PATERNITY TEST

Ref. Paternity PCR (4 practices)

1. EXPERIMENT OBJETIVE

This experiment introduces students to the use of DNA and PCR to simulate a paternity determination.

Students will learn how agarose gel electrophoresis separates different dye molecules by their charge that will mimic DNA fragments.

Students will learn how these fragments form a unique pattern for each person, which is basic for **DNA fingerprinting analysis** used in paternity determinations.

2. BACKGROUND INFORMATION

The DNA present in the nucleus of any cell is the genetic material that acts as a translator for the synthesis of proteins of each cell. However, in mammals, a long fraction of the total DNA does not code for proteins and its function is not very clear. Polymorphic DNA refers to the regions of the chromosome that varies from one individual to another. By examining several of these regions within the genomic DNA obtained from a person, one can determine the **DNA fingerprinting** for this person.

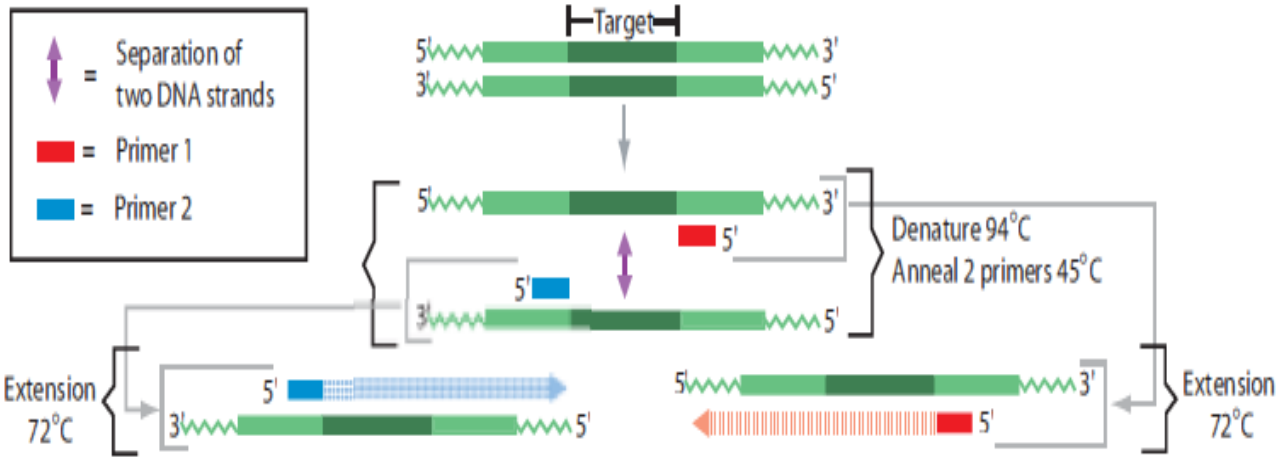
DNA polymorphisms are widely used to determine paternity, kinship, identification of human remains and the genetic basis of various diseases.

DNA fingerprinting allows the identification of the origin of a DNA sample, this is very important in many forensic cases, since it can allow a positive identification with very precision, matching the DNA obtained from a scene of a crime against the DNA of suspected suspects.

PCR is currently being used for DNA forensics. This technique requires much less DNA (less than 500 times) than RFLP analysis and is a much faster method.

PCR amplification uses an enzyme known as **Taq polymerase**, which was originally purified from bacteria that live in high temperature (near-boiling) locations. The PCR reaction includes 2 synthetic oligonucleotides (15-30 nucleotides), known as **primers**; Taq; nucleotides; and extracted DNA, known as **template**.

The region of DNA to be amplified is called **target**. 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 exponentially the object sequence. The PCR is performed with a **thermocycler**, an instrument that is programmed for rapid heating, cooling and maintenance of the samples for several times.

In these cases, PCR is used to amplify and examine highly variable DNA regions, these regions varying in length from one individual to another are classified into 2 categories:

1. **VNTR (variable number of tandem repeats)**, a variable region composed of sequences of 15-70 base pairs, typically repeated 5-100 times.
2. **STR (short tandem repeats)**, similar to the VNTR but the repeat sequence is only 2-4 base pairs.

By examining several different VNTRs or STRs of the same person, researchers can obtain a unique DNA pattern for each personl that is different from another person (except for identical twins). For example, the FBI uses 13 different markers so the hit percentage is 99.9%.

In this experiment, DNAs (represented by dyes) will be analyzed simulating the analysis of several VNTRs. In this hypothetical case, the dyes represent DNA fragments.

The DNA pattern obtained is very simple to analyze directly on an agarose gel. DNA extracted from 2 prospective parents and the mother is analyzed at several polymorphic sites (VNTRs), and then compared with the same study of VNTRs performed on the child to see if the parent can be determined.

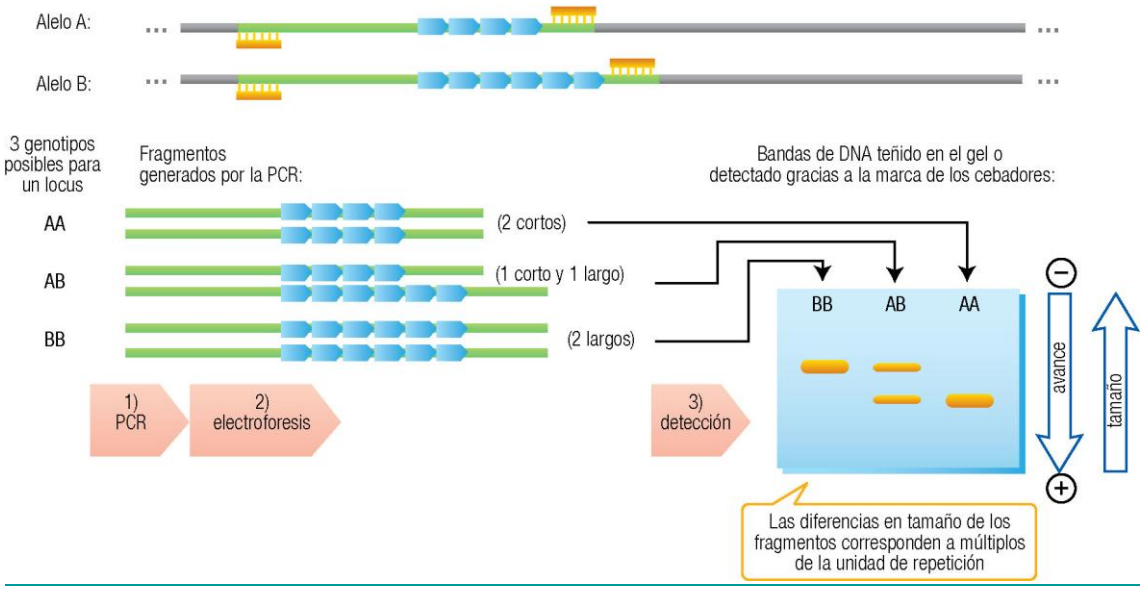


Diagram explaining the process for a single locus or VNTR

This particular VNTR produces an A allele which by PCR amplification yields a short fragment and a B allele which, having 2 repeat units more as seen in the drawing will produce a larger fragment when amplified by PCR.

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

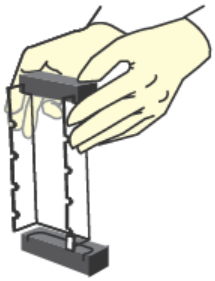
Add 450 ml of distilled water to each 10x Electrophoresis Buffer container to make 2 x 500 ml of 1x Electrophoresis Buffer which is the Working Buffer.

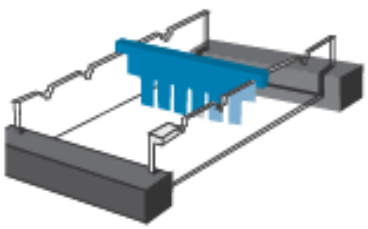
4. EXPERIMENT PROCEDURES

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.

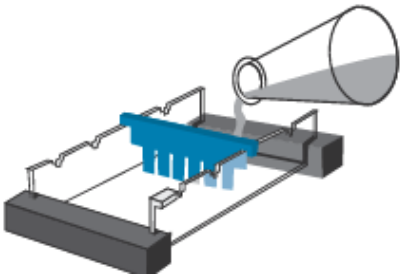
For 7 x 10 cm gels: Add **42 ml of 1x electrophoresis buffer** plus **0.40 g of agarose**, stir the mixture to dissolve the agarose clumps.

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.

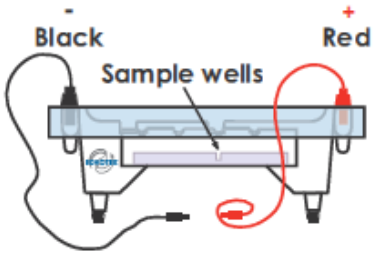


6.b) Allow the gel to solidify. To accelerate the process, the gel can be planted and then put it in a refrigerator (if the electrophoresis is performed the next day, keep the gel at 4ºC).

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.

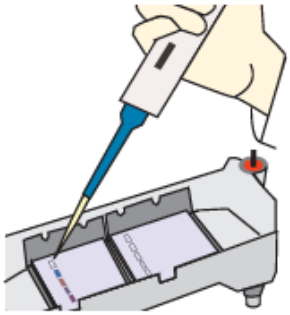
A) Electrophoresis samples

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 one are supplied, loading the samples in the following order:

WELL	SAMPLE	DESCRIPTION
1	**GREEN**	MOLECULAR WEIGHT MARKER
2	**RED**	FATHER 1
3	**LILAC**	FATHER 2
4	**BLUE**	SON
5	**YELLOW**	MOTHER

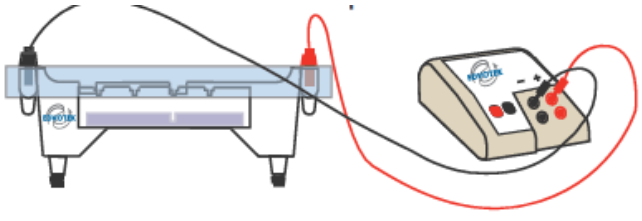
2.b) Load 20 microliters of each sample, using the fixed volume micropipette with a pipette tip supplied.



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).



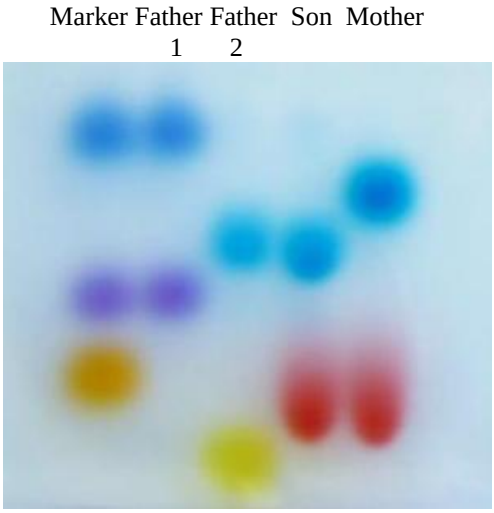
3.b) Set the power supply **at 75 volts (30 minutes) or 150 volts (20 minutes)**. **Watch that the dyes do not come out of the gel.**

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



Each dye stain simulates a genetic marker (VNTR). From the rather simplified simulation of several VNTRs, since normally 13 different markers are usually used so that the percentage of success is 99.9% and its pattern in the agarose gel:

It is shown that the father of the child is Father 2 because they share the blue marker and with the mother shares the orange marker (remember that each parent provides half of genetic material), instead does not share any marker with the father 1.

6. QUESTIONS AND ANSWERS ABOUT THE PRACTICE

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

1. **Who is the father?**

It is shown that the father of the child is Father 2, as they share the blue marker and with the mother share the orange marker (remember that each parent provides half of genetic material).

2. **What do the different dyes of the electrophoresis represent?**

The different dyes represent different length DNAs obtained from the possible parents, child and mother.

3. **Why do different person, like siblings, have a different (individual) genetic footprint?**

The chromosomes are in pairs, one is acquired from the mother and the other from the father. There are 2 copies of a specific gene at a chromosome locus and represent the only genotype for the given offspring, so it is possible that copies of the same gene in siblings have been received differently.

4. **What is the basis of the genetic fingerprint analysis of mitochondrial DNA?**

Mitochondrial DNA is inherited from the mother as the egg contains a large amount of mitochondria compared to that of sperm.

5. **What is the difference of the genetic fingerprint analysis using mitochondrial DNA and cellular DNA?**

Unlike mitochondrial DNA and cellular nuclear DNA, it is found in equal proportions in both progenitors. In each pair of chromosomes one is inherited from the mother and another from the father.

6. **What determines that each person has a unique pattern of their DNA?**

Variations in individual DNA sequence (VNTR, SRT) called **polymorphisms** will produce different patterns for each person.

7. **What do you think if 2 people have the same pattern of DNA?**

They are genetically identical twins.

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