# Polymerase chainreaction (PCR)

## Theory

PCR is a simple, yet elegant, enzymatic assay, which allowsfor the amplification of a specific DNA fragment from a complex pool of DNA. Its principle is based on the use of DNA polymerase which is an in vitro replication of specific DNA sequences. By this method we can generate tens of billions of copies of a particular DNA fragment (the sequence of interest, DNA of interest, or target DNA) from a DNAextract (DNA template). The power of PCR is based on the fact that the amount of matrix DNA is not, in theory, a limiting factor. We can therefore amplify nucleotide sequences from infinitesimal amounts of DNA extract. There are many applications of PCR. It is a technique now essential in cellular and molecular biology. The technique is currently widely used by clinicians and researchers to diagnose diseases, clone and sequence genes, and carry out sophisticated quantitative and genomic studies in a rapid and very sensitive manner. One of the most important medical applications of the classical PCR method is the detection of pathogens. In addition, the PCR assay is used in forensic medicine to identify criminals. It is alsoused to make genetic fingerprints, whether it is the genetic identification of a person in the context of a judicial inquiry. PCR makes it possible to obtain, by in vitro replication, multiple copies of a DNA fragment from an extract. Matrix DNA can be genomic DNA as well as complementary DNA obtained by RT-PCR from a messenger RNA extract (poly-A RNA), or even mitochondrial DNA. It is a technique for obtaining large amounts of a specific DNA sequence from a DNA sample.

This amplification is based on the replication of a double-stranded DNA template. It is broken down into three phases:

**Denaturation: -** It is the separation of the two strands of DNA, obtained by raising the temperature. The first period is carried out at a temperature of 94°C, called the denaturation. At this temperature, the matrix DNA, which serves as matrix during the replication, is denatured: the hydrogen bonds cannot be maintained at a temperature higher than 80°C and the double-stranded DNA is denatured into single stranded DNA (single-stranded DNA).

**Annealing: -** The second step is annealing. The annealing temperature (Ta) chosen for PCR relies directly on length and composition of the primers. Generally, you should use an annealing temperature about 5°C below the Tm of your primers. Decreasing the temperature allows the hydrogen bonds to reform and thus the complementary strands to hybridize. The primers, short single-strand sequences complementary to regions that flank the DNA to be amplified, hybridize more easily than long strand matrix DNA.

**Elongation: -** The third period is carried out at a temperature of 72°C, called elongation temperature. It is the synthesis of the complementary strand. At 72°C, Taq polymerase binds to primed single-stranded DNAs and catalyzes replication using the deoxyribonucleoside triphosphates present in the reaction mixture. It takes 20–40 cycles to synthesize an analyzable amount of DNA (about 0.1 µg). Each cycle theoretically doubles the amount of DNA present in the previous cycle. It is recommended to add a final cycle of elongation at 72°C, especially when the sequence of interest is large (greater than 1 kilo base), at a rate of 2 minutes per kilo base. PCR makes it possible to amplify sequences whose size is less than 6 kilobases. The PCR reaction is extremely rapid, it lasts only a few hours (2–3 hours for a PCR of 30 cycles).

The polymerase chain reaction is carried out in a reaction mixture which comprises the DNA extract (template DNA), Taq polymerase, the primers, and the four deoxyribonucleoside triphosphates (dNTPs) in excess in a buffer solution. Free divalent cations are needed for the activity of thermo stable polymerases. We also use magnesium ions. Taq polymerase is a magnesium-dependent enzyme and determining the optimum concentration to use is critical to the success of the PCR reaction. Excessive magnesium concentrations also stabilize double stranded DNA and prevent complete denaturation of the DNA during PCR reducing the product yield.

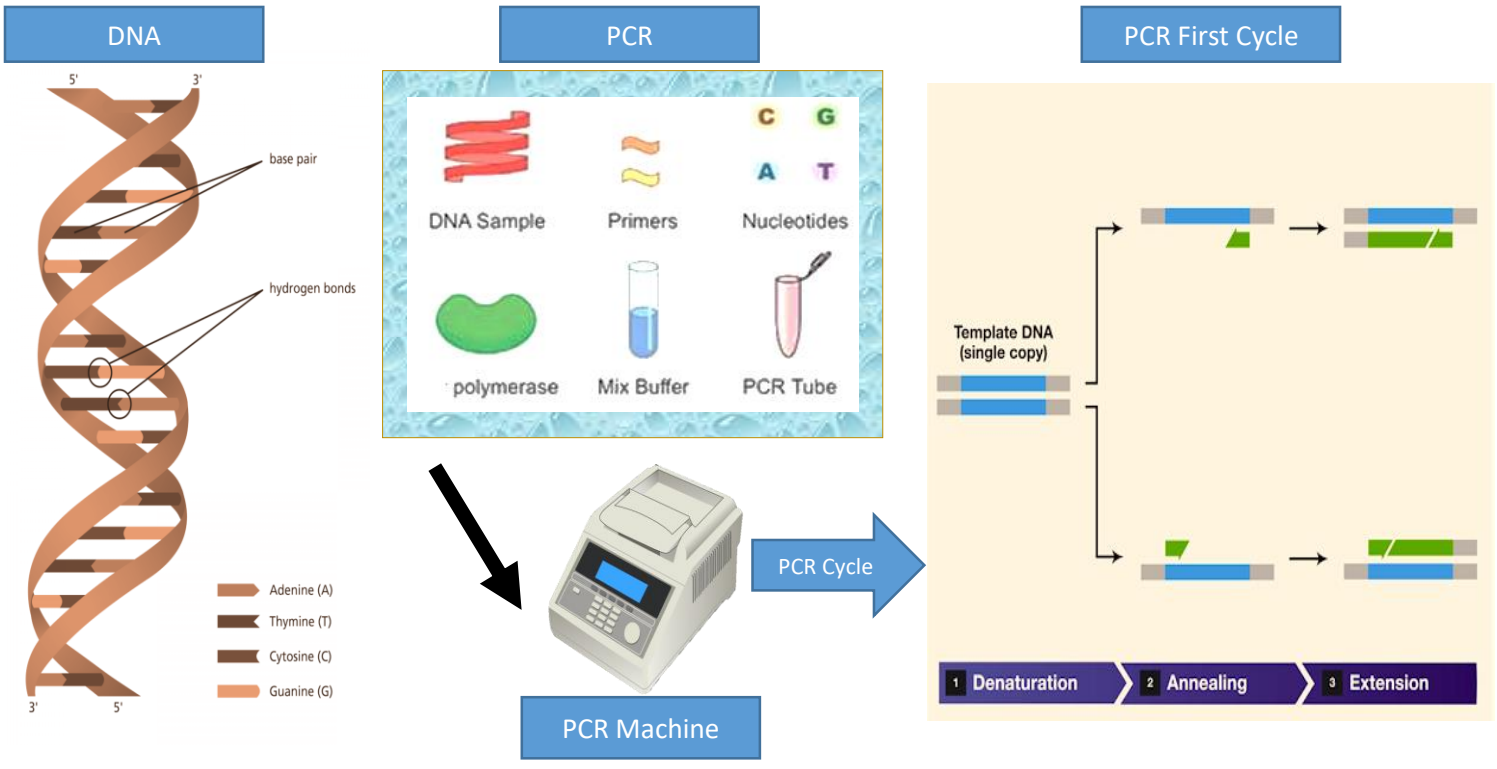


Figure 1, PCR in Nutshell

# Protocol

| Components | 25 uL reaction |
|---|---|
| Reaction Buffer (10X) | 2.5 |
| 10 mM dNTPs | 0.5 |
| 10 uM Forward Primer | 0.5 |
| 10 uM Reverse Primer | 0.5 |
| Template DNA | variable |
| DNA Polymerase | 0.25 |
| Nuclease Free Water | upto 25 |

## Thermocycling

| Step | Temperature | Time |
|---|---|---|
| Initial Denaturation | 95 ° C | 5 min |
| Denaturation | 95 ° C | 30 sec |
| Annealing | 55-70 ° C (Depends on primer Tm) | Depend on length of DNA generally (30-45 sec) |
| Extension | 72 ° C | Depend on length of DNA and polymerase generally (30 sec – 1.5 min) |
| Final Extension | 72 ° C | 5 min |
| Hold | 4 ° C | infinite |

# References: -


- Research Techniques Made Simple: Polymerase Chain Reaction (PCR). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4102308/pdf/nihms593299.pdf
- Polymerase Chain Reaction (PCR): Principle and Applications. https://www.intechopen.com/books/synthetic-biology-new- interdisciplinary-science/polymerase-chain-reaction-pcr-principle- and-applications
- PCR Revisited! - A Review https://juniperpublishers.com/jojcs/pdf/JOJCS.MS.ID.555624.pdf