PCR HUMAN 18S ARNr GENE

Ref. PCR18S

1. EXPERIMENT OBJETIVE

The objective of this experiment is to introduce students to the principles and practice of Polymerase Chain Reaction (PCR) by amplifying a human 18S rRNA gene fragment using the PCR technique.

It is not necessary to isolate the DNA of the students, since a sample of human genomic DNA is supplied to carry out the amplifications.

2. BACKGROUND INFORMATION

2.1 PCR

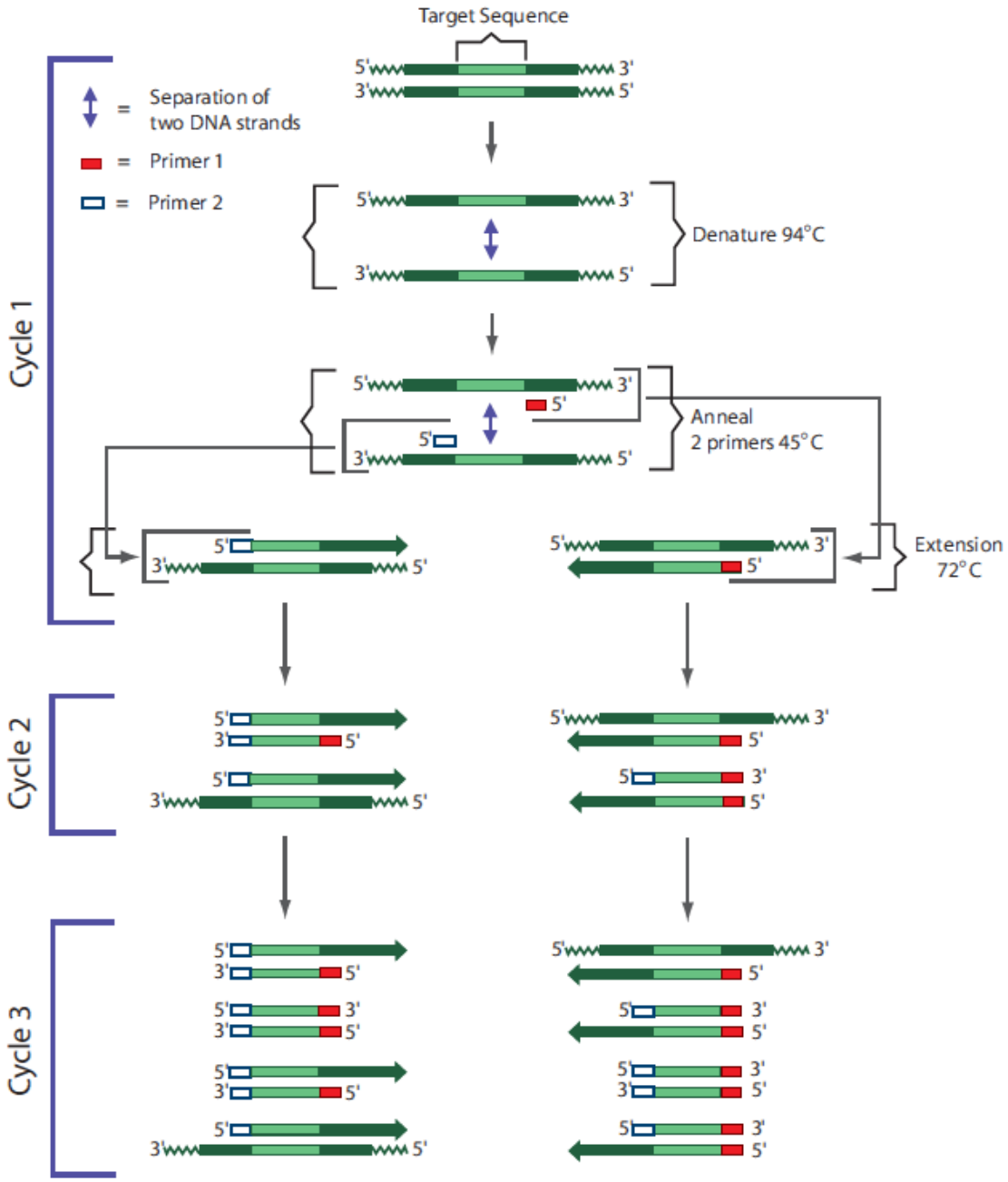
PCR has revolutionized research and diagnosis based on Molecular Biology. PCR is a simple, accurate and highly reproducible process that provides the advantage of starting with a small amount of DNA and being able to amplify it so that it has enough to perform experiments.

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 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.



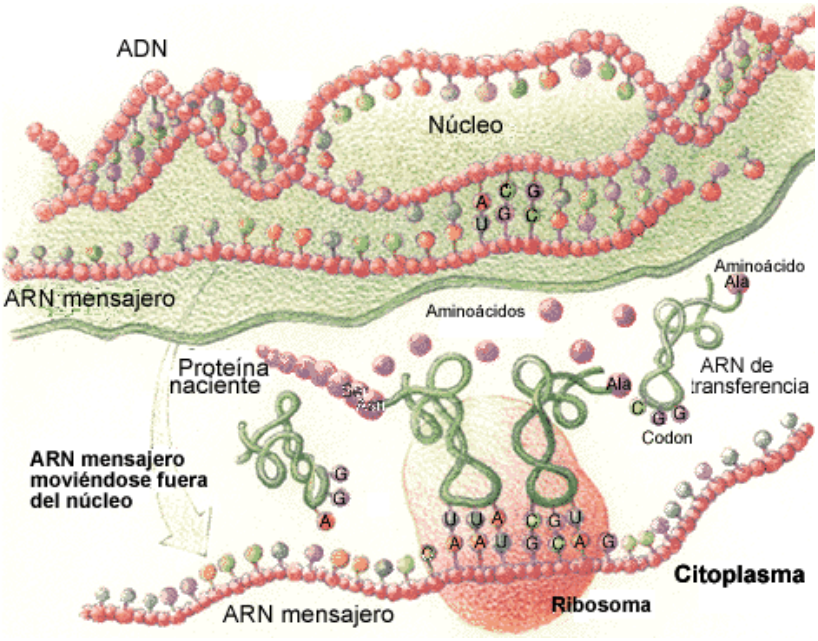
These three steps, denaturation-annealing-extension, constitute a PCR cycle. This process is repeated for 20-40 cycles by amplifying the object sequence exponentially. The PCR is performed on a **thermocycler**, an instrument that is programmed for rapid heating, cooling and maintenance of the samples for several times. The amplified product is then detected by removal of the reaction mixture by agarose gel electrophoresis.

2.2 18S ribosomal ARN

The **nucleolus** is the place where transcription and processing of rRNA and the assembly of the pre-subunits of the **ribosomes** take place, the nucleolus is the factory of production of ribosomes. The ribosomes of eukaryotic cells contain four different ribosomal RNA molecules: 28S, **18S**, 5.8S, and 5S. The major 60S subunit of the ribosome contains the ribosomal RNAs 28S, 5.8S and 5S, while the minor subunit 40S contains the 18S rRNA. The three rRNA molecules, 18S, 5.8S and 28S are synthesized in the nucleolus, whereas 5S rRNA is synthesized by **RNA polymerase III** outside the nucleolus in another region of the nucleoplasm. RNAs constitute 80% of the RNA molecules found in a eukaryotic cell.

The cells contain multiple copies of the genes for the rRNAs to be able to satisfy the transcription demand of high number of rRNA molecules that are necessary to synthesize the ribosomes. For example, continuously growing mammalian cells contain 5 and 10 million ribosomes, which must be synthesized each time the cell divides. The cells therefore contain multiple copies of the rRNA genes. The human genome, for example, contains approximately two hundred copies of the gene coding for the 28S, 18S, 5.8S rRNA arranged sequentially (in tandem) with a spacer DNA that is not transcribed by separating each repeating unit into five different human chromosomes (13,14,15,21,22) and approximately 200 copies of the gene encoding the 5S rRNA in chromosome 1.

The nucleolar rRNA 18S, 5.8S and 28S are synthesized (transcripts) by **RNA polymerase I** from the rRNA-encoding genes (rDNA). What allows the transcription to be easily visualized by electron microscopy, each of the rRNA genes are placed in series and are surrounded by densely packed growing RNA (linked to different processing proteins and ribosomes) giving rise to structures in typical form of "Christmas tree".

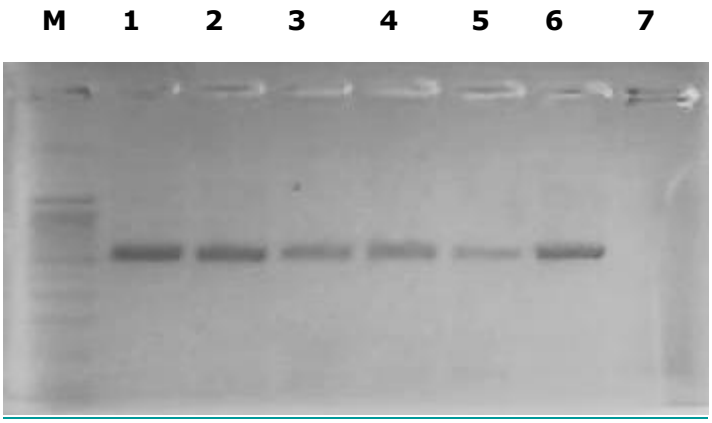


Messenger RNA	Acts as a template and conveys information for protein synthesis. Presents codons, group of 3 nucleotides.
Transfer RNA	Carries the amino acids towards the ribosomes for protein synthesis. It is found in the cytoplasm and contains anticodones.
Ribosomal RNA	It is located in the ribosome, organelle where proteins are synthesized. Receive genetic information and translate proteins.
Heteronuclear RNA	It is the precursor of RNAs.

Mature RNAs 5.8S, 18S and 28S and 5S rRNA are combined in the nucleolus with ribosomal proteins (imported from the cytoplasm) to form the pre-40S and pre-60S ribosomal subunits. These pre-subunits are exported through nuclear pore complexes (NPCs) into the cytoplasm where maturation is complete.

The genes coding for the different ribosomal proteins are transcribed out of the nucleolus by **RNA polymerase II**, originating mRNA that are transported through NPCs into the cytoplasm where they are translated into ribosomal proteins in cytoplasmic ribosomes. The ribosomal proteins are then transported back through the NPCs to the nucleolus where they are assembled with the mature rRNA to form the pre-ribosomal particles.

This kit allows the amplification of a 554 base pair fragment of the 18S rRNA gene.



PCR analysis of different individuals for the amplification of the human 18S rRNA gene.

A 2% agarose gel stained with DanaGlue-Bioted DanaBlue is used for the detection of amplified PCR products using genomic DNA from different individuals.

Marker: DanaMarker Shumman.

Well 1 to 6: Amplification from different individuals.
Well 7: Negative control.

3. EXPERIMENT COMPONENTS

Enough reagents are provided for carrying out 25 individual PCRs and the production of 4 gels of **2% agarose electrophoresis***.

COMPONENT		STORE
10x Concentrated electrophoresis buffer	100 ml	
Agarose	2.5 gr	
PCR MIX	750 µl	at -20ºC
Human DNA	75 µl	at -20ºC

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

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

3.1 PCR Mix

Ready for use, it allows amplifying any fragment from DNA, so that the user only has to add the sample of the isolated DNA.

4. EXPERIMENT PROCEDURES

4.1 DNA Extraction

The step prior 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, bacteria, etc.).

For the accomplishment of this practice it is not necessary the extraction of the DNA of the students, since it supplies a sample of human genomic DNA.

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 the human genomic DNA for each PCR reaction.

   IMPORTANT: **Prepare a negative amplification control** by placing **2.5 µl of nuclease-free water** instead of DNA, to see if the reagents, micropipettes or tips may be contaminated with DNA.

REAGENTS	VOLUME
PCR MIX	**22,50 µl**
Human DNA (100-250 ng)	2,5 µl
Total Volume	**25 µl**

2. Mix well.
3. For those thermal cyclers that do not have a heated lid, add 25 µl of mineral oil to prevent evaporation.

Sex determination PROGRAM

STEP	TEMPERATURE	TIME
Initial denaturation	**96ºC**	**1 minutes**
PCR cycles **Carry out 35 cycles**	**94ºC** **58ºC** **72ºC**	**1 minutes** **1 minutes** **1 minutes**
Final extension	**72ºC**	**10 minutes**
Final	**4ºC**	

4. The PCR product can be seeded directly onto an agarose gel after PCR, since the MIX contains red fill buffer.
5. Use the method of detection or staining of the DNA used in the laboratory. We recommend the use of **DANABLUE** or GELSAFE, our non-toxic methods.
6. A similar result to that observed in the figure should be obtained.

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