Components of the PCR Mixture

1. Buffer

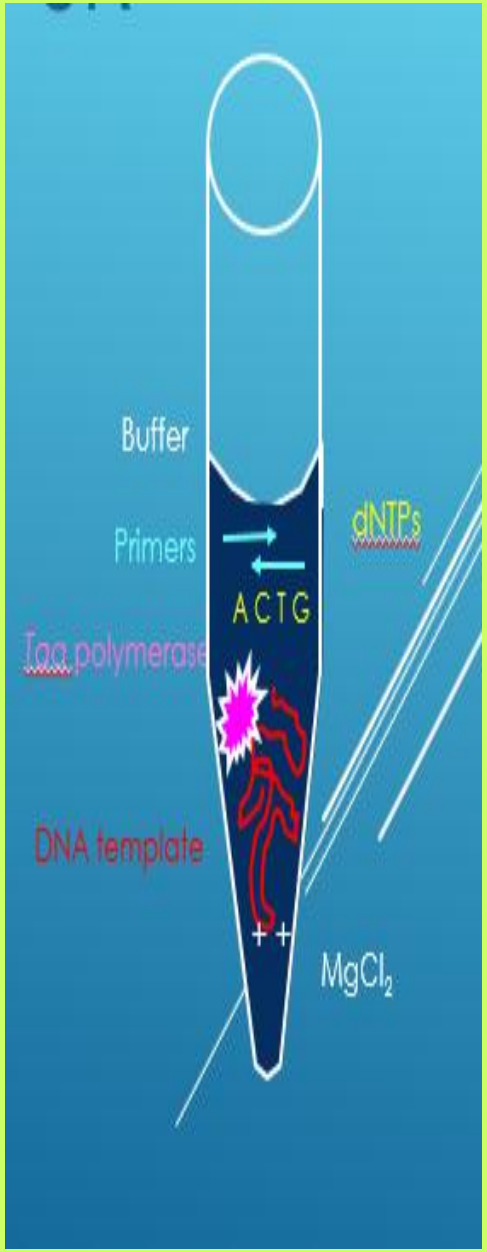
buffers contain Tris at 10 mM and KC1 at 50 mM concentrations; the pH varies from 8.2 to 9.0 (at 25°C). The pH of Tris drops as the temperature increases), pH 7.0-7.5 is considered optimal for *Tag* polymerase.

2. Magnesium Chloride

Magnesium is acation that binds to the polymerase and is an essential cofactor for polymerase activity, the optimal amount (usually between 1.0 and 3.0 mM. The lower of the magnesium concentration, the more stringent the conditions for primer annealing.

3. Primers

Primers are synthetic oligonucleotides, generally 15-30 nucleotides in length, which are complementary to a particular sequence in the genome of the organism of interest. The optimal primer concentration for PCR is usually between 0.1 and 1.0 \M.



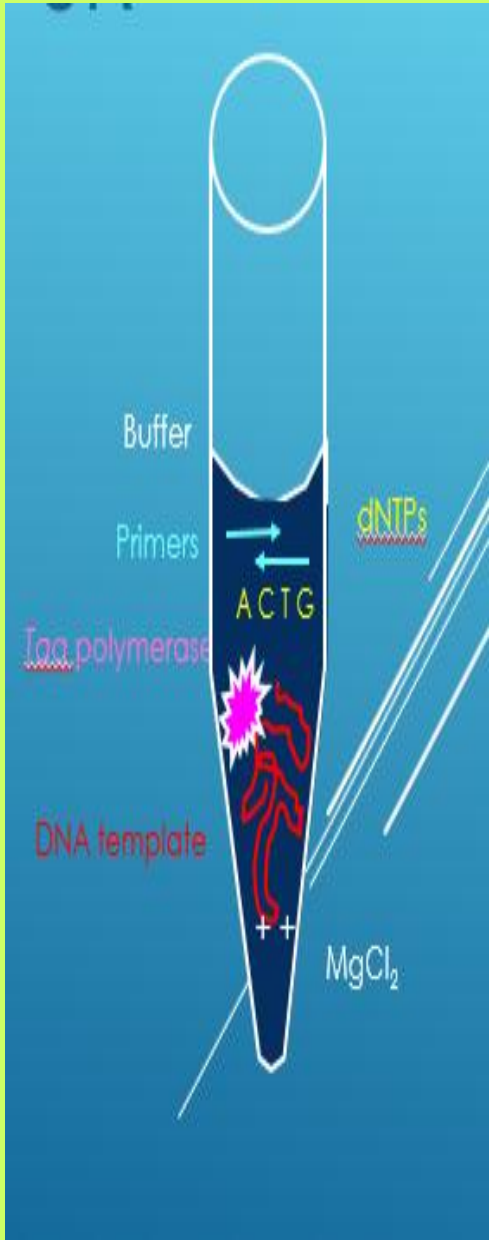
Components of the PCR Mixture

4. *Deoxy nucleotide Triphosphates (dNTPs)*

The building blocks of the DNA replicated during PCR. Equal amounts of each of the four dNTPs (dATP, dCTP, dGTP, dTTP) are included in the reaction, usually at a concentration of 0.2 *mM* (200 *M)* each, Inorder to increase the stringency of reactions that require heightened specificity, the nucleotide concentration can be lowered (e.g., to 1.5 mM).

5. Thermostable Polymerase

The most common thermostable polymerase and the first to be widely used for PCR is Taq DNA polymerase, its purified from bacteria that grow naturally at even higher temperatures in the ocean floor (e.g., *Thermococcus litoralis, Pyrococcus*).



Components of the PCR Mixture

6. Stabilizers

In order to stabilize the polymerase, bovine serum albumin (BSA; 0.01 %), glycerol (1-2%) are sometimes included in the reaction mix.

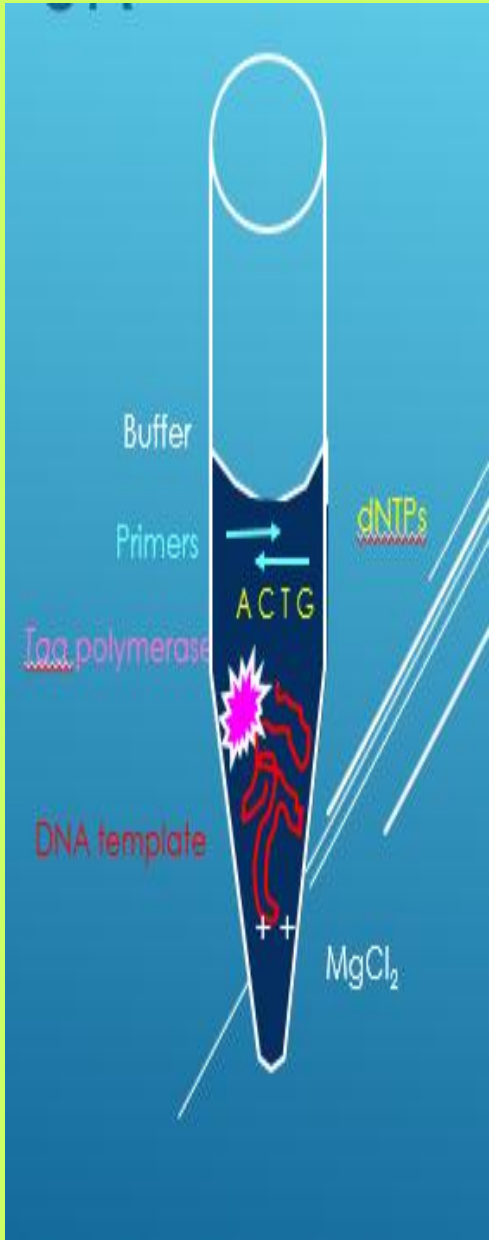
7. Enhancers

A number of chemicals can be used to optimize the performance and sensitivity of PCR, including

A- dimethyl sulfoxide (DMSO)

B- tetramethylammonium chloride (TMAC)

Most of these compounds function by destabilizing the hydrogen bonds between the bases in opposite strands of the double helix, thereby making it easier for the two strands to be denatured and become accessible for primer binding and subsequent strand replication. In effect, this lowers the Tem. of the annealing step.



Components of the PCR Mixture

8. DNA Template

The choice of sample preparation method depends on the desired sensitivity. Procedures range from protocols that yield purified DNA/RNA, such as standard organic extraction/ethanol precipitation or nucleic acid separation using silica particles, to crude lysates generated with detergents and proteases, chloroform, chelating resins, boiling or autoclaving, or direct extraction.