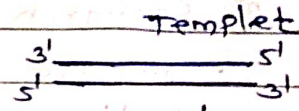
- Kasar R.N

* PCR : Polymerase Chain Reaction
- Karry mulis - invented PCR
- In-vitro - DNA amplification
- <5Kb - DNA can be amplify well in PCR.

* Requirements of PCR:
- Template DNA
- DNA polymerase - (Thermostable)
- Primers - length 18-20 nt (DNA) — forward primer, Reverse primer
- Mg^{2+} - Cofactor of DNA Polymerase.
- Buffer
- dNTP (dATP, dCTP, dGTP, dTTP)

- Taq - DNA Polymerase : optimum activity at 72°C
  ↳ Isolated from Thermus Aquaticus

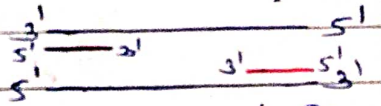


- Every cycle - 3 Important steps:

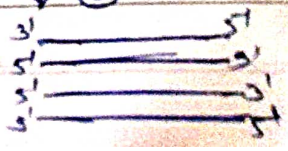
1) Denaturation: Temp - 96°C , Time - 0.5 min. ①
- H-Bond break , due to temp↑ of double stranded DNA to single stranded DNA.

2) Primer Annealing : Temp - 51-54°C , Time - 1.5 min
- Primer length 15-20nt.
- Avoid repetative seq?
- Provid starting point of DNA synthesis.
- Primer GC content = 60%.
  AT content = 40%.

3) Primer extension: Temp - 72°C , Time - 1 min.
- Taq Polymerase extends the DNA chain by adding nucleotides to the 3' ends of primers. ②



③



- PCR - limitation : Flanking sequences known

* Some Types of PCR.

① Real-time PCR

② Nested PCR

* Application:

① Medicine

- Testing of genetic disease mutations.
- Monitoring the gene in gene therapy.
- Detecting disease-causing genes in the parents.

② Forensic Science

- Used as a tool in genetic fingerprinting.
- Identifying the criminal from millions of people.
- Paternity tests.

③ Research and Genetics:

- Compare the genome of two organisms in genomic studies.
- Analysis of gene expression.