Polymerase Chain Reaction (PCR)

Principle of the experiment: The PCR method, known as ”Molecular Photocopy” was developed by Kary Mullis (Nobel Prize in Chemistry in 1993) by Cetus Company researcher in 1985. PCR, which allows the DNA to be obtained in large amounts by taking advantage of the replication feature, has opened a new era in DNA analysis. The polymerase chain reaction is the *in vitro* expression of a specific DNA fragment by enzymes using primers.

Preparation: PCR is designed according to the basic characteristics of DNA replication. A specific region of DNA is amplified *in vitro* by PCR reaction. For the synthesis of DNA;

1. Template DNA molecule,

2. The primers, are synthetic oligonucleotides which are 20-25 base pairs in length complementary to the ends of the target DNA sequences 3.

3. DNA polymerase enzyme (Taq polymerase),

4. Deoxynucleoside triphosphates (dNTPs),

5. The buffer solution, provides the appropriate chemical environment for the stability and optimum activity of DNA polymerase

6. Divalent ions such as magnesium or magnesium ions, usually some components, such as Mg^{2+}, must be present.

PCR occurs in three steps: denaturation, annealing and extension/elongation. However, the initial step required for the activation of the DNA polymerase called ”initialization step is needed before proceeding to the main steps. Likewise, a final elongation / extension step is needed to ensure that the single chain DNA remaining in the medium in any way after the final PCR cycle is fully extended.

1. Denaturation: Denaturation of the DNA molecule at 94-96 °C, thus, a single chain structure is formed.

2. Annealing: At 50-64 °C the primers are bound to the template DNA.

3. Elongation: The DNA chain is ligated to the primer by dNTPs using DNA polymerase at 68-72 °C.

As a result of the denaturation of the DNA by heat, the primers which are complementary to the template DNA adhere and the DNA polymerase allows the addition of appropriate dNTPs to the primers. Thus, the new DNA chain begins to grow. This 3-steps of the PCR reaction is continued for 20-40 cycles, resulting in a doubling of the amount of DNA in each cycle.

Preparation of the PCR mixture:

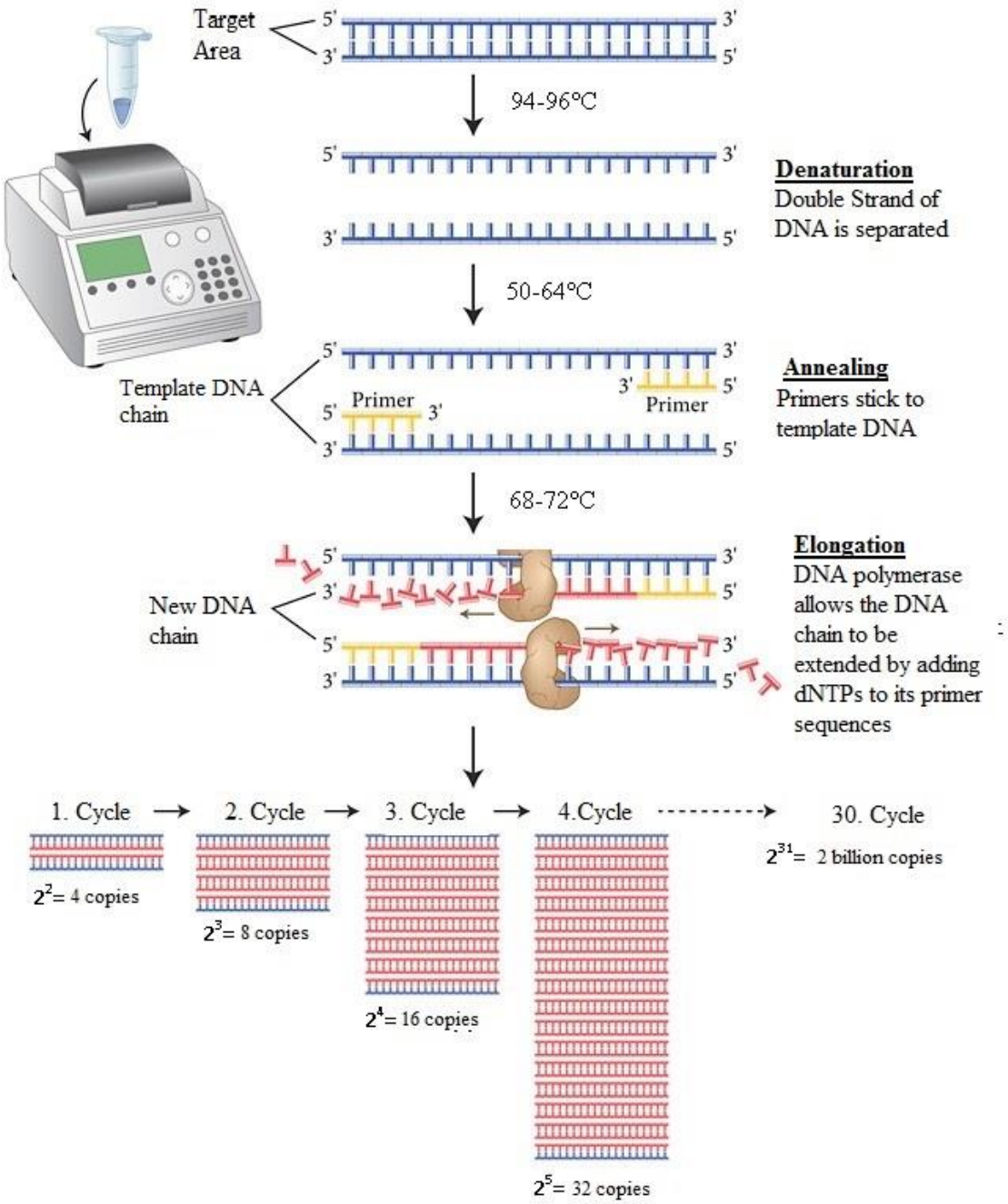
Primers (0.1-0.5 µM)	
Forward	0.5µl
Revers	0.5µl
dNTP (20-200 µM)	5 µl
Mg^{2+} (0.5-2.5 mM)	1.5 µl
10X Buffer	2.5 µl
Bidistilled water (ddH_2O)	14.2 µl
Taq DNA polymerase (1-2.5 U)	0.3 µl
DNA (50-200 ng)	0.5 µl

Note: 10X Buffer: 500 mM KCl, 100 mM Tris-HCl (pH 8.3), 15 mM $MgCl_2$

(Final concentrations of these substances in the PCR mixture are as follows: 50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl2)

Sample PCR protocol:

95°C	3	1 cycle
94°C	45	35 cycles
56°C	45	35 cycles
72°C	45	35 cycles
72°C	7	1 cycle



DNA replication by PCR method

https://www.neb.com/applications/dna-amplification-and-pcr