

POLYMERASE CHAIN REACTION

Several thin, white, parallel diagonal lines extending from the bottom right towards the top right of the slide, adding a modern, dynamic feel to the background.

Technical overview

DNA consists of four elements: A, C, G and T

DNA molecule

- Double stranded DNA strands
- Bound together by chemical forces
 - Exception: single stranded DNA/RNA viruses

What you need for PCR

- ❑ Two short DNA fragment that stick specifically to each of the DNA strands at some distance of each other

Primers

- Can be specific for: – Bacterial species – Genes (e.g., toxin gene)

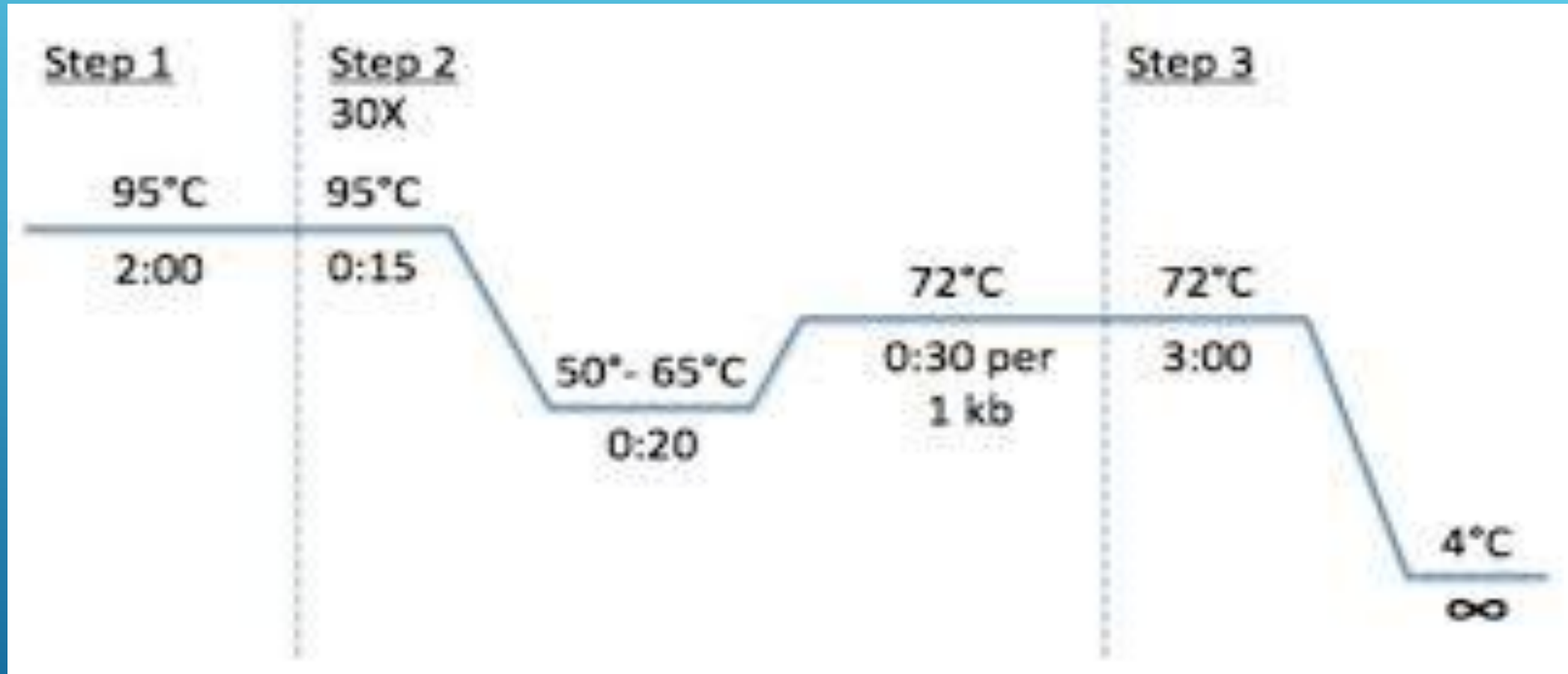
- ❑ Apparatus to perform about 35 cycles of a three temperature procedure

- 95 °C (denaturation of DNA)
- 50-60 °C (annealing of primers)
- 72 °C (extension of the primers)

- ❑ Put into one reaction tube:

- Sample (+/- target DNA)
- Primers for the specific detection
- Nucleotides
- Enzyme

TEMPERATE PROCEDURE



PCR MASTER MIXES

Basic Components of PCR

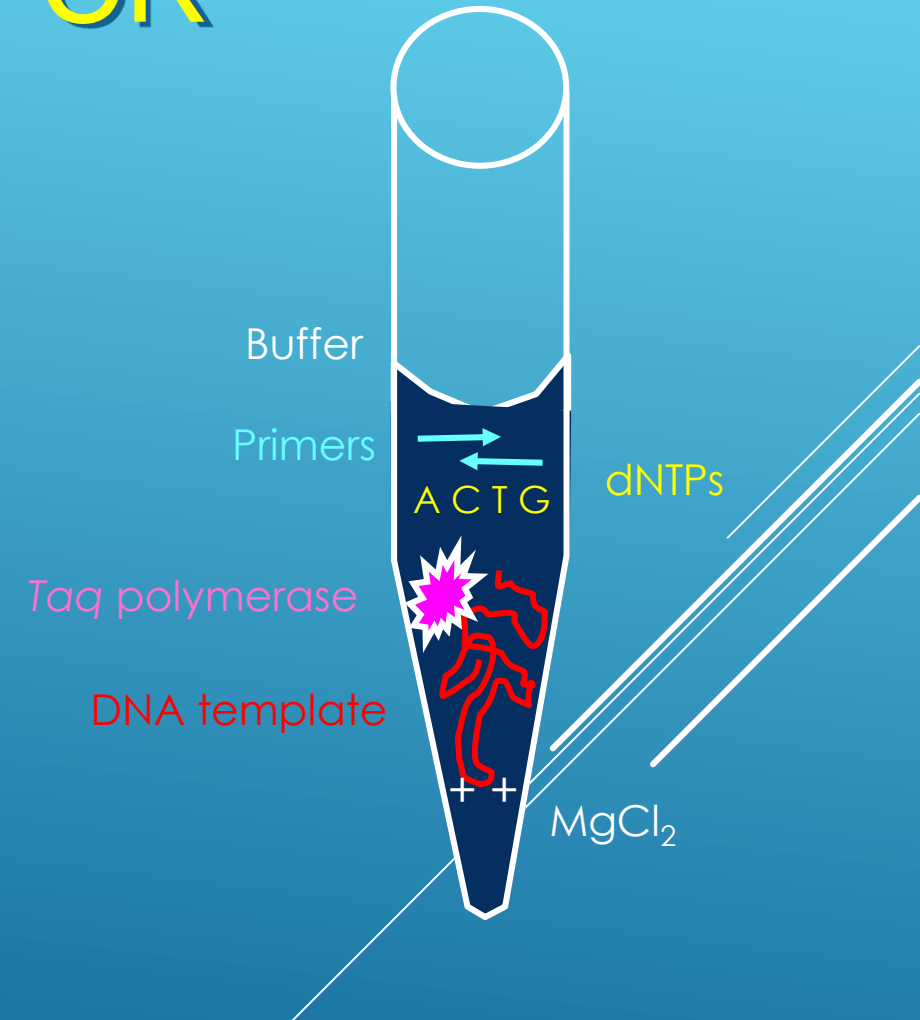
- Template DNA (0.5 - 50 ng)
- Oligonucleotide primers (0.1 – 2.0 μM)
- dNTP's (20 – 250 μM)
- Thermostable DNA pol (0.5 – 2.5 U/rxn)
- MgCl_2 (1 – 5 mM)
- Buffer (usually supplied as 10X)

Working concentrations

KCL (10 – 50 mM)

Tris-HCl (10 mM, pH 8.3)

NaCl_2 (sometimes)



PCR MASTER MIXES

The lyophilised Master Mixes contain all reagents which are necessary for a successful PCR:

- Taq DNA Polymerase
- dNTP's
- primers for the specific infection
- Stabilizers and enhancer

You have to add only a dilution buffer and your DNA sample to start thermocycling.



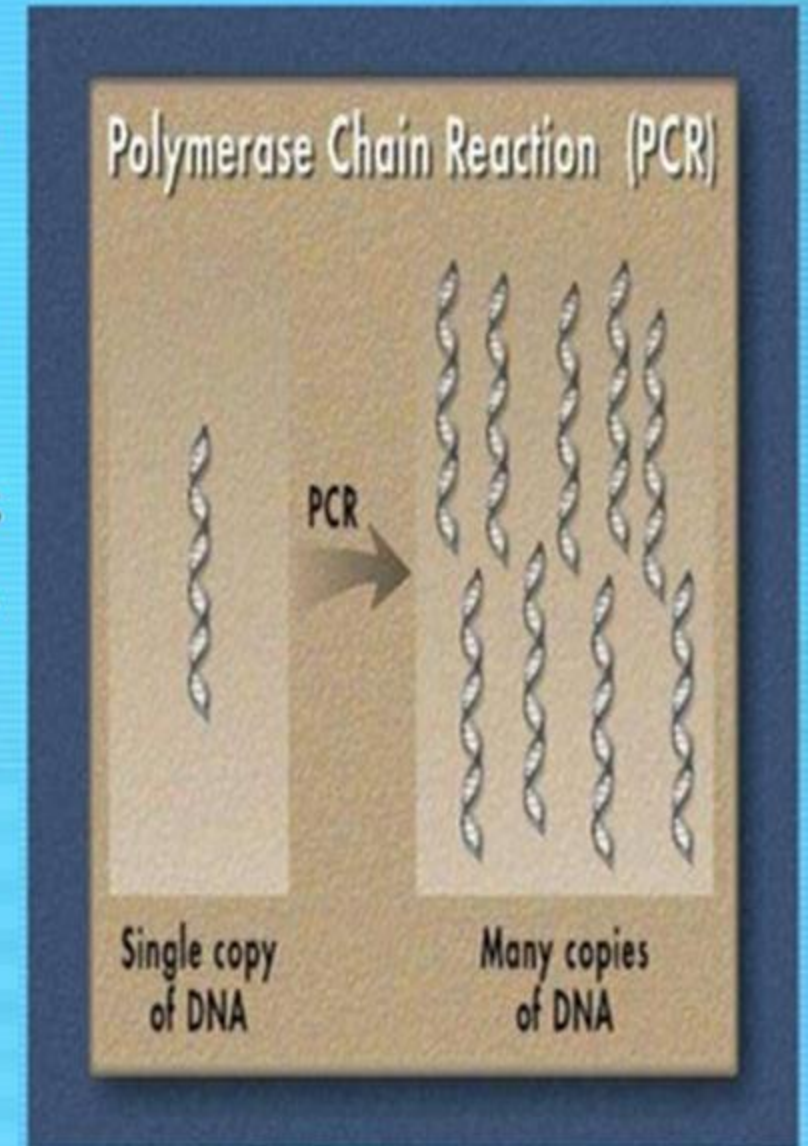
PCR MASTER MIXES

Reagents	1X (μ l)	5X (μ l)	Final conc.
2X <i>Taq</i> Master Mix	10	50	1X
10 μ M Forward primer	2.0	10	1 μ M
10 μ M Reverse primer	2.0	10	1 μ M
Nuclease free water	4	20	-
DNA template (25 ng/ μ l)	2.0	-	50 ng (2.5 ng/ μ l)
Total	20	แบ่งใส่ tube ละ 18 μ l + 2 μ l template DNA	



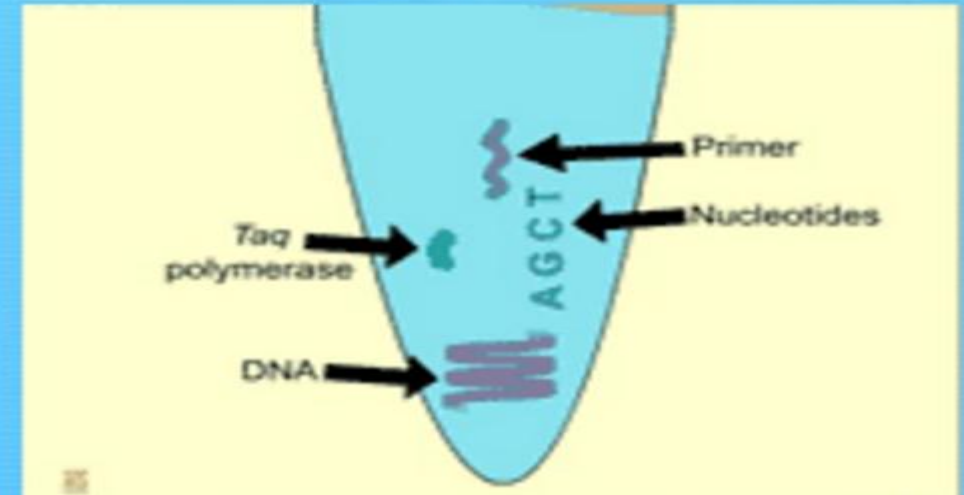
Polymerase Chain Reaction(PCR)

- PCR targets and amplifies a specific region of a DNA strand.
- It is an invitro technique to generate large quantities of a specified DNA.
- Often, only a small amount of DNA is available eg. A drop of blood, Semen strains, Single hair, vaginal swabs etc.



REQUIREMENTS OF PCR

- DNA Template
- Primers
- Taq polymerase
- Deoxynucleoside triphosphates(dNTPs)
- Buffer solution
- (Mg^{2+})

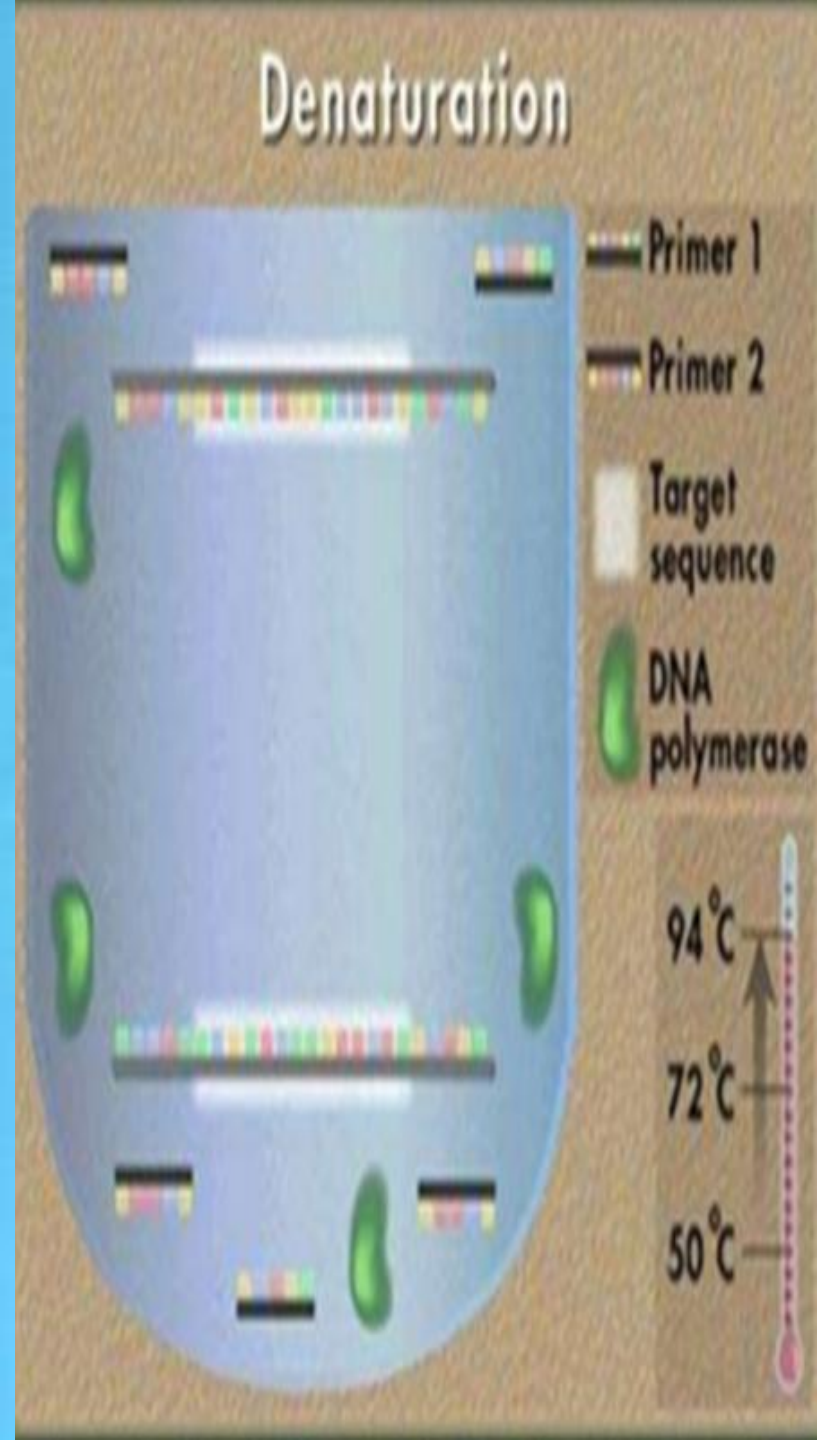


- ***Taq* polymerase:-** is a thermostable DNA polymerase named after the thermophilic bacterium *Thermus aquaticus* from which it was originally isolated by Chien et al. in 1976. Its name is often abbreviated to *Taq* Pol or simply *Taq*. It is frequently used in the TaqMan version of polymerase chain reaction (PCR), a method for greatly amplifying short segments of DNA.

STEPS INVOLVED:

DENATURATION:

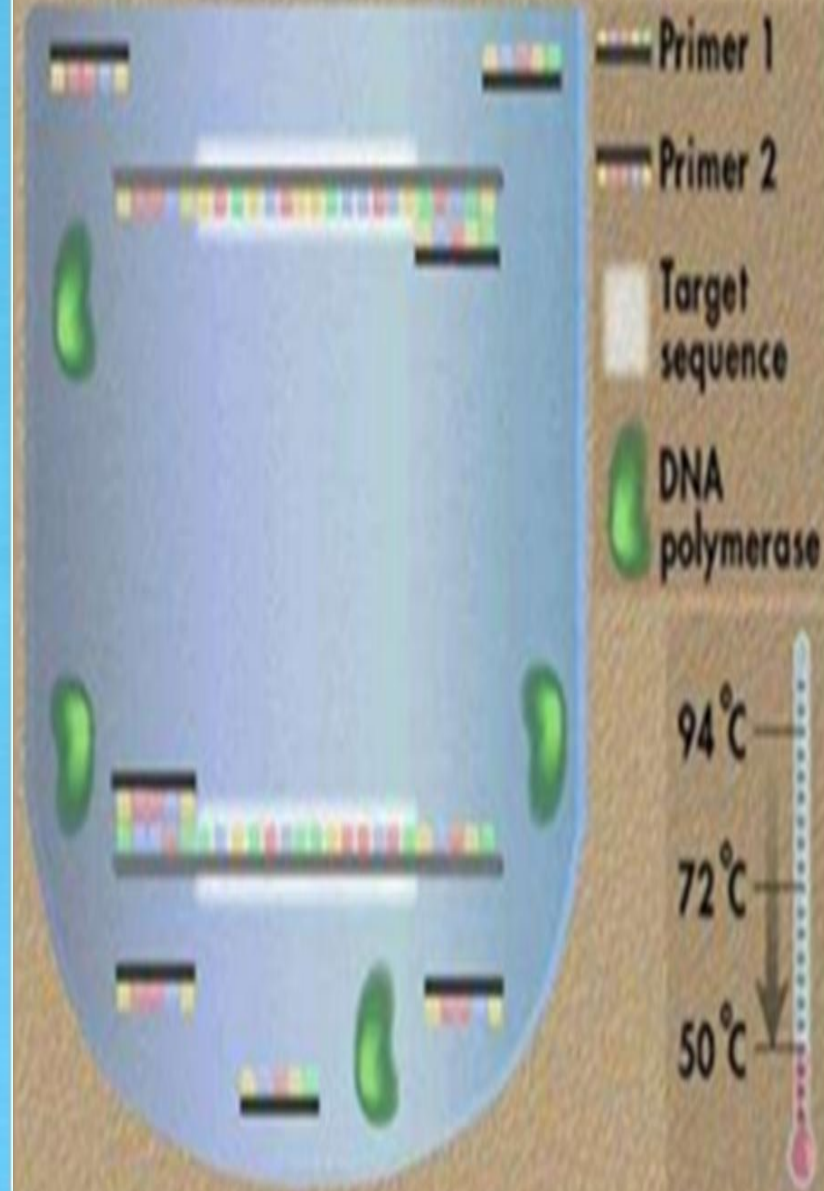
- The reaction mixture is heated to a temperature between 90-98° C so that the ds DNA is denatured into single strands by disrupting the hydrogen bonds between complementary bases.
- Duration of this step is 1-2 mins.



ANNEALING:

- Temperature of reaction mixture is cooled to 45-60° C
- Primers base pair with the complementary sequence in the DNA.
- Hydrogen bonds reform.

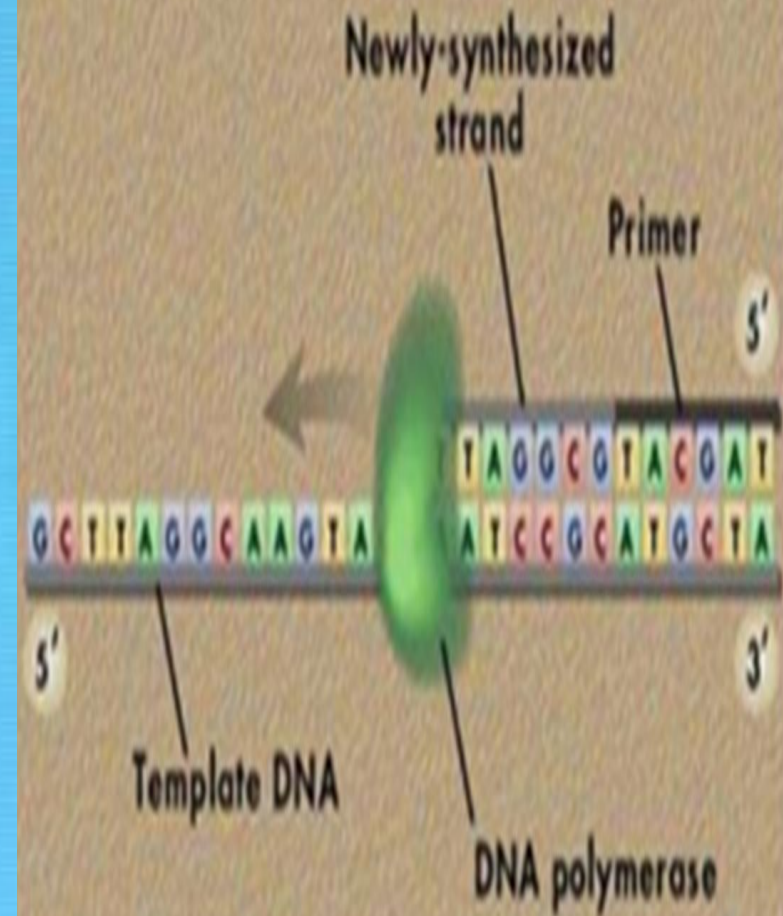
Annealing Primers



EXTENSION:

- The temperature is now shifted to 72°C which is ideal for polymerase.
- Primers are extended by joining the bases complementary to DNA strands.
- Elongation step continues where the polymerase adds dNTP's from 5' to 3', reading the template from 3' to 5' side, bases are added complementary to the template.
- Now first cycle is over and next cycle is continued, as PCR machine is automated thermocycler the same cycle is repeated upto 30-40 times.

Direction of DNA Synthesis



Performing PCR

1. Put your tube in the apparatus
2. Let the program run (35 cycles)
3. If primers fit, there is amplification of target DNA
4. If primers do not fit, no amplification product
=> the DNA (micro-organism) was not in the sample
5. Detect if there is PCR product

Step	Temperature (°C)	Time	No. of Cycle
Initial Denature	95	7 min	1
Denature	95	30 sec	35
Annealing	58	30 sec	
Extension	72	2 min 30 sec	
Final Extension	72	5 min	1

Step	Temperature (°C)	Time	No. of Cycle
Initial Denature	98	5 min	1
Denature	98	10 sec	31
Annealing	55	20 sec	
Extension	72	40 sec	
Final Extension	72	5 min	1



Thermocyclers

Conventional PCR



Advantages of PCR

Quick

Reliable

Sensitive

Relatively easy

Specific



Disadvantage of PCR

Need for equipment

Taq polymerase is expensive

Contamination

False reactions

Internal control

Cross-reaction

Applications of PCR

1. Appointment of DNA
2. Detection of genetic mutations
3. Virus detection and Genotyping of the hepatitis virus
4. Help in the diagnosis of certain diseases .
5. Used in cloning and Sequencer of DNA
6. Detection the length of DNA
7. Technology of c DNA (complementary DNA)
8. Determine the desired gene from a mixture of genes
9. Human genome project
10. Diagnosed of cancer
11. Organ transplantation

Restrictions of PCR

Contamination of reagents or lab results in false positive results

Failure due to a mistake in the protocol

Different materials/parts of the sample can inhibit the PCR process