CSI TEST

Ref. PCR detectives (4 practices)

1. EXPERIMENT OBJETIVE

This practice introduces students to using DNA and PCR to simulate how DNA obtained from a hair or saliva sample from a crime scene can be used to identify a criminal. To do this they use dyes that migrate in the agarose gel as if they were fragments of DNA.

Students will learn how restriction enzymes cut DNA molecules into specific DNA sequences by producing DNA fragments of different lengths.

Students will learn how agarose gel electrophoresis separates different sizes of DNA fragments.

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

2. BACKGROUND INFORMATION

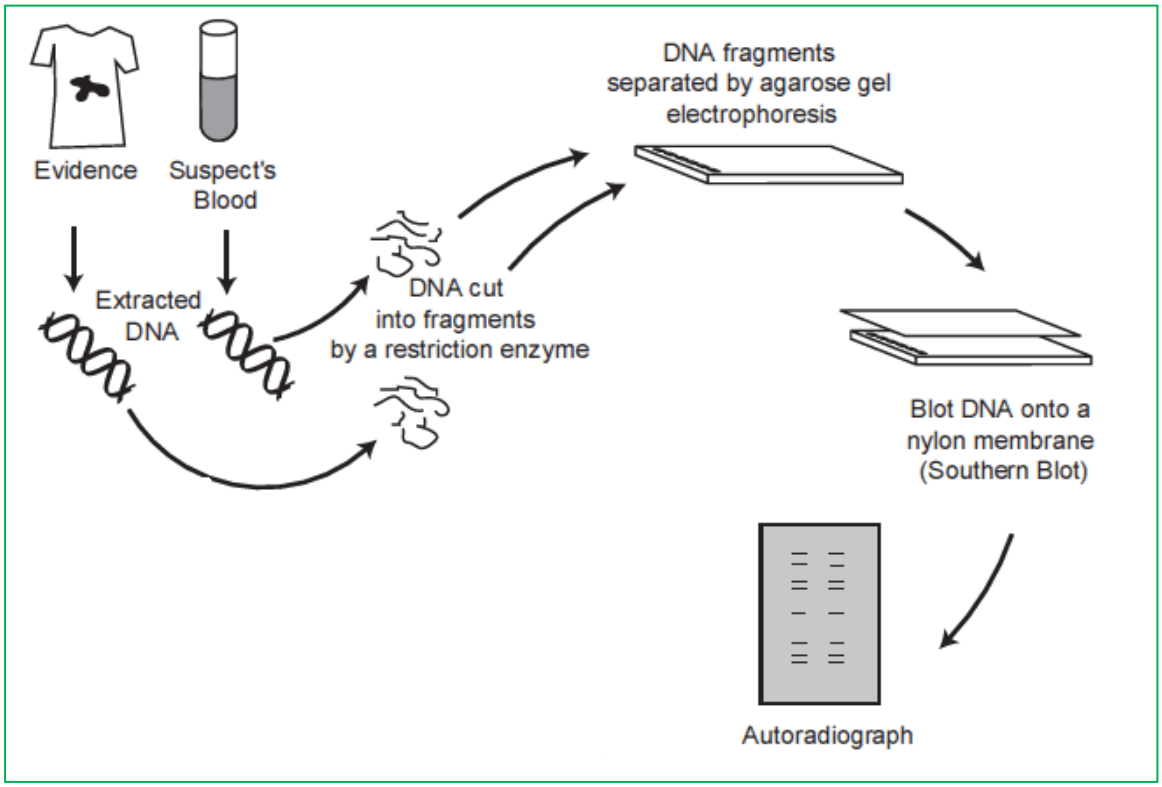
The DNA present in the nucleus of any cell is the genetic material that acts as a translator for the synthesis of proteins of each cell. However, in mammals, a long fraction of the total DNA does not code for proteins and its function is not very clear. Polymorphic DNA refers to the regions of the chromosome 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.

Several steps are involved in DNA fingerprinting. First, a sample must be obtained, forensics must be very careful in getting evidence of the scene of a crime where the DNA is allegedly not damaged, such as blood or hair. The DNA is then extracted and digested with restriction enzymes, or subjected to a PCR (**Polymerase Chain Reaction**).

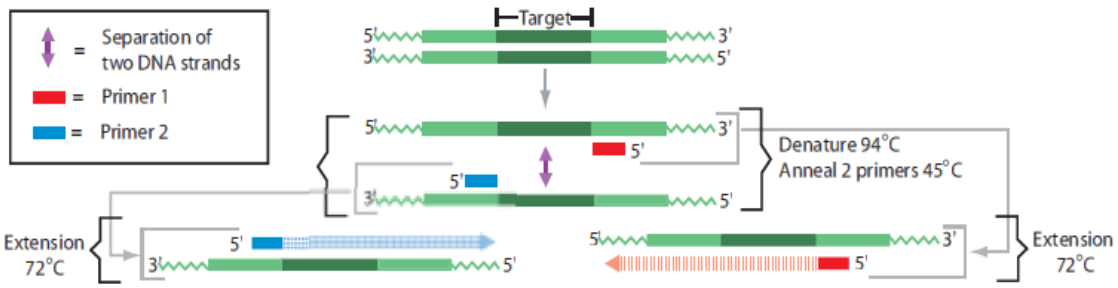
The method called **Restriction Fragment Length Polymorphism** (RFLP) involves the digestion of DNA with restriction enzymes, transfer of DNA to a membrane (**Southern blot**), and membrane hybridization with a probe of polymorphic regions (**autoradiography**). This method takes several days and requires a lot of DNA, which is sometimes quite difficult to obtain in evidence of a crime. In spite of this, it is a method with a very high precision.



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).

In this experiment, DNAs (represented by dyes) will be analyzed simulating the analysis of 2 VNTRs. In this hypothetical case, the dyes represent DNAs obtained from the scene of a crime and 2 suspects.

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	6	at 4ºC

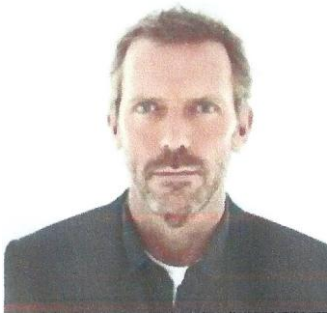
Add 450 ml of distilled water to each 10x Electrophoresis Buffer container to make 2 x 500 ml of 1x Electrophoresis Buffer which is the Working Buffer.

STATEMENT OF FACTS

Peter Polimerasa, 50, has been found dead in his residence in Gataca. There have been signs of a fight and a blood-stained candlestick with which he is believed to have been beaten and has finally killed him. The main motive of the crime seems to be a robbery, since Pedro Polimerasa is a wealthy businessman. But soon the interrogations of the police cause to have 2 suspects:

Malena Drosofila, 47, wife of the victim and heiress of the entire empire of her husband, since they have no children.

Carla Ecoli, 28, lover of the deceased during the last 4 years. He said that the deceased had promised several times that he would leave his wife to marry her, which he never did.



Murdered: Peter Polimerasa

Wife: Malena Drosofila

Lover: Carla Ecoli

Evidences have been collected from the scene of the crime, including blood and hairs samples that will be evaluated if they belong to one of the suspects.

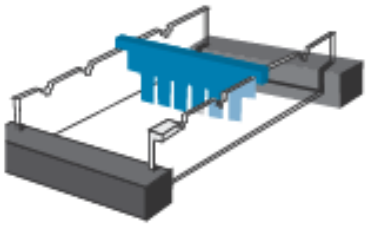
The DNA pattern obtained is very simple to directly analyze on an agarose gel. The aim is to analyze and compare the pattern of DNA fragments after electrophoresis and to determine whether Malena Drosofila or Carla Ecoli was at the scene of the crime.

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.

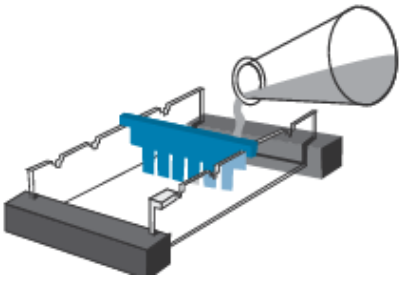
For 7 x 10 cm gels: Add **42 ml of 1x electrophoresis buffer** plus **0.40 g of agarose**, stir the mixture to dissolve the agarose clumps.

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.

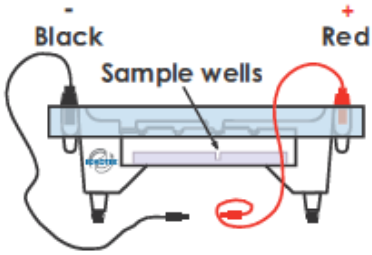


6.b) Allow the gel to solidify. To accelerate the process, the gel can be planted and then put it in a refrigerator (if the electrophoresis is performed the next day, keep the gel at 4ºC).

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.

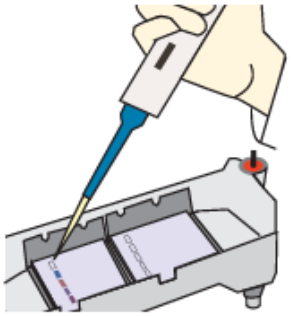
A) Electrophoresis samples

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

Well	Sample	Descripción
1	**Green**	DNA 1 crime scene sample (hair)
2	**Red**	DNA 2 crime scene sample (blood)
3	**Lilac**	Malena Drosofila DNA 1
4	**blue**	Malena Drosofila DNA 2
5	**Yellow**	Carla Ecoli DNA 1
6	**White**	Carla Ecoli DNA 2

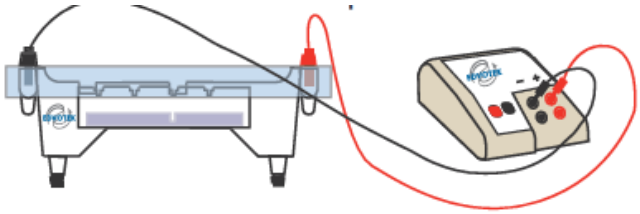
2.b) Load 20 microliters of each sample, using the fixed volume micropipette with a pipette tip supplied.



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).



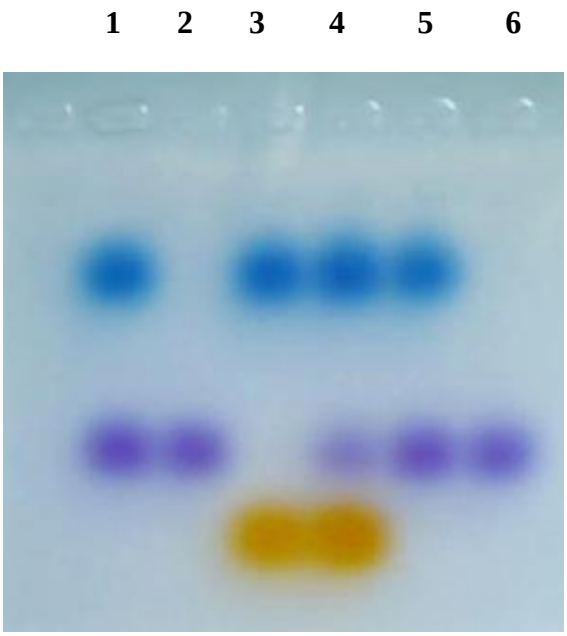
3.b) Set the power supply **at 75 volts (30 minutes) or 150 volts (20 minutes)**. **Watch that the dyes do not come out of the gel.**

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. DNA 1 crime scene sample (hair).
2. DNA 2 crime scene sample (blood).
3. Malena Drosofila DNA 1.
4. Malena Drosofila DNA 2.
5. Carla Ecoli DNA 1.
6. Carla Ecoli DNA 2.

From the rather simplified simulation of several of 2 VNTR or STR (since normally the **FBI** uses 13 different markers so that the percentage of success is of 99.9%) and its pattern in the agarose gel: It is shown that the DNA found in the crime scene belongs to **Carla Ecoli**.

6. QUESTIONS AND ANSWERS ABOUT THE PRACTICE

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

1. Why is the position of the samples near the negative electrode important?

The DNA is negatively charged and will travel to the positive pole.

2. What kind of evidence can be observed in a crime scene to obtain DNA?

E. g. Hair, blood, skin.

3. How will you be able to determine who perpetrated the crime?

The criminal's DNA pattern must match that found at the crime scene.

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

Variations in DNA sequence at individual level (VNTR, SRT) will produce different patterns for each person.

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

They are genetically identical twins.

6. Who is the suspect who has been at the scene of the crime and possibly may have committed the crime?

Carla Ecoli.

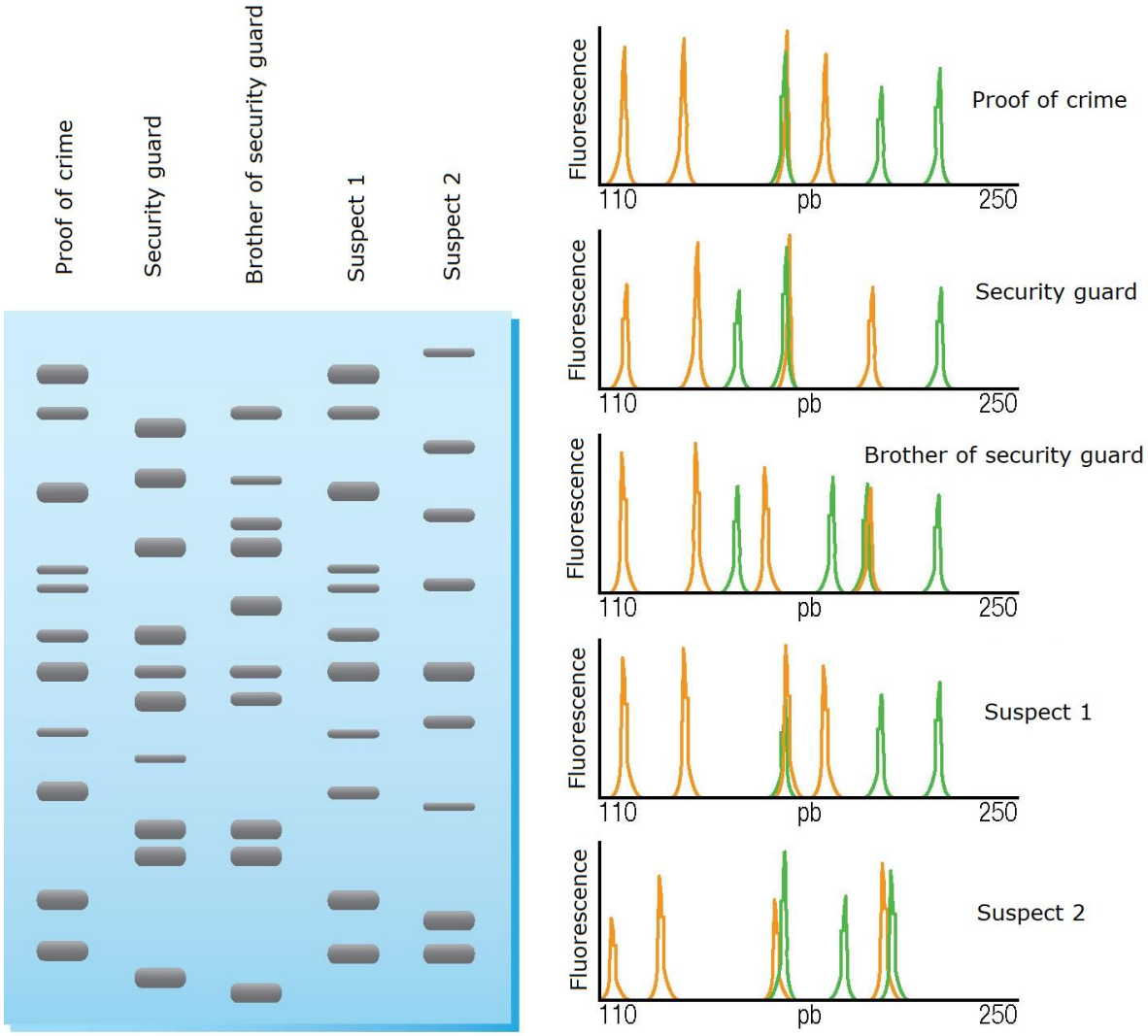
7. Classic Method vs Modern Method

In the following diagram we can see how it is actually done, using many genetic markers (VNTR or STR) that allow giving reliability in the results of incriminate someone. We see on the left the traditional method practiced by practically all the laboratory technician and the modern method where everything is done by modern laboratory equipment.

According to the results, what is the suspect of DNA isolated from the blood stain?

An example of application of the genetic fingerprint (DNA fingerprint) at the investigation of a crime:

There has been a theft of a work of art in a museum. Breaking the case, the thief left a blood stain from which the researchers have isolated the DNA. Police interrogated the security guard, a brother of his and two habitual criminals, and obtained DNA samples from all of them. Aside from identifying the likely offender, it can be seen how the footprint is more similar between family members than between unrelated individuals.



Traditional analysis: VNTR polymorphism at multiple loci of the same repetitive sequence. Detection by Southern hybridization or by PCR and gel electrophoresis.

Modern analysis: Polymorphism of several different STR markers (4 in the example, from 8 to 13 in reality). Multiplex PCR detection with fluorescent primers and capillary electrophoresis.

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