STUDY OF ALU HUMAN POLYMORPHISMS BY PCR

Ref. PCR3

1. EXPERIMENT OBJECTIVE

The objective of this experiment is to introduce students to the principles and practice of Polymerase Chain Reaction (PCR) by studying Alu polymorphisms between individuals using the PCR technique.

2. BACKGROUND INFORMATION

2.1 PCR

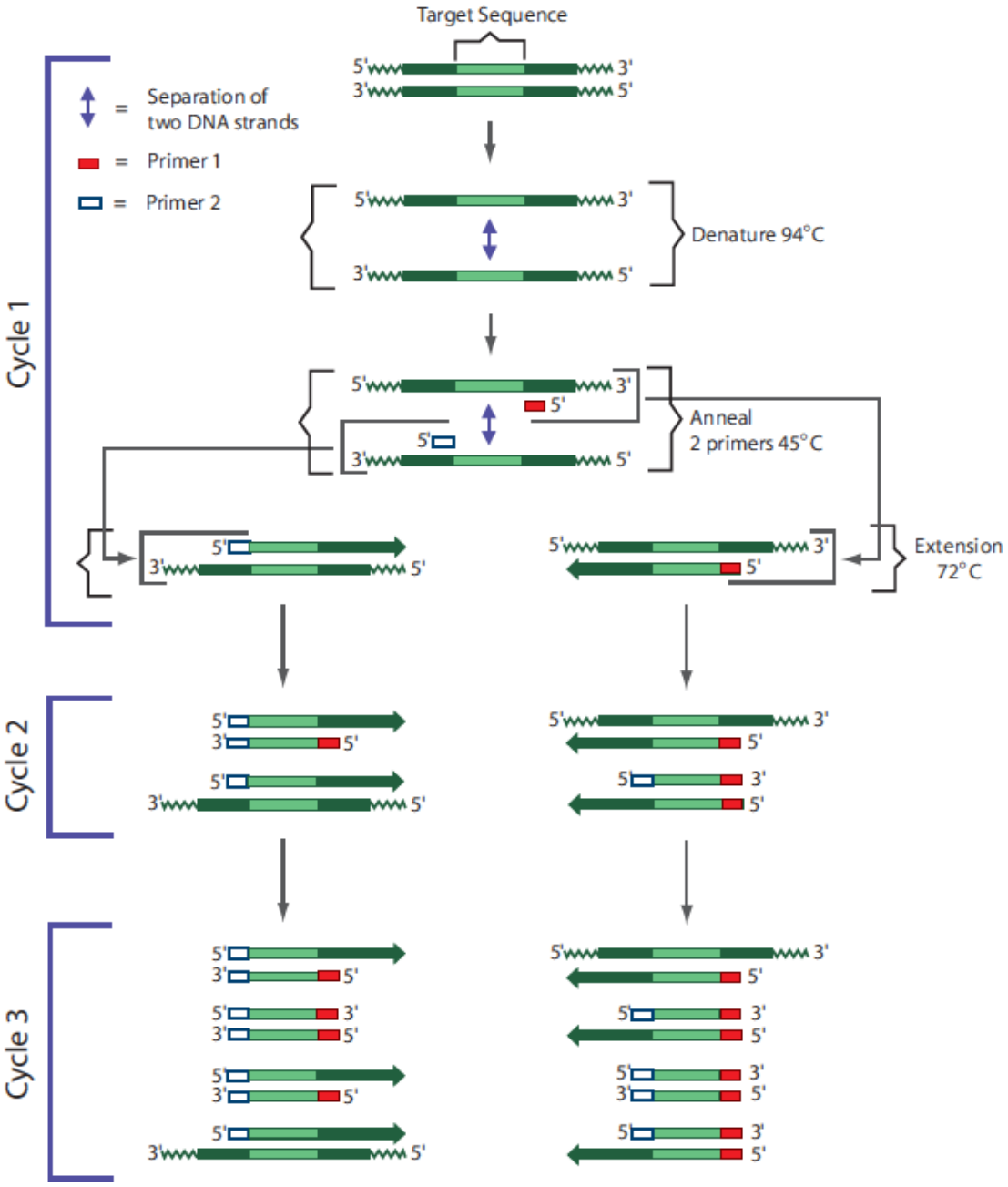
PCR has revolutionized research and diagnosis based on Molecular Biology. PCR is a simple, accurate and highly reproducible process that provides the advantage of starting with a small amount of DNA and being able to amplify it so that it has enough to perform experiments.

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 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 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 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, whereas 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 carried out in a thermocycler, an instrument that is programmed for rapid heating, cooling and maintenance of the samples several times. The amplified product is then detected by removal of the reaction mixture by agarose gel electrophoresis.

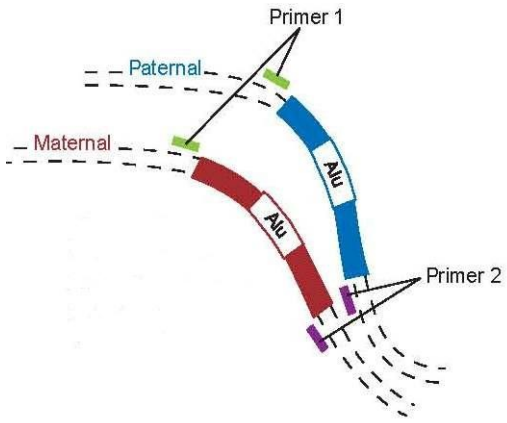
2.2 Alu human polymorphisms

The human genome consists of 2.9 billion pairs of DNA base. Of this total, only 5% consists of exons which code for proteins. Introns and other non-coding sequences may have functions that are yet to be discovered, though many others do not. Many of these sequences are repeated hundreds or thousands of times across the genome representing just over 20% of the human genome.

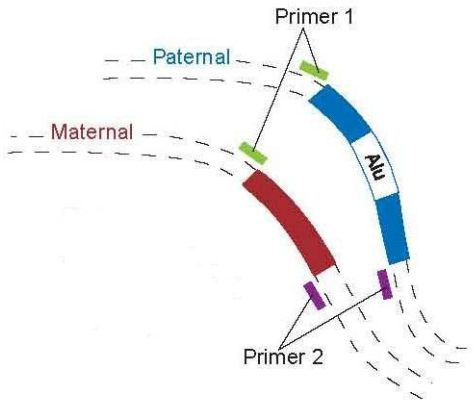
In 1979, human DNA was found to contain a 300 bp repeating element. Copies of this element contain a recognition site for the restriction enzyme Alu I, so they were called **Alu Elements**. These elements have been found in exons, introns and in other non-coding regions. When the Alu sequences are inserted into a coding region for a protein could promote the breakdown of the gene and to lead damages in the organism.

Although all humans and other primates possess hundreds of thousands of Alu elements, variations in their location can be observed. DNA sequences that vary between individuals are known as **POLYMORPHYMS**. For example, a person has an Alu insert at a specific locus of DNA, while another individual does not. In addition, the insert may be present or absent in each of the homologous chromosomes. **The Alu polymorphism we are going to study in this practice is the insertion found in intron 8 of the tissue plasminogen activator gene TPA Alu**. This section is about 260-270 nucleotides in length and the insert is about 300 base pairs in length, so that the insertion will result in an increase in length by 570 base pairs.

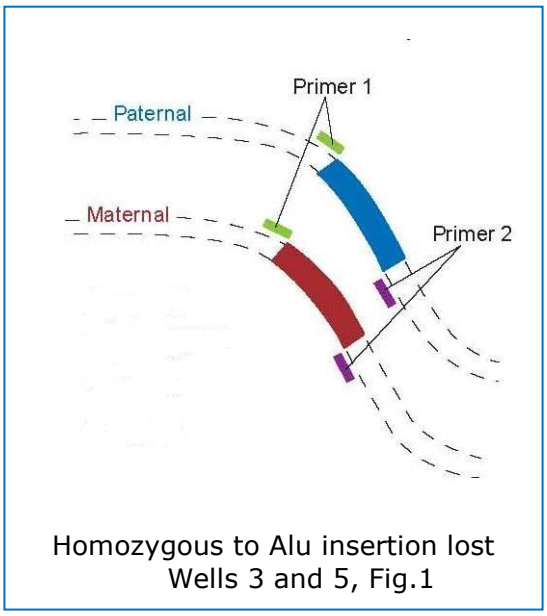
One can test whether a person possesses an Alu insert at the TPA locus by locus amplification using PCR. If a person is **homozygous** for insertion, the agarose gel electrophoresis of the PCR product will produce **a single band of 570 base pairs**. If a person is **heterozygous**, it has the insertion in one of the homologous chromosomes but not in the other, **2 bands will appear in the gel, one of 570 base pairs and another of 260 base pairs**. If a person loses insertion in both homologous chromosomes, the PCR will result in a single band of 260 base pairs.



Homozygous to ALU insertion
Wells 1 and 7, Fig.1



Heterozygous to Alu insertion
Wells 2, 4 and 6, Fig.1



Homozygous to Alu insertion lost
Wells 3 and 5, Fig.1

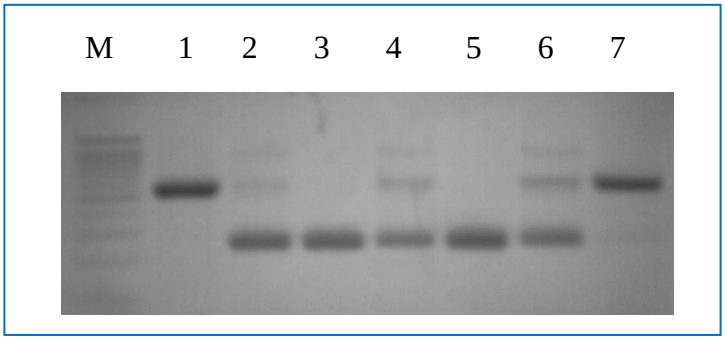


Figure 1

Fig.1: PCR analysis of different individuals for the study of the ALU insertion in the intron 8 of the tissue plasminogen activator gene.

*A 2.5% agarose gel stained with the **DanaBlue of DanaGen-Bioted** is used for the detection of amplified PCR products using genomic DNA from different individuals and using primers PLAT.A and PLAT.B.*

Marker: DanaMarker Shuman, contains 11 bands of 1,250 bp up to 100 bp

Homozygous for the Alu (+) allele produces a 570 bp fragment. (Wells 1 and 7)

Homozygous for the Alu (-) allele produces a 260 bp fragment. (Wells 3 and 5)

Heterozygous produce fragments for both sizes 570 and 260pb (wells 2, 4 and 6), in these cases also a slight formation of heteroduplex bands is observed that are formed by the annealing of Alu (+) and Alu (-) chains.

3. EXPERIMENT COMPONENTS

Enough reagents are provided for the performance of 25 individual PCRs and the production of **4 gels of 2.0-2.5% agarose electrophoresis**.

Electrophoresis buffer 10x concentrate	100 ml	
Agarose	3.0 gr	
PCR MIX	2 x 350 µl	Store at -20ºC
Heterozygous positive control	20 µl	Store at -20ºC

Electrophoresis buffer 10x to make 2 x 500 ml of electrophoresis buffer 1x which is the working buffer.

*** The 2% agarose concentration allows a better separation of fragments less than 1000 base pairs.**

3.1 MIX POLYMERASE HOT STAR

The polymerase is 2x concentration ready for use, which allows amplification of any fragment from DNA. The user only has to add water. **A 10 minute activation step is required at 95°C** so that non-specific products as "primers-dimers" are removed. It also contains a **red dye** that allows easy visualization and direct seeding into the gel without the need to mix with a loading buffer.

3.2 PRIMERS (PLAT.A + PLAT.B)

The primers flanking the TPA locus were generated from the sequences published by Frienzer-Degen et al. 1986.

4. PRACTICE

4.1 DNA extraction

The previous step to any genetic study is usually the isolation of genomic DNA; this can be done in different ways (home methods, commercial kits, etc.) and from different samples (blood, tissue, etc.).

For the practice of this practice it is recommended that the source of the DNA comes from the **student's saliva**, since it is the most accessible source of DNA and does not pose any risk, such as blood draw. To this end, the use of **DANAGENE SALIVA KIT** is recommended, which allows the genomic DNA to be obtained from a saliva sample or oral smear.

4.2 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 each student's DNA for each PCR reaction.
IMPORTANT:
a) **Prepare a negative amplification control** by placing **2.5 µl of nuclease-free water** instead of DNA, this is to know if reagents or micropipettes and tips may be contaminated with DNA. In the negative control, nothing is to be amplified.
B) **Prepare a positive amplification control** by placing **2.5 µl of the heterozigous positive control** instead of the 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 Volumen	**25 µl**

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

4. For thermocyclers that do not have a heated lid, add 25 µl of mineral oil to prevent evaporation.

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

ALU PROGRAM

STEP	TEMPERATURE	TIME
Denaturation HOT STAR	**95ºC**	**10 minutes**
PCR cicles **Perform 35 cicles**	**95ºC** **65ºC** **72ºC**	**30 seconds** **30 seconds** **45 seconds**
Final extension	**72ºC**	**10 minutes**
Final	**4ºC**	

6. The PCR product can be loaded directly in an agarose gel after PCR, as the red dye acts as a loading buffer.

7. Use the method of DNA detection or staining used in the laboratory. We recommend the use of **DANABLUE or GELSAFE**, our non-toxic methods.

8. A result similar to that observed in Figure 1 is to be obtained.

9. The observed frequency of the different polymorphisms in the class can be calculated.

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