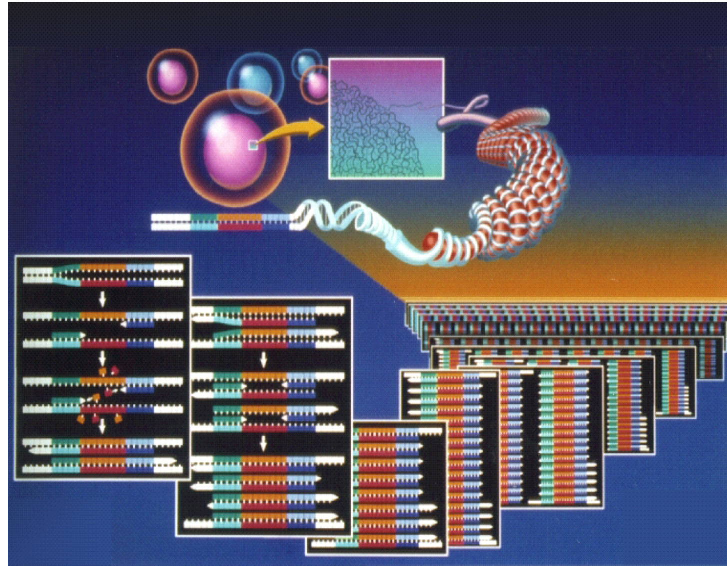
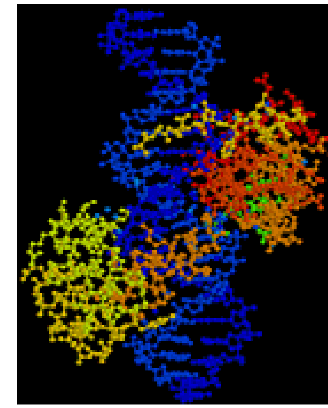


Polymerase Chain Reaction

Lecture Content

- *Definition*
- *History*
- *Intracelluler DNA replication*
- *PCR contents*
- *PCR basic principles*
- *Where to use PCR?*
- *Advantages and disadvantages*
- *PCR optimisation*
- *PCR inhibitors and enhancers*
- *Troubleshooting*

PCR definition



- *in-vitro* enzymatic synthesis of the copies of a specific DNA fragment by the help of small oligonucleotides called “primers”
- *in-vitro* amplification of nucleic acids
- DNA photocopy
- samanlıkta iğne aramak yerine samanlıktaki iğnelerin sayısını çoğaltmak

Short History of PCR:

- **1971** *Khorana et al.* Developed a method replicating a specific region of an double-stranded DNA by the help of two DNA synthesis primers 3' ends of which are opposed to each other
- **1983** Kary Mullis (Cetus firm) developed PCR

Kary B. Mullis

- **1985** First report of *PCR with a DNA polymerase I Klenow fragment (Saiki et al., 1988).*
- **1988** First application of PCR by the help of first time use of Taq polymerase
- **1993** *Kary Mullis awarded Nobel Prize in Chemistry*
- **1993** Licences of PCR technology and Taq polymerase enzymes were bought by the world leader commercial firms

Intracellular DNA replication

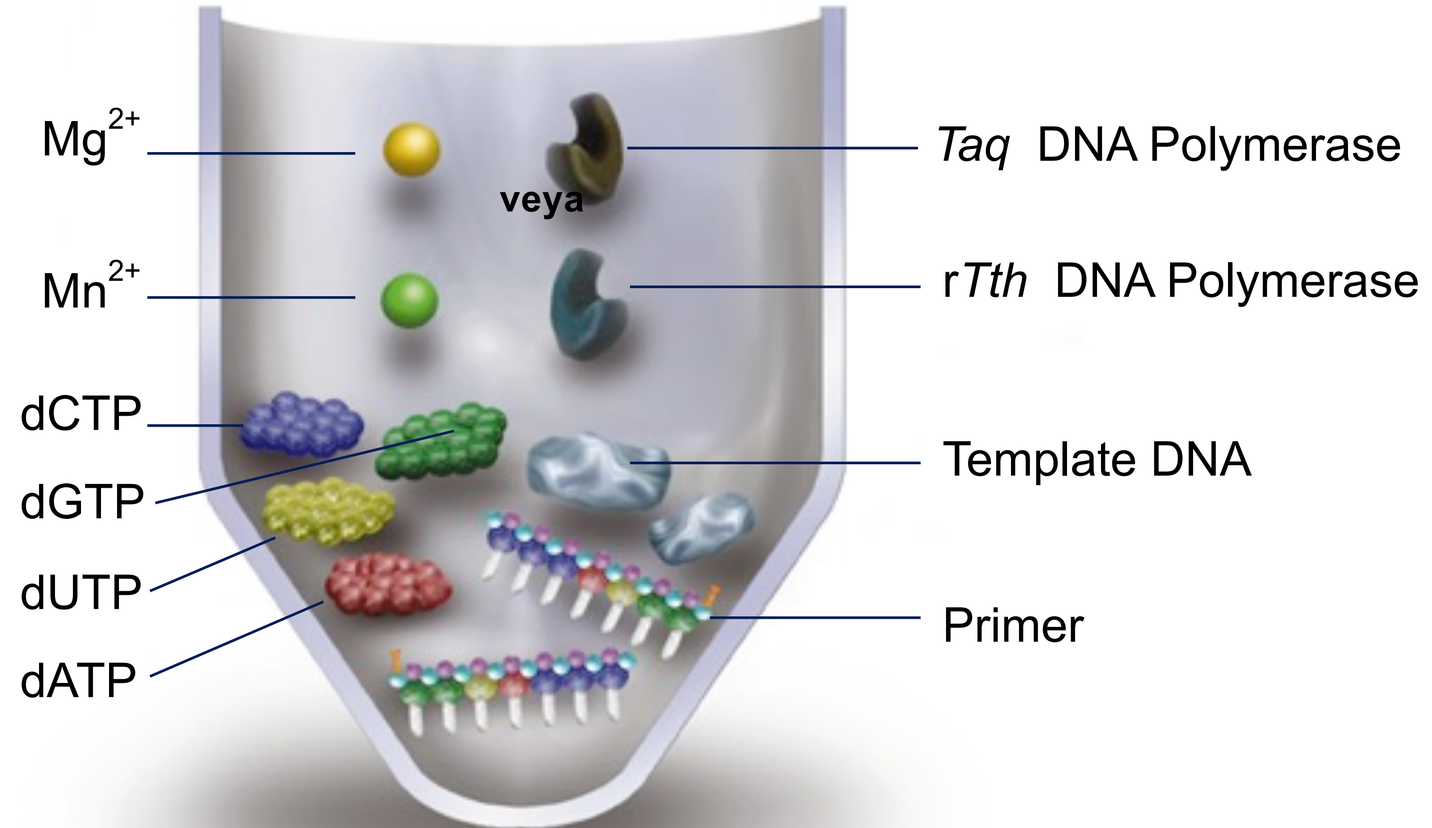
- *DNA replication occurs at 37° C*
- Helper proteins like single strand binding proteins are used in replication
- *A RNA primer of 12 nucleotide are formed by an enzyme "primase" in the origin of replication*
- DNA polymerase binds to this enzyme and synthesis DNA by binding nucleotides to 3' end

PCR

- **Reaction content**
  - Reaction buffer
  - Template DNA
  - Primers
    - Forward ve reverse
  - Nucleotides
    - DNA synthesis
  - Polymerase
    - Taq DNA polymerase
  - $MgCl_2$
    - Enyzme activity

- **Temperature control**
  - PCR equipment (thermal cycler)
  - Automatic regulation of temperature by steps and cycles

PCR mix contents

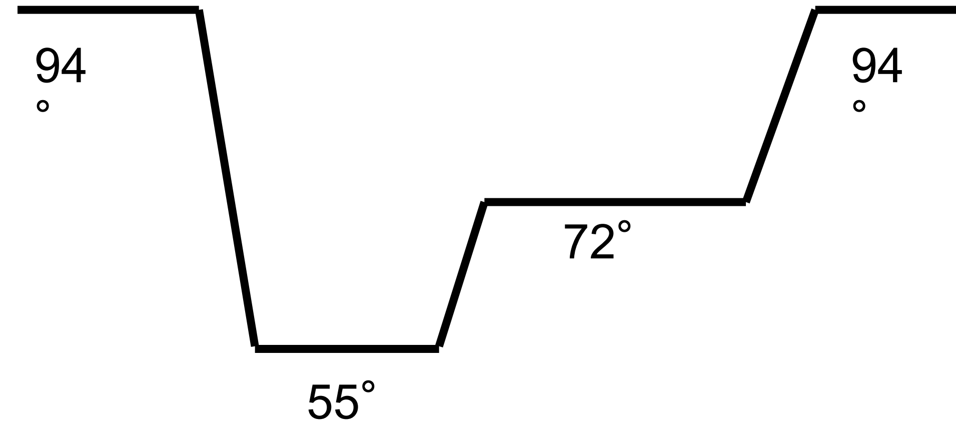


Characteristics of Primers

- 18-30 nucleotides
- F and R primers should have similar Tm values
- G+C content ~ 50%
- Primer should end with G or C at the 3′ end
- Primers should not end with A or T
- Sequences those could form hairpins should be inhibited
- Primers should not be complementary to each other

PCR

- **How does PCR work?**:
  - Separation of two strands from each other (94°C)
  - Annealing of Primers (55°C)
    - Beginning of Replication
  - Extension (polymerisastion) (72°C)
    - = replication
  - Repeat for 20-30 times (cycles)



CYCLE PARAMETRES

Denaturation; 93° C - 95° C

30 sec – 1 min

Annealing; 37° C - 65° C

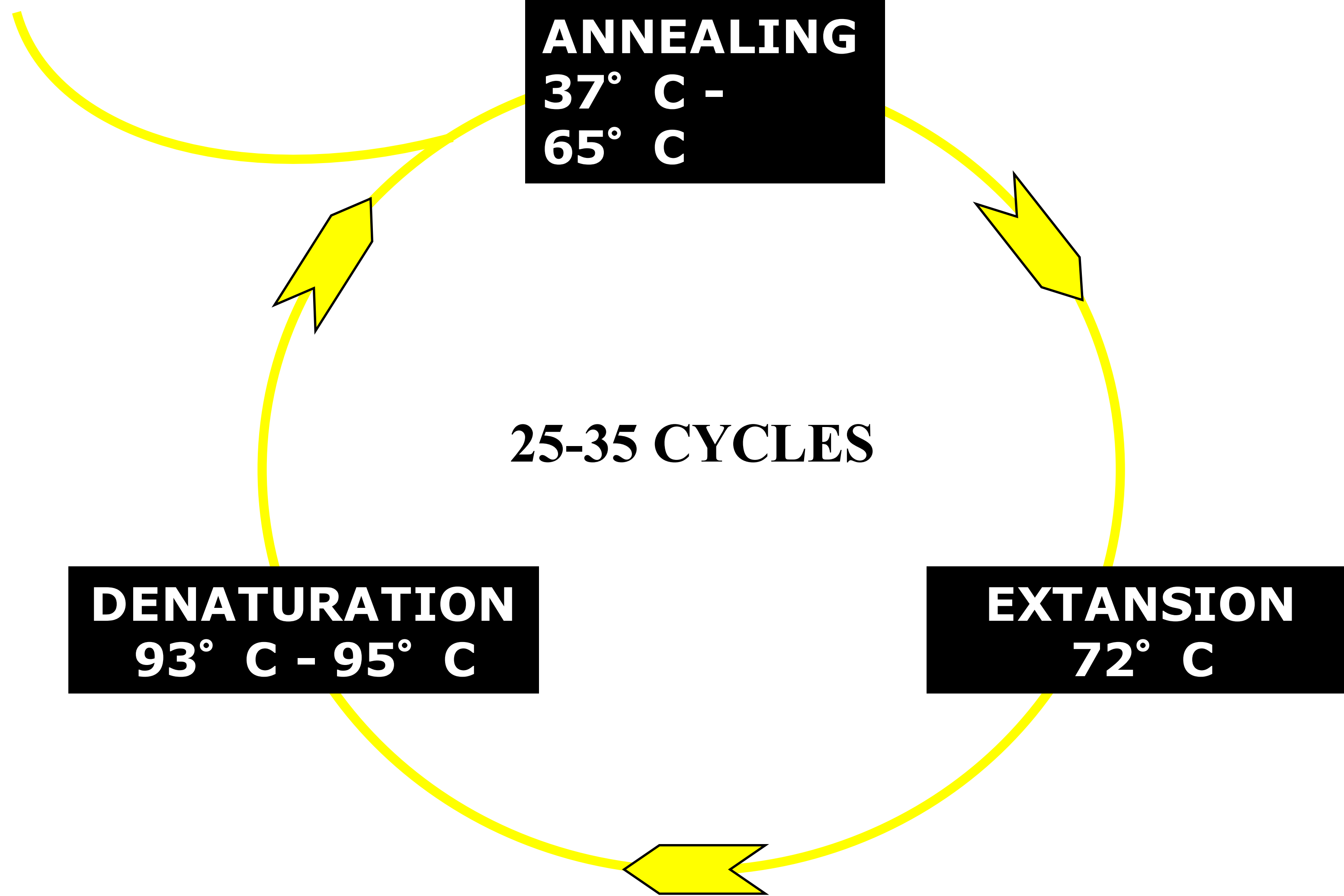
30 sec – 1 min

Extension; 72° C

1 min
(For every 500 bp DNA add + 30 sec)

25-35 cycles

Final exstantion 2-10 min


DENATURATION
93° C - 95° C
ANNEALING
37° C -
65° C
25-35 CYCLES
DENATURATION
93° C - 95° C
EXTANSION
72° C

Basic PCR conditions

- 25-50-100 μl final reaction volumes
- Template DNA 1-1000ng
- Primers 10-20 pmols
- 10 mM Tris-CL pH 9.0, 50 mM KCl (10xPCR buffer)
- $MgCl_2$ 0.5-3.0 mM
- final concentration of each dnTPs would be 200 μM dNTP'ler: dATP, dGTP, dTTP, dCTP
- 1 unit Taq polymerase enzyme

REACTION MIXTURE

25 or 50µl final volume Eppendorf (0.2 ml) tube

COntents	Volumes	Final Concentration
10 X PCR Buffer	**5µl**	**1X**
10 X dNTPs (2mM)	**5µl**	**200µM**
Forward primer (10pmols/µl)	**5µl**	1µM (50pmols/50µl)
Reverse primer (10pmols/µl)	**5µl**	1µM (50pmols/50µl)
Genomic DNA template	**2µl**	**1µg**
Taq polymerase (2U/µl)	**0.5µl**	**1 unit**
H_2O (to 50µl Final volume)	**27.5µl**	