Biology Department,
College of Education,
Salahaddin University - Erbil

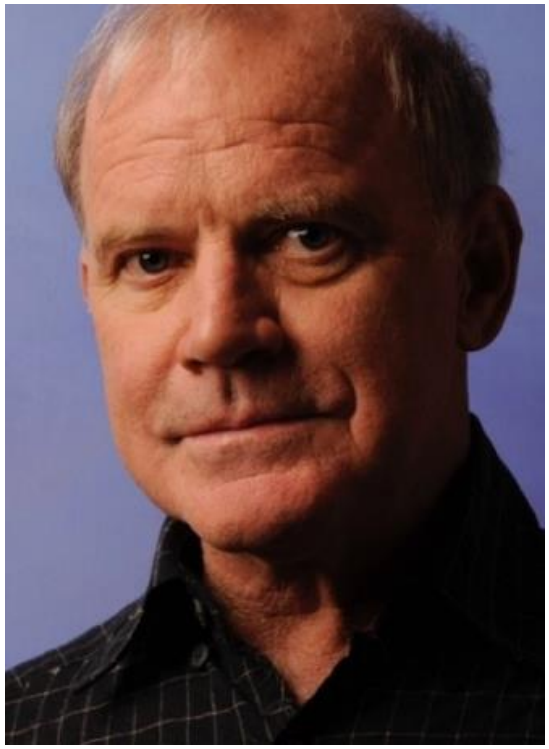
Polymerase Chain Reaction (PCR)

Lecturer: Asst. Prof. Dr. Sevan O Majed
sevan.majed@su.edu.krd

Lab-4

Polymerase Chain Reaction (PCR)

- In vitro technique for generating large quantities of a specific DNA sequence.
- Automated version of DNA replication
- Invented by Kary Mullis in 1983 who received Nobel price in Chemistry in 1993.



Kary Mullis, 1983

Polymerase Chain Reaction (PCR)

DNA polymerase is used for replication of the target sequence.

DNA sequence is repeatedly replicated

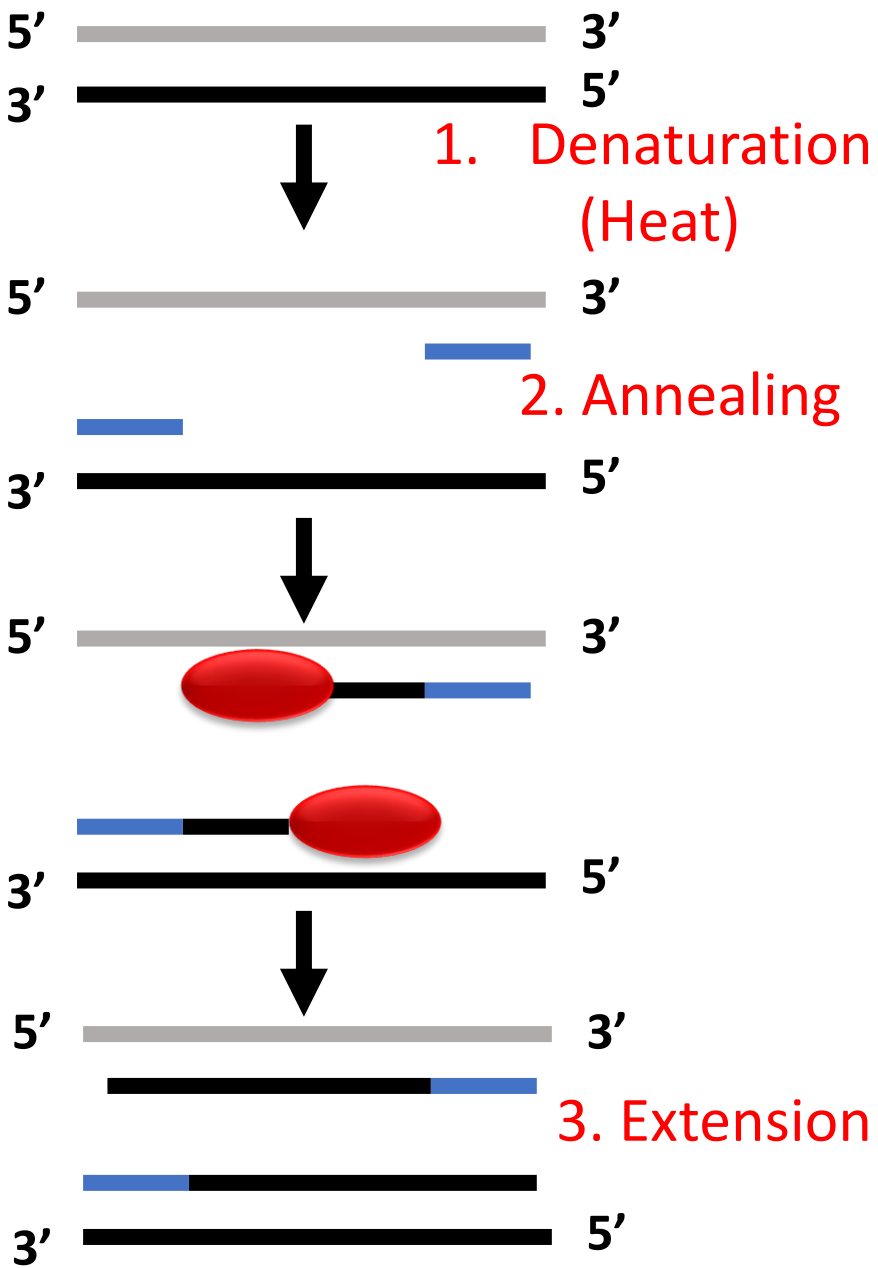
Polymerase Chain Reaction (PCR)

The basic idea behind the technique

Primers

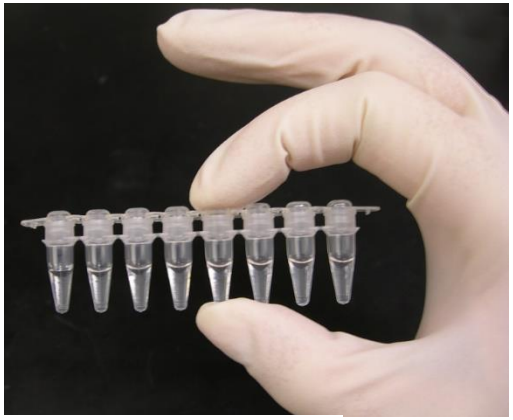
dNTPs (dATPs, dCTPs, dGTPs, dTTPs

DNA polymerase (Taq DNA polymerase)



Polymerase Chain Reaction (PCR)

- PCR is a cycle process.
- Consists of a series of 30-35 cycles
- Each cycle is 3 steps: 1. Denaturation 2. Annealing 3. Extension
- Each cycle lasts from 3-5 minutes.
- Takes place in a small polypropylene tubes Known as PCR tube.



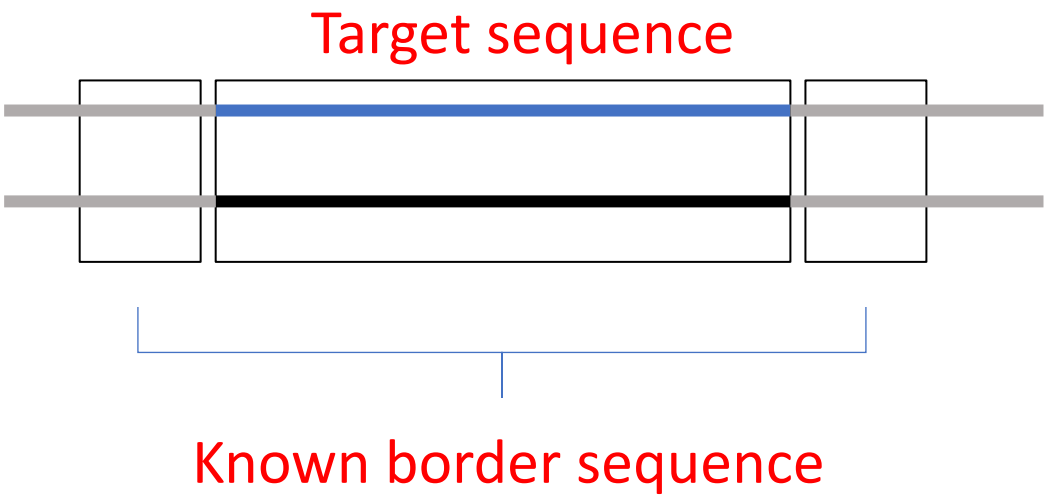
- Reaction is automated by using an instrument known thermal cycles

PCR requirements

Requires 6 basic components

1. Double stranded DNA seq or target sequence.

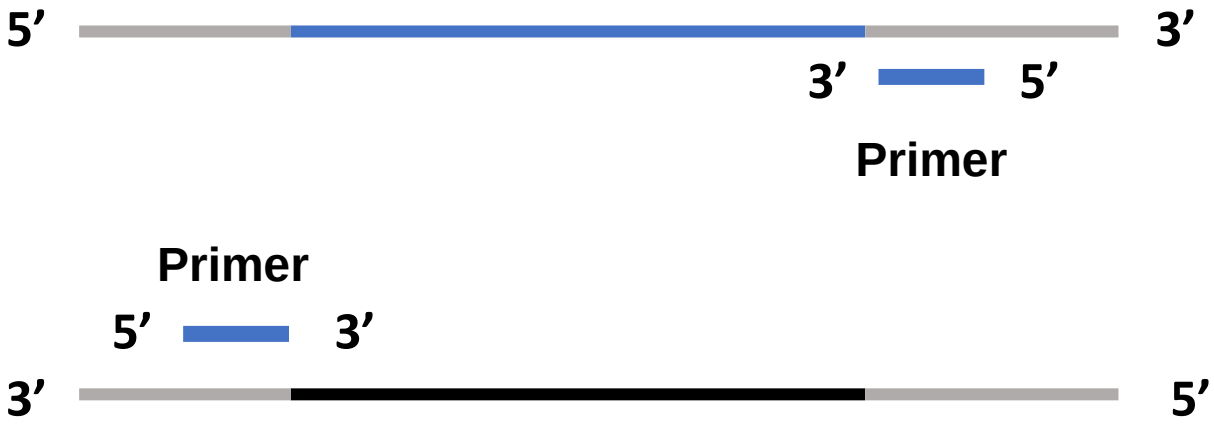
In PCR, both the stands of DNA act as template



PCR requirements

2. Two different single-stranded primers known as (Forward & Reverse primers)

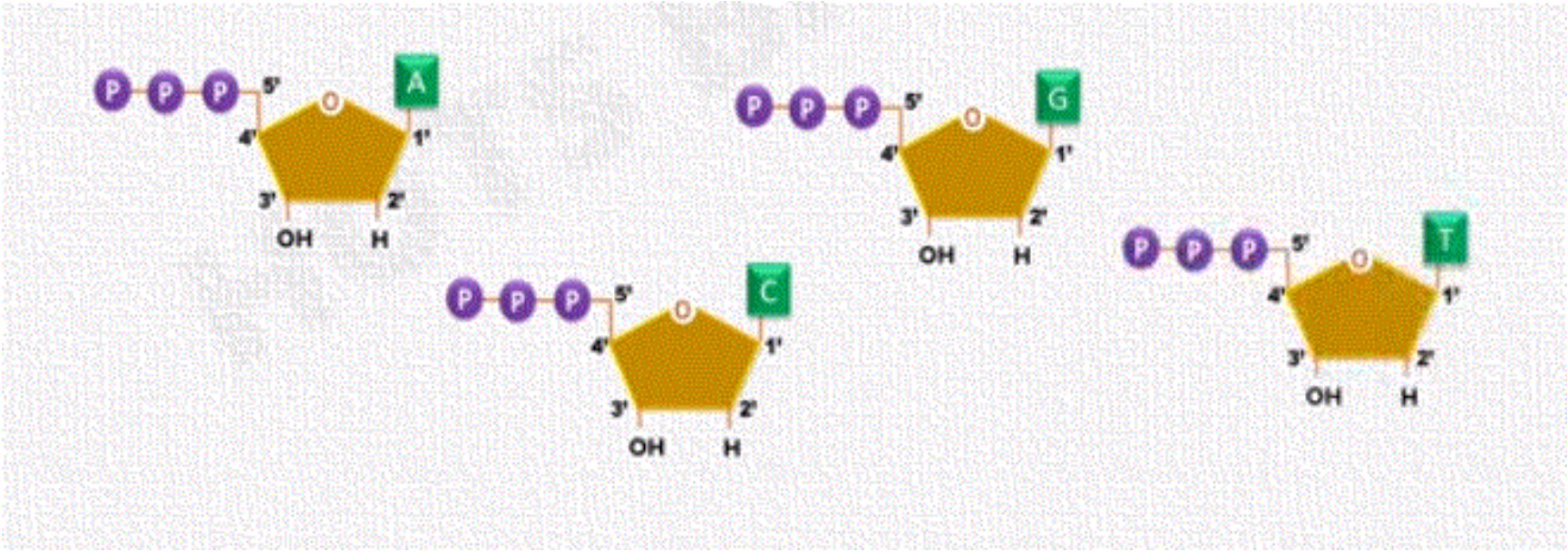
- These are short about 12-24 nucleotides long
- Chemically synthesized DNA sequence



PCR requirements

3. A supply four dNTPs **dATP, dCTP, dGTP & dTTP**

They are using by DNA polymerase to synthesize new strands during replication process



PCR requirements

4. A heat stable DNA Polymerase

- **Taq DNA polymerase**

  (from thermophilic bacterial species *Thermus aquatics*)

- **Nowadays, there a number of more efficient DNA polymerase**

- **Pfu polymerase *(Pyrococcus furiosus)***
- **Tli polymerase *(Thermococcus litoralis)***
- **Tth polymerase *(Thermus thermophilies)***

PCR requirements

5. Mixture buffer

- **10xBuffer and salt**

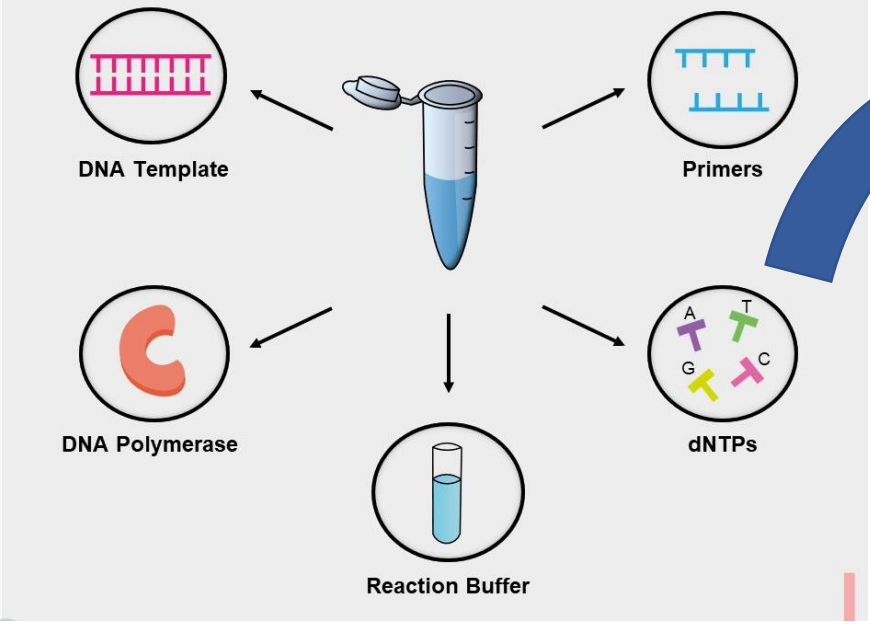
  It results in high yield of PCR products and minimizes the need for optimization of Mg2+ concentrations or the annealing temperatures.

- **MgCl2 solution**

  Acting as a cofactor, it enhances the enzymatic activity of DNA polymerase, thereby boosting DNA amplification.

6. Thermocycler

PCR components



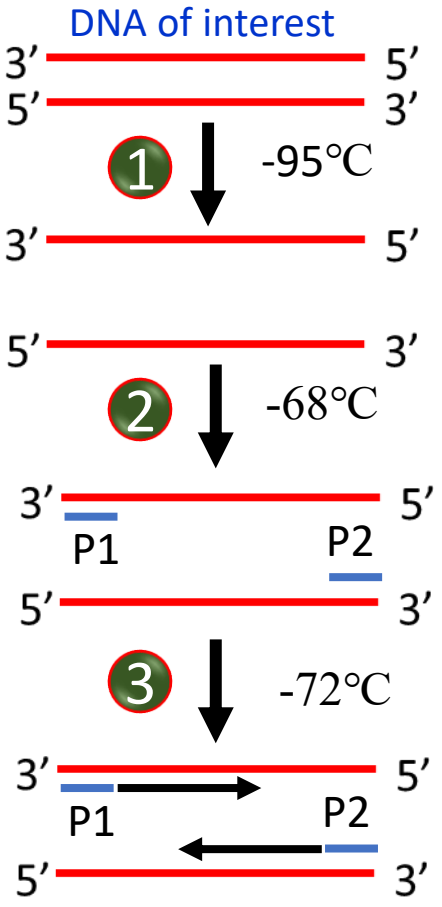
How PCR works?

❖ **Predenaturation (Hot start cycle): 95℃ for 5min**

1. **Denaturation** where a DNA solution is heated to **94-96˚C** for **45s** to break the hydrogen bonds between the two DNA strands and thus produce the single-stranded templates.

2. **Annealing** where the DNA solution is quickly cooled to **50-70˚C** for **45s** or less to allow the primers to attach to their complementary sequences.

3. **Extension** where the solution is heated to **72˚C** for **1 90s**. DNA polymerase can produce new DNA strands by adding nucleotides to the primers. Two new stranded DNA are produced for each original template of target DNA.



Video

Typical PCR cycling profile

(Hot start cycle) (Activity of enzymatic reaction)	**step 0**	**95° C**	**5 min**	**Predenaturation**
Cycle 1	**step 1**	**95° C**	**45s**	**Denaturation**
	step 2	**55° C**	**45s**	**Annealing**
	step 3	**72° C**	**1 min**	**Extension**
Cycle 2 to 29	**step 1**	**95° C**	**45s**	**Denaturation**
	step 2	**55° C**	**45s**	**Annealing**
	step 3	**72° C**	**1 min**	**Extension**
Cycle 31	**step 1**	**72° C**	**10 min**	**Polishing**
	step 2	**4° C**	**30 min**	**Incubation**

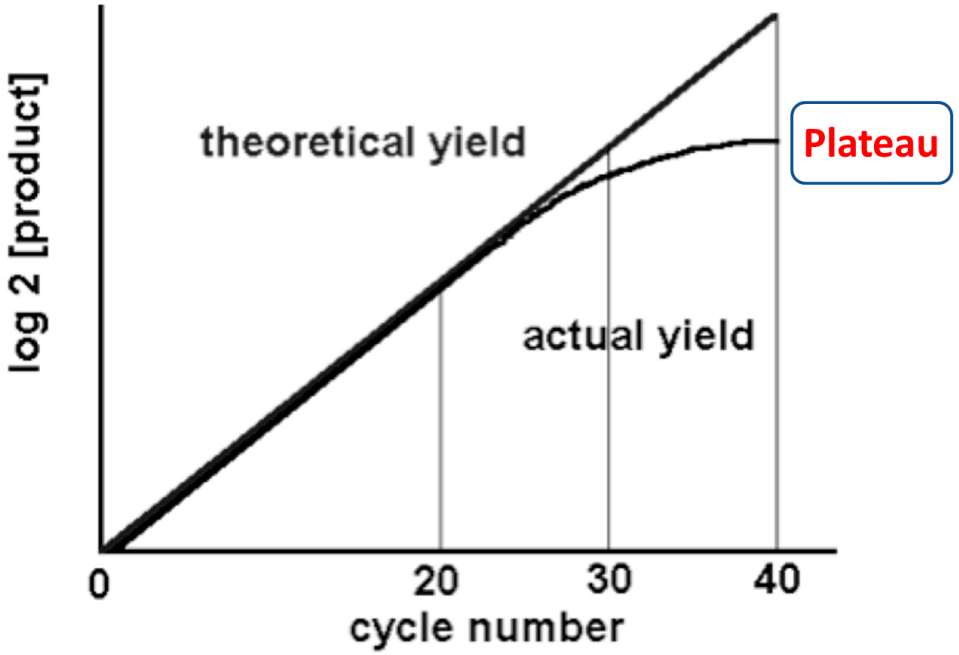
PCR applications

- New gene identification (amplification of specific genomic DNA sequences (100 pb to > 20 kbp).
- Diagnosis of infectious diseases (viral, bacterial & parasitic infections).
- Detection of hereditary diseases (β-thalassaemia, cystic fibrosis, Haemophelia.....), also cancer disease.
- Applied in DNA sequencing.
- DNA fingerprinting to identify paternity testing.
- Trace forensic evidence in crime investigations.

The 'Plateau Effect' in PCR Amplification

Plateau effect caused by:

- Degradation of reactants (dNTPs, enzyme).
- Reactant depletion.
- Competition for reactants by non-specific products.



PCR protocol

Prepare the following reaction mix in 0.2 ml PCR tube:

Template DNA (60 ng/ µl)			**5 µl**
Forward primer (1 µM)			**2.5 µl**
Reverse Primer (1 µM)			**2.5 µl**
MasterMix	**(25mM)**		**15 µl**
ddH_2O		**upto**	**30.0 µl**
	Total volume		**50.0 µl**

- Keep all reactions on ice.
- Centrifuge the mixture briefly (5 sec).
- Put the tube in PCR machine and start the reaction.
- Run the sample in 1.5% agarose gel then visualised the result under UV transilluminator.

Visualisation of PCR products

Usually visible as a single band (amplicon) on agarose gel electrophoresis