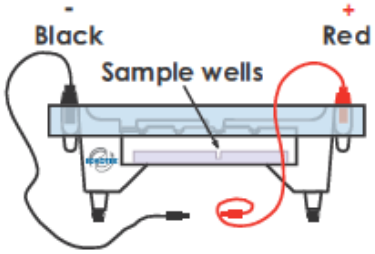


6.b) Allow the gel to solidify. To accelerate the process, the gel can be planted and then put it in a refrigerator (if the electrophoresis is performed the next day, keep the gel at 4ºC).

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.

4.2 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.

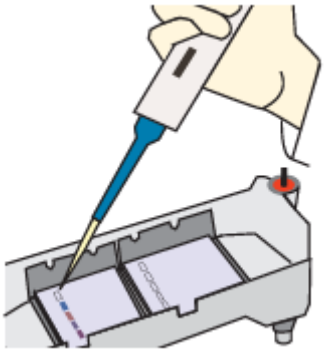
A) Electrophoresis samples

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) Five different samples presented in 5 tubes of a different color each are supplied, loading the samples in the following order:

WELL	SAMPLE	DESCRIPTION
1	**Green**	Molecular weight marker
2	**Black**	Sample after 10 cycles
3	**Lilac**	Sample after 20 cycles
4	**White**	Sample after 30 cycles
5	**Yellow**	Sample after 40 cycles

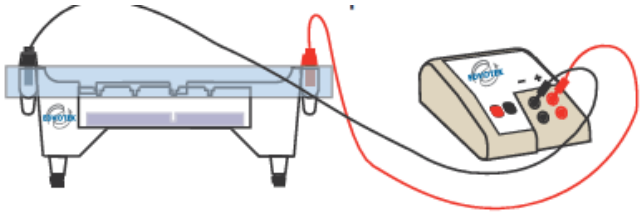
2.b) Load 20 microliters of each sample, using the fixed volume micropipette with a pipette tip supplied.



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).



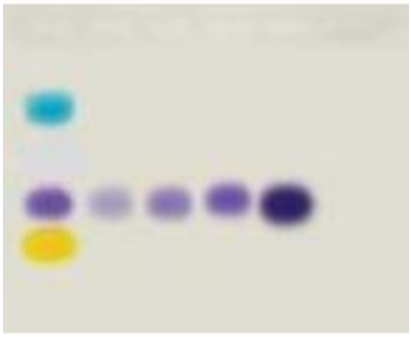
3.b) Set the power supply **at 75 volts (30 minutes) or 150 volts (20 minutes)**. **Watch that the dyes do not come out of the gel.**

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).

5. PRACTICE RESULTS



1. In this simulated PCR it is possible to observe how the intensity of the dye band increases with the number of cycles, this is what happens to a larger number of cycles, more quantity of our amplified DNA fragment and that is what observed on an agarose gel with DNA fragments.
2. The molecular weight marker is made up of DNA fragments of known size and serves as a control because you know the size of your fragment that you want to amplify. In our experiment, we are amplifying a fragment that must match the deep blue fragment.

6. QUESTIONS AND ANSWERS ABOUT THE PRACTICE

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

1. **Why is DNA amplification important?**

A large number of diagnostic tests have been developed, it has also been used in DNA mapping and sequencing in genome projects and is being used forensic determinations, paternities, etc. In addition, when a initial sample is limiting (low quantity), the PCR process can produce enough DNA to make the analysis possible.

2. **What is the difference between the PCR reaction and cells replication in cells?**

In replication (DNA synthesis in cells) a large amount of proteins are involved in all steps of cell division and synthesis and the reactions are carried out at 37°C (body temperature). In the PCR, only the synthesis is carried out and it is realized at non-physiological temperatures.

3. **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.

4. **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).

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