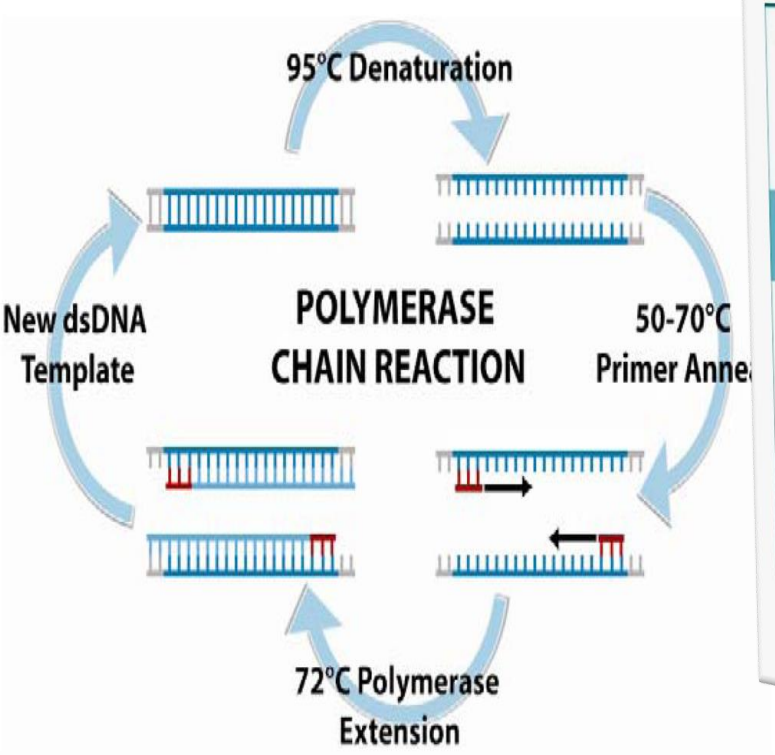
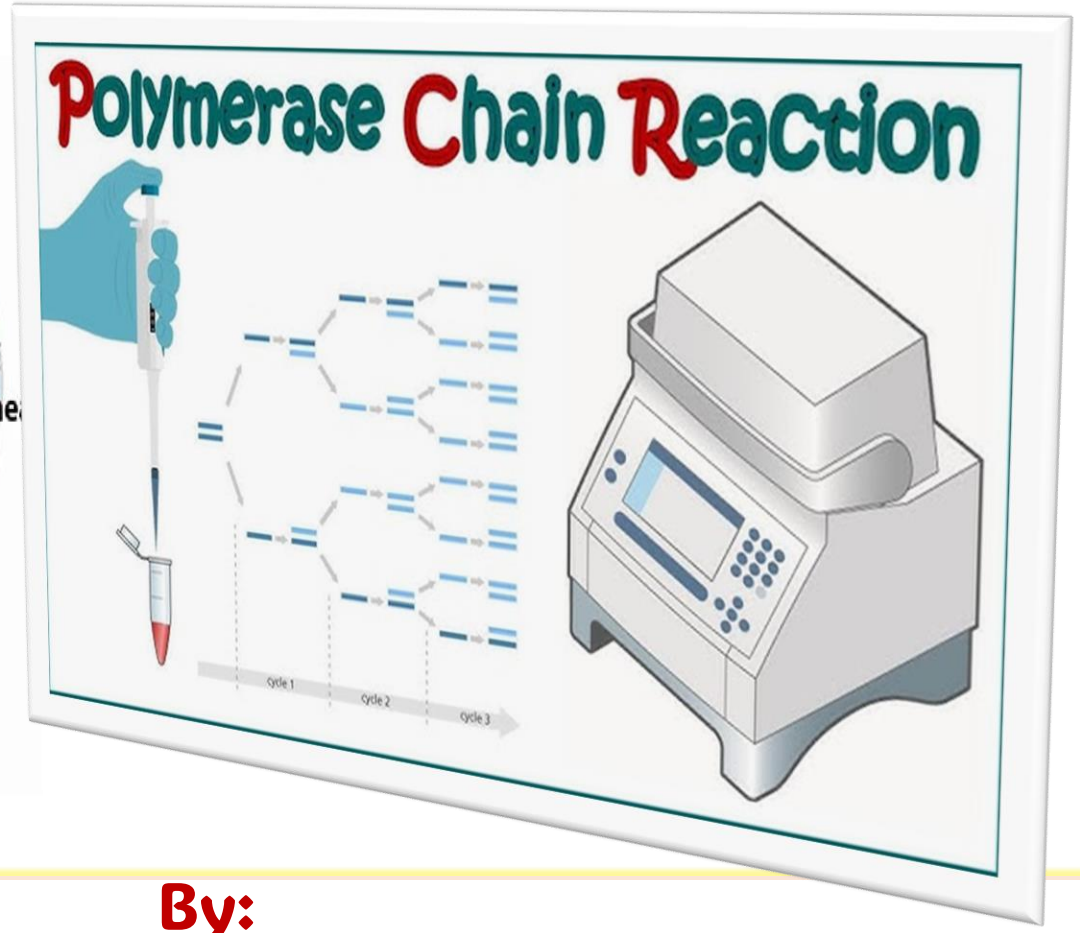
DNA amplification techniques





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Learning outcomes

At the end of this lecture, the student should be able to

1. Recognize different DNA amplification techniques including PCR.
2. Demonstrate components, steps of PCR technique (virtual lab)
3. Recognize different variants of PCR
4. Identify different applications of PCR

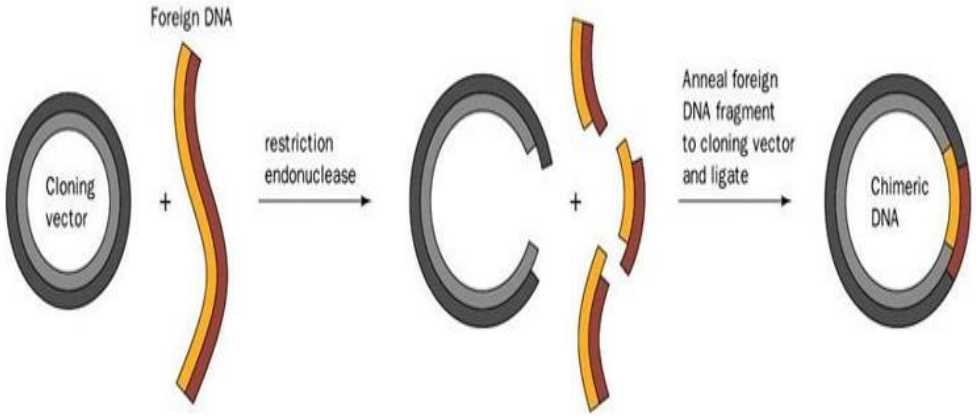
DNA Amplification Techniques

1. PCR

In vitro laboratory technique for rapidly producing millions to billions of copies of a specific segment of DNA.

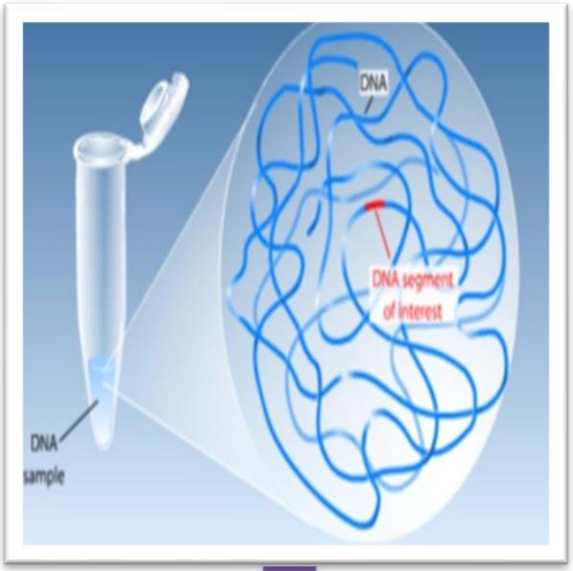
2. Cloning of DNA

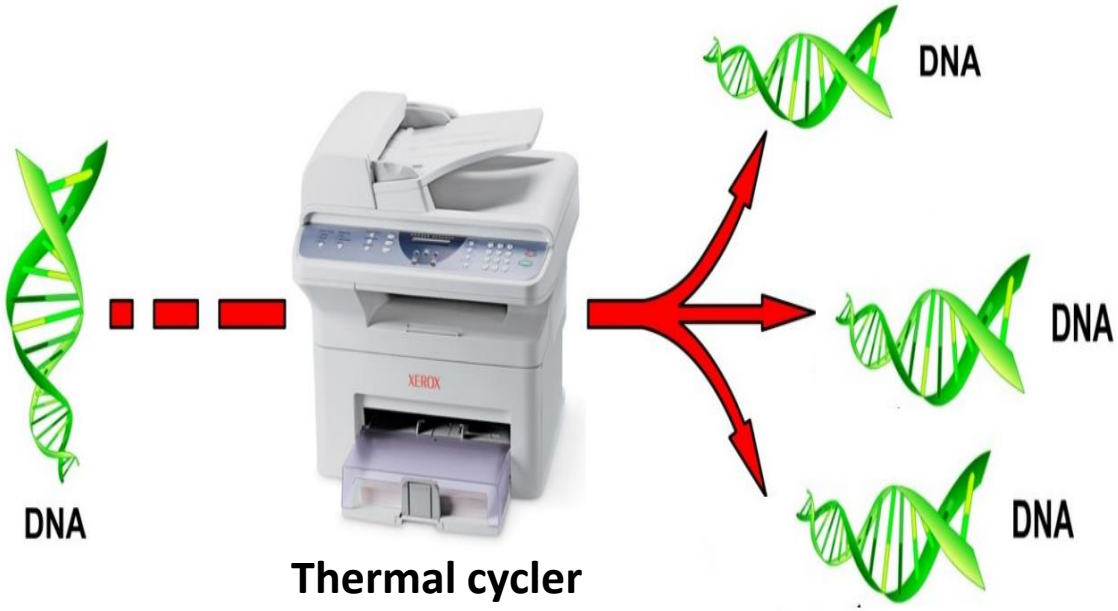
Producing many copies of a gene (DNA segment) **in vivo** for example, by replicating it in a culture of bacteria

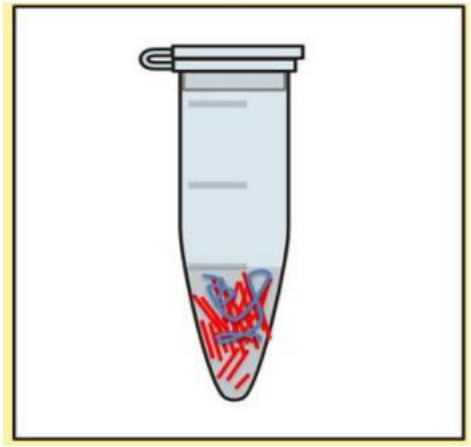


Polymerase Chain Reaction (PCR)

- In vitro laboratory technique for rapidly producing millions to billions of copies of a specific segment of DNA.



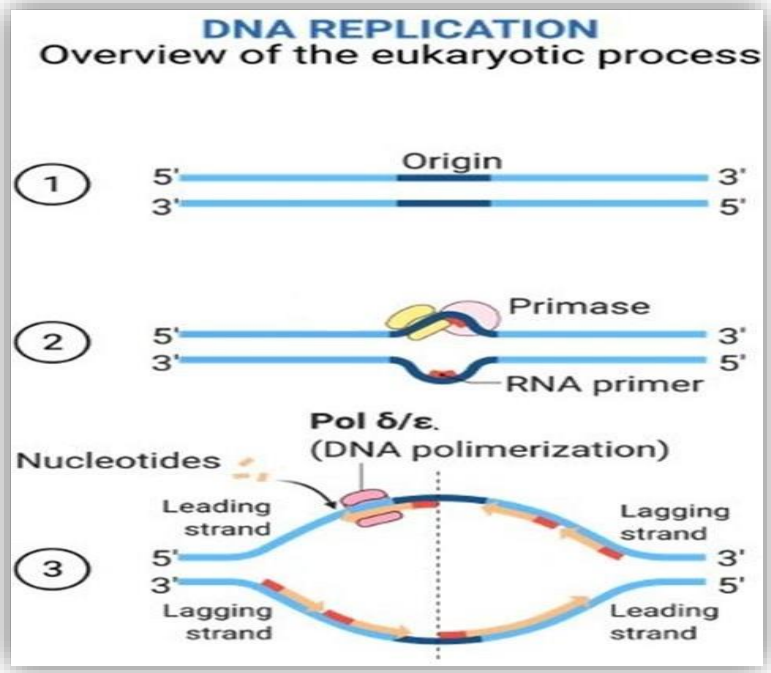


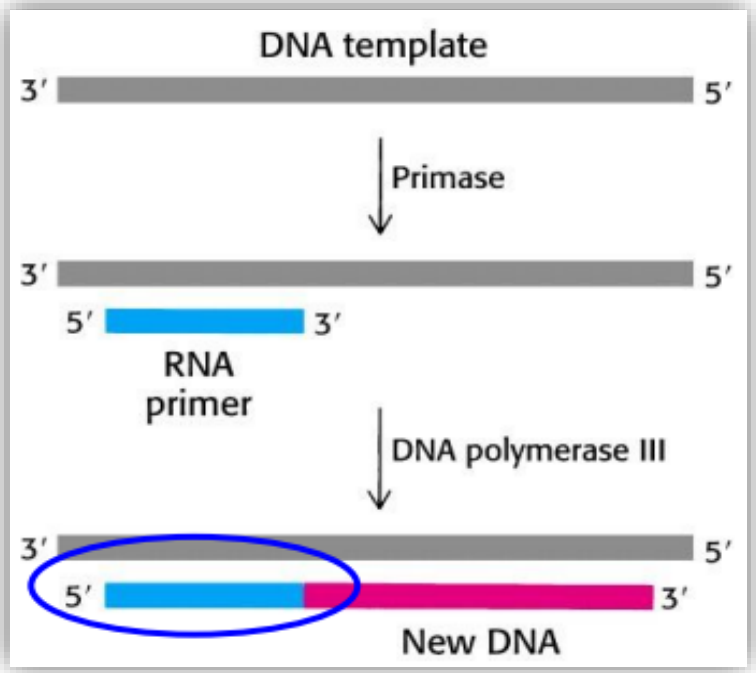


How PCR Works

- PCR is an **artificial way of doing DNA replication**
- As in replication, PCR involves:

  -Melting DNA -Priming -Polymerization





- Instead of replicating all the DNA present, **only a small segment is replicated**, but this small segment is replicated **many times.**

PCR reaction components

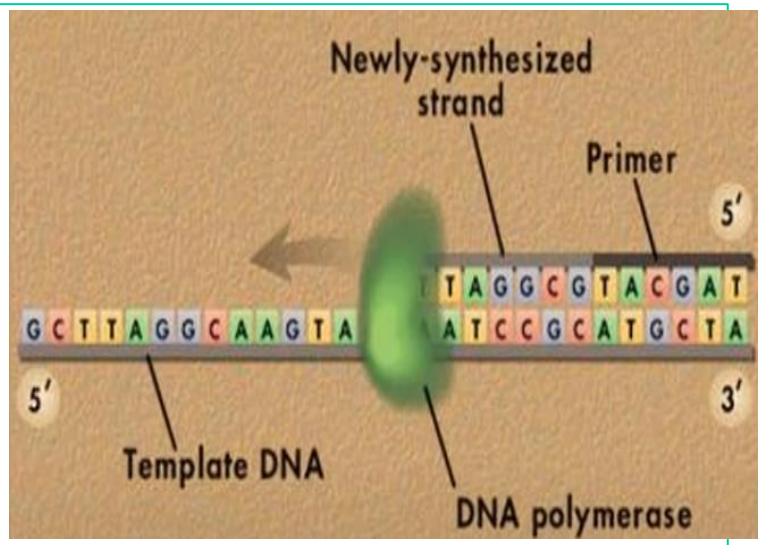
1) Target DNA

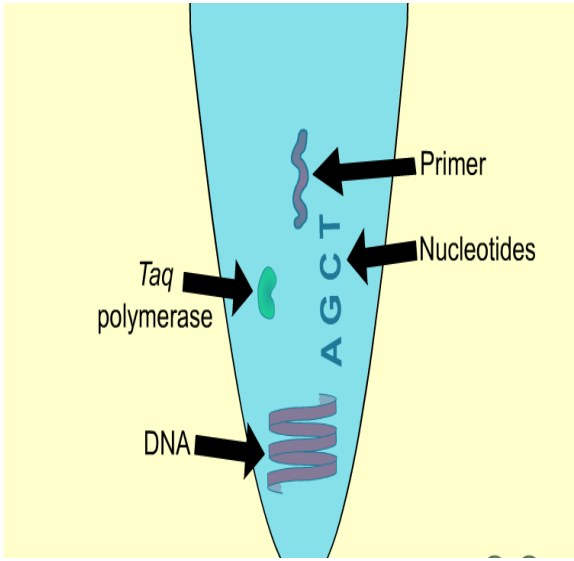
2) Pair of Primers
(flank DNA segment to be amplified)

3) dNTP

4) A Thermostable DNA Polymerase
(Taq polymerase)

5) Buffer solution
(containing Mg++, maintains pH suitable for the enzyme activity)

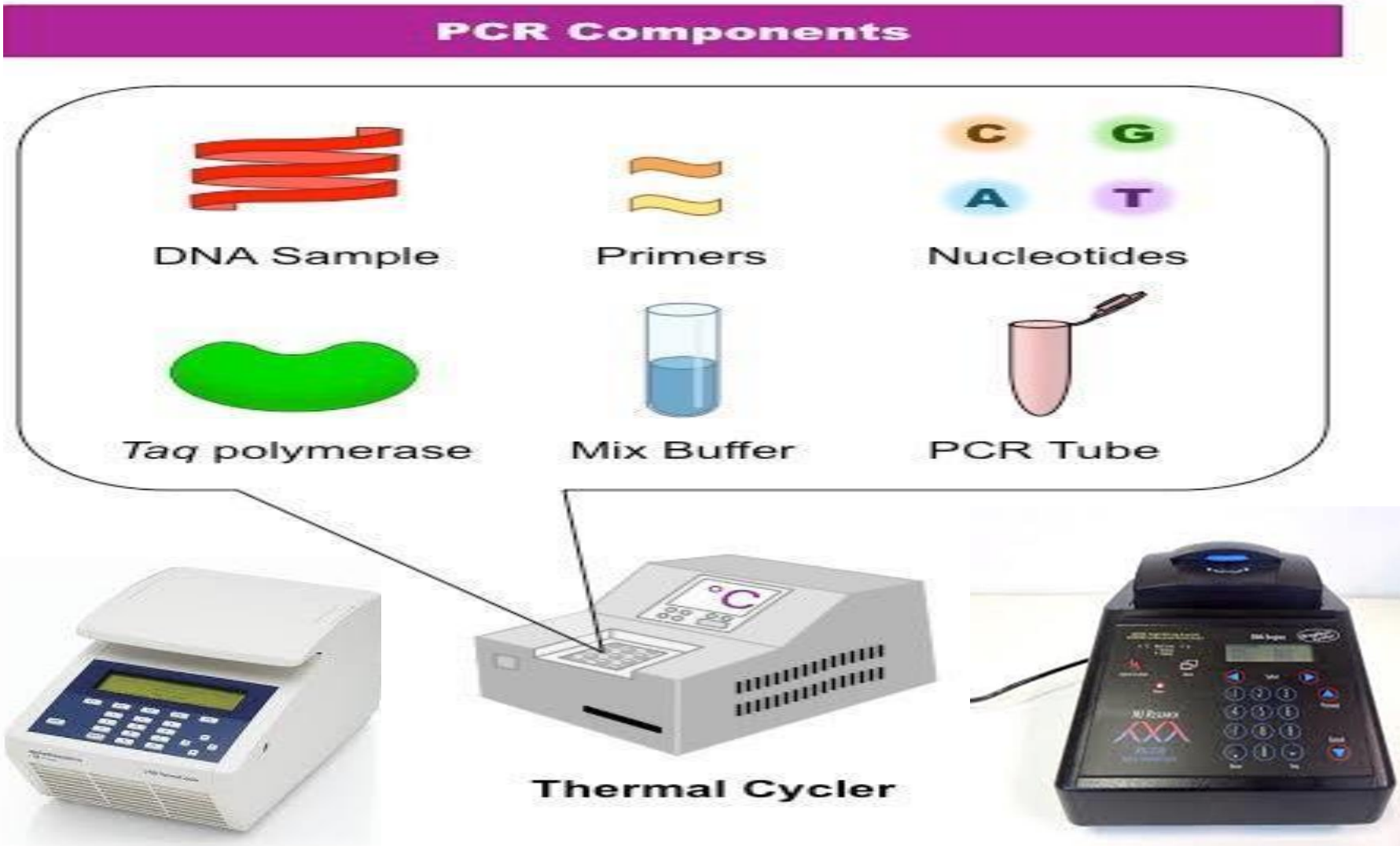




Taq polymerase

- **In PCR: Thermostable DNA polymerase is needed, because of high temperature used in PCR**
- **Called Taq polymerase because it was isolated from Thermus aquaticus, a bacterium that lives in hot springs.**

PCR Requirements



Thermal cycler

- Laboratory apparatus used to amplify segments of DNA via the polymerase chain reaction (PCR)
- The device has a thermal block with holes where tubes holding the reaction mixtures can be inserted
- The cycler then raises and lowers the temperature of the block in pre-programmed steps

PCR Technique (1st cycle)

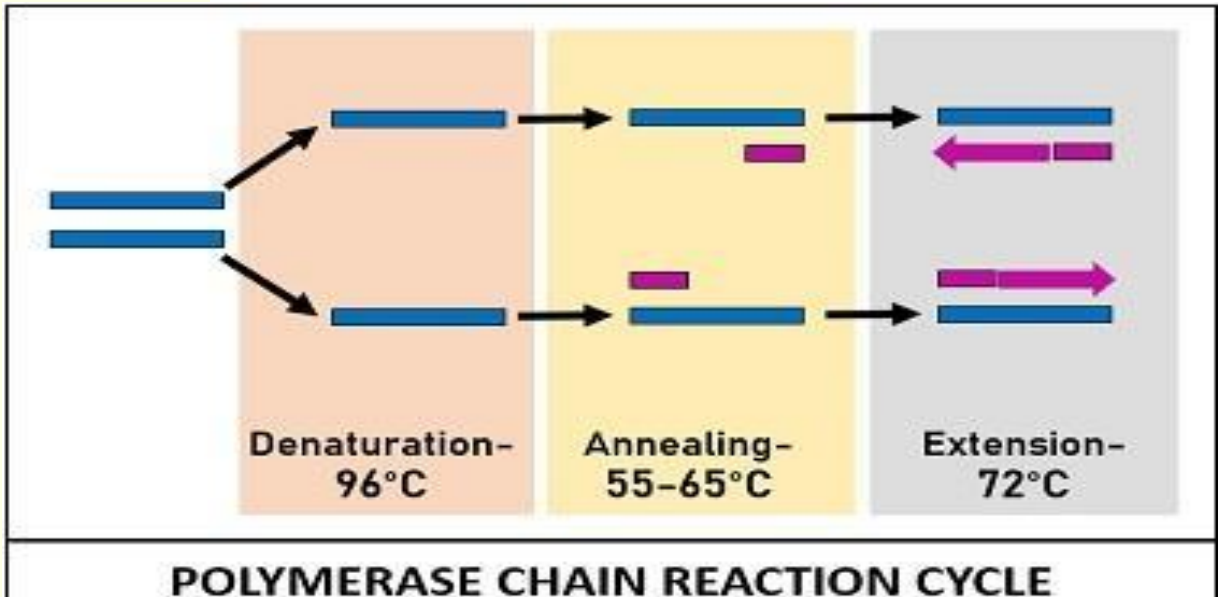
The PCR is a cyclic process:

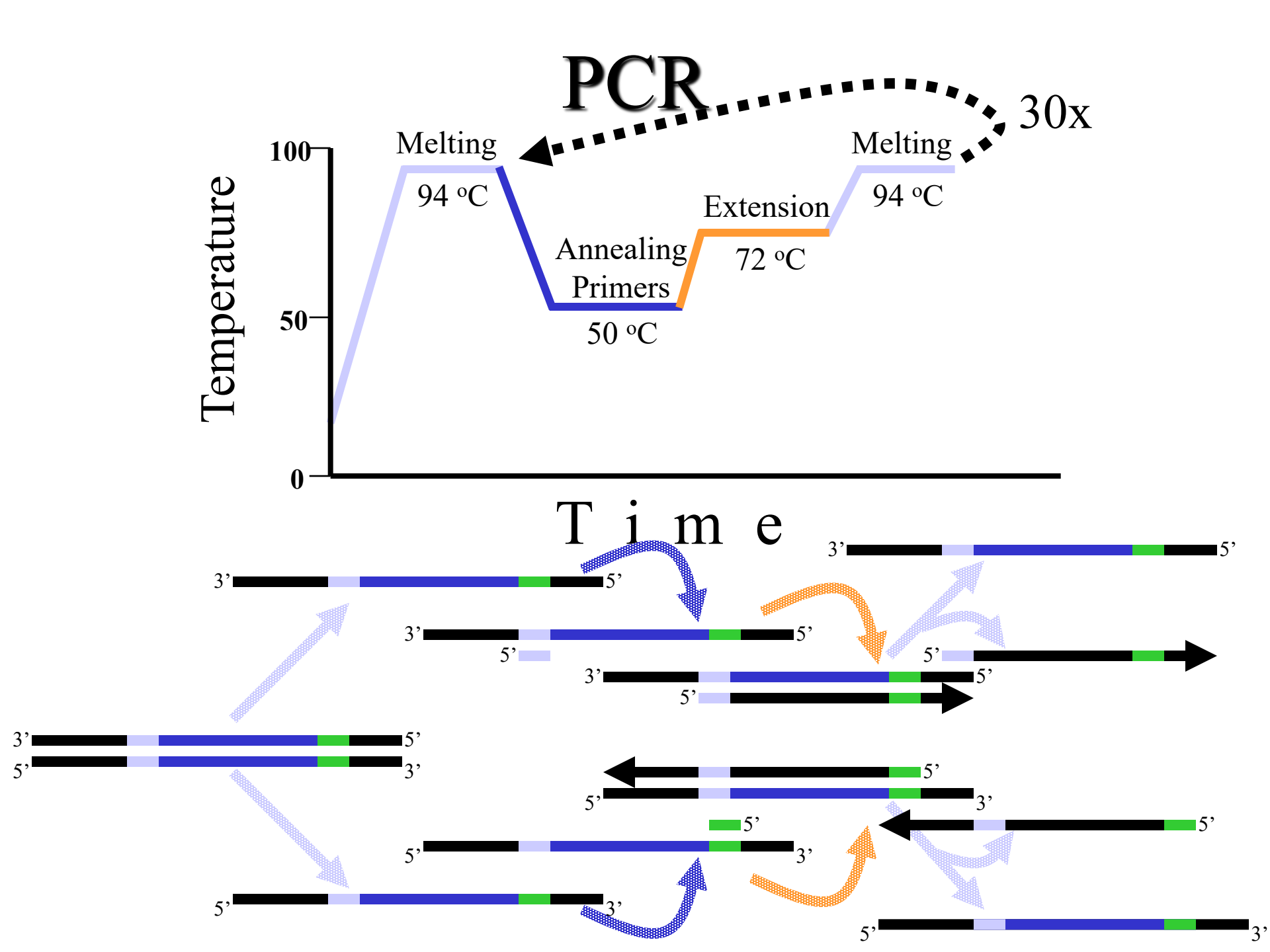
1. **Melting (Denaturation) (94-95 °C): The double stranded DNA is denatured by heat to two single strands.**

2. **Annealing (50-65°C): The primers anneals with the single-strand templates by base pairing.**

3. **Extension (72°C): by Taq polymerase in the presence of dNTP.**

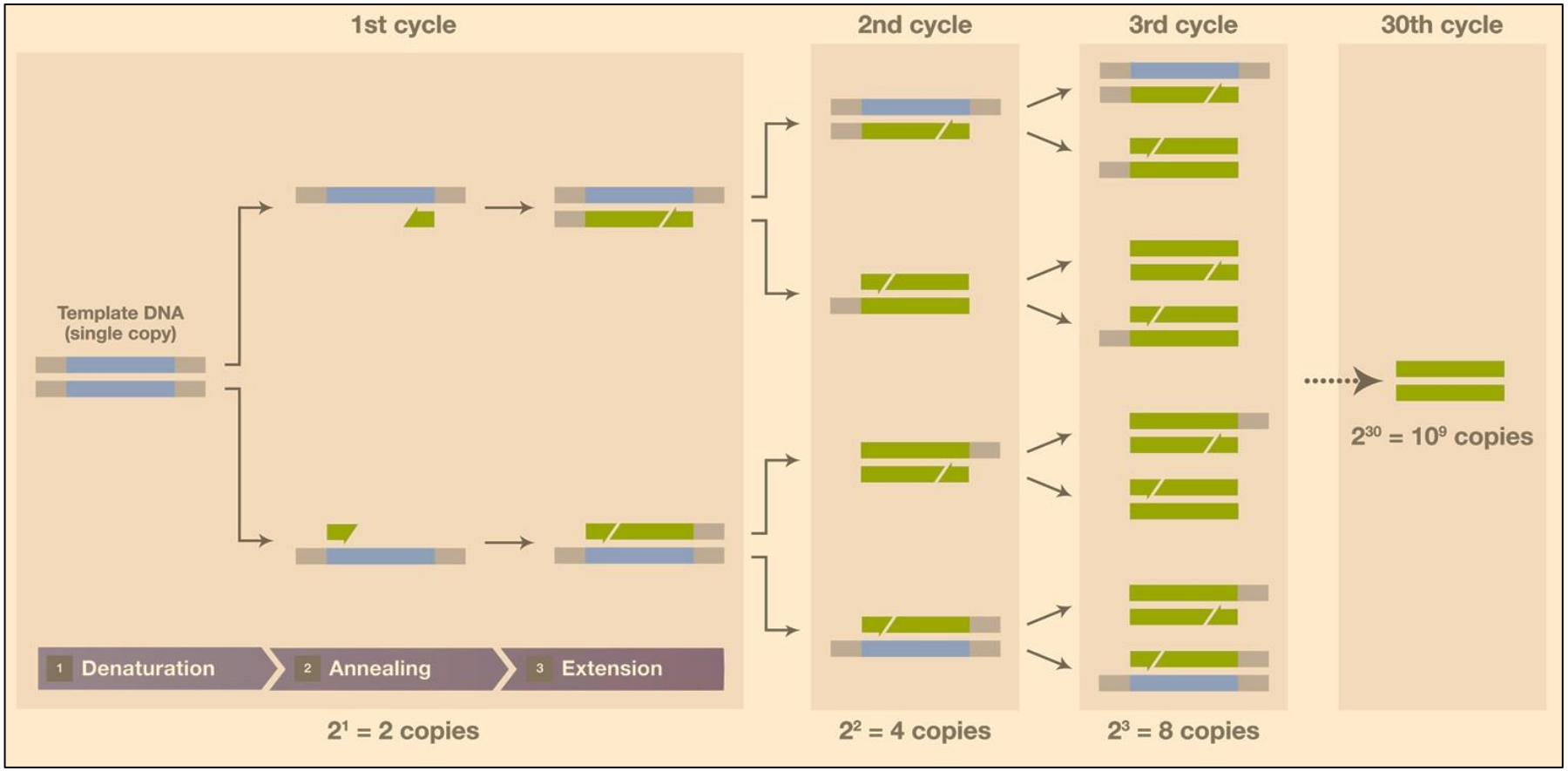
So, A copy of the original sequence is formed



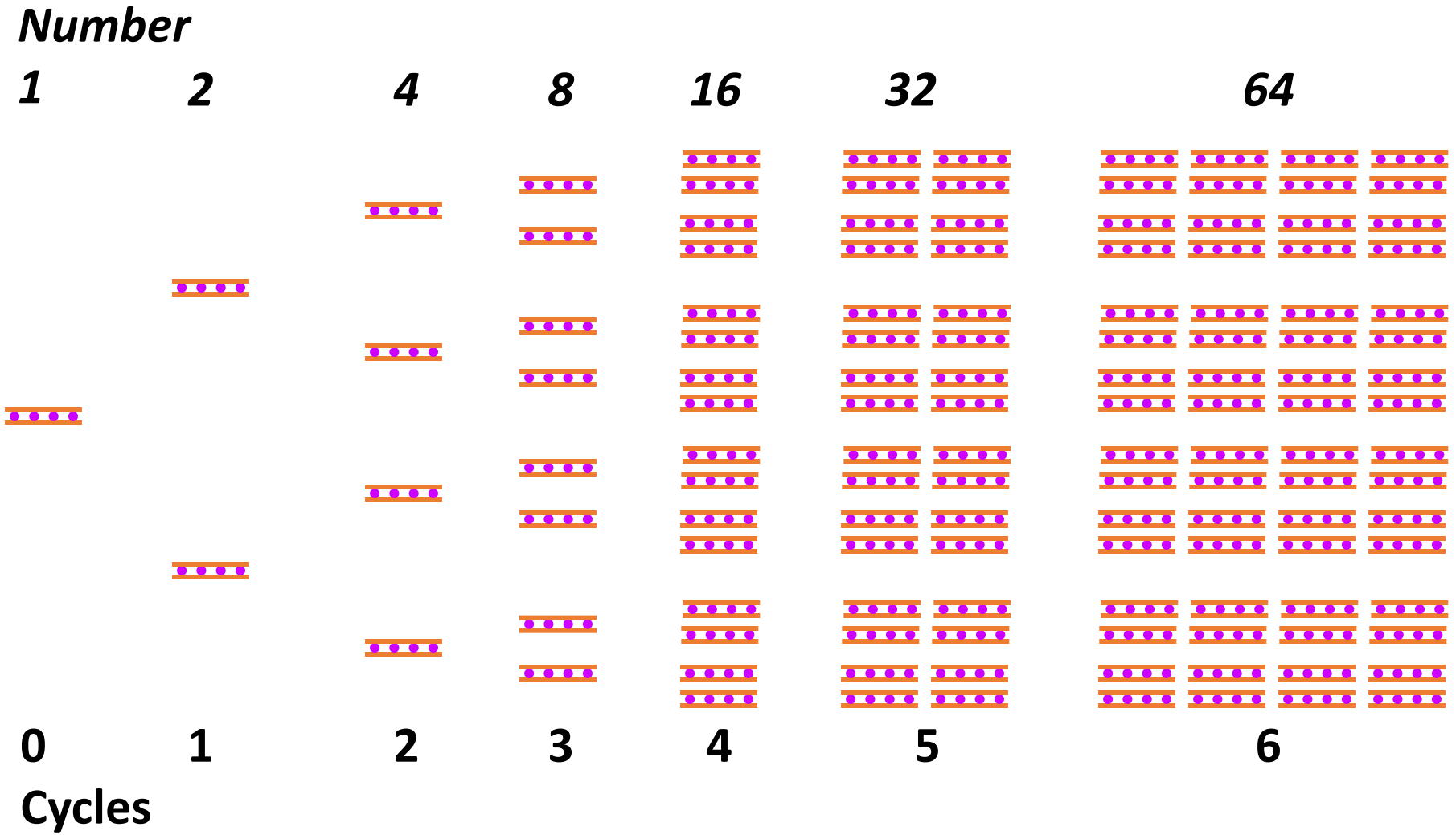

PCR
30x
Melting
94 °C
Annealing
Primers
50 °C
Extension
72 °C
Melting
94 °C
100
50
0
Temperature
T i m e
3’
5’

PCR Technique (Next cycles)

- Subsequent cycles contain the same three steps
- After 20-30 cycles of amplification, million/ billion copies of DNA can be generated from a single copy.



DNA Between The Primers Doubles With Each Thermal Cycle

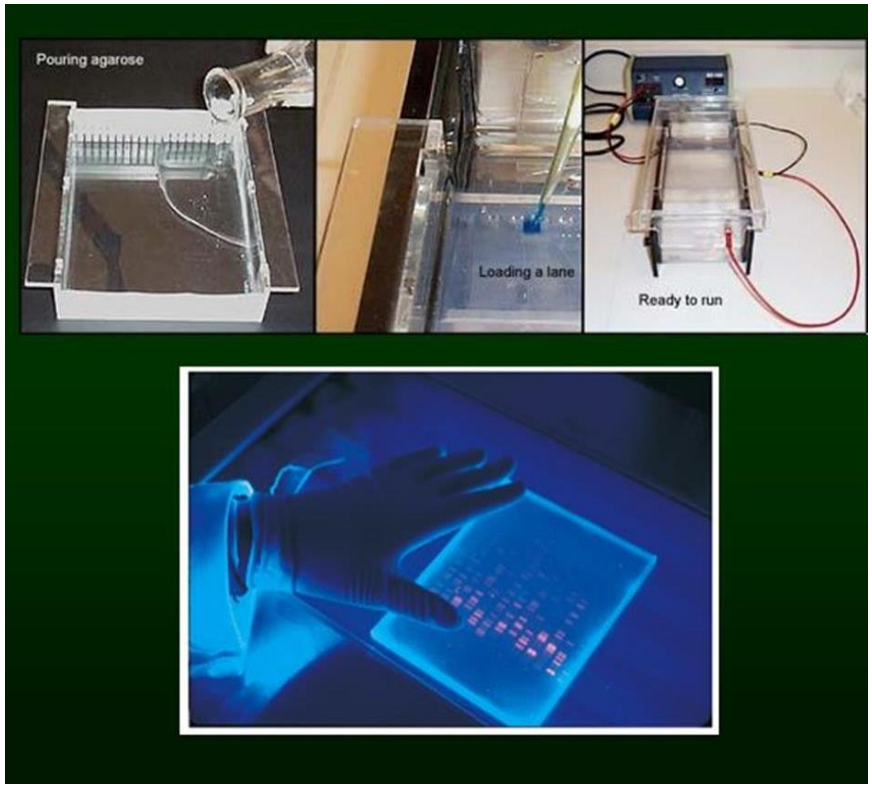


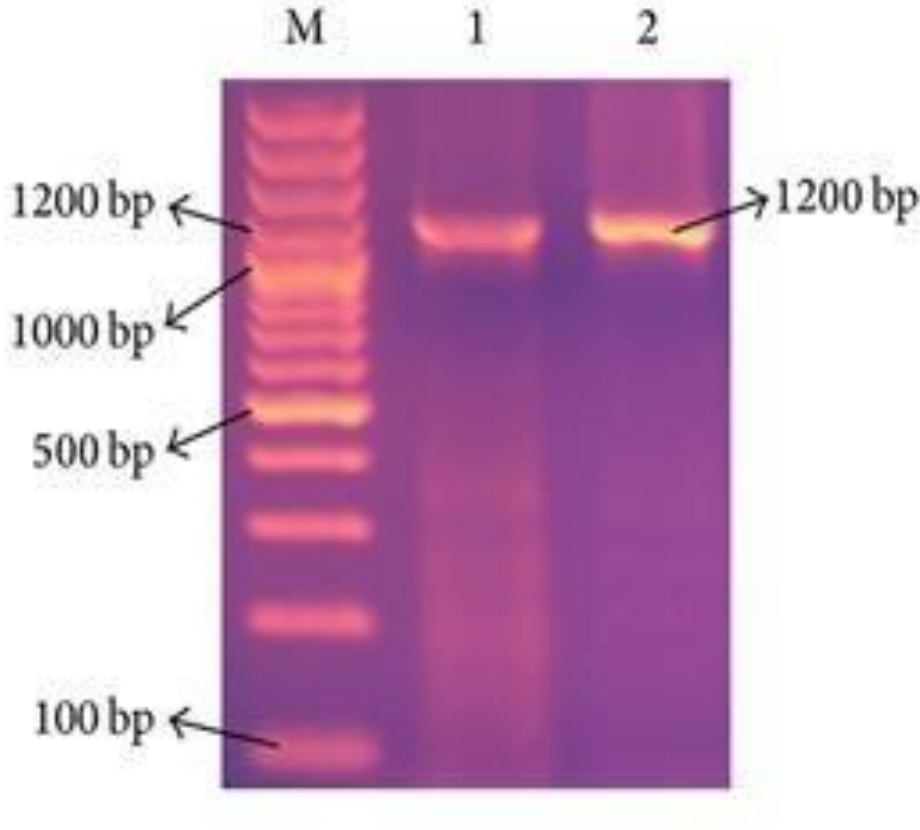
PCR Demo

https://www.youtube.com/watch?v=2KoLnIwoZKU

Detection of PCR products

- After amplification, the PCR product is analyzed using agarose gel electrophoresis to check the size of the DNA fragments produced.

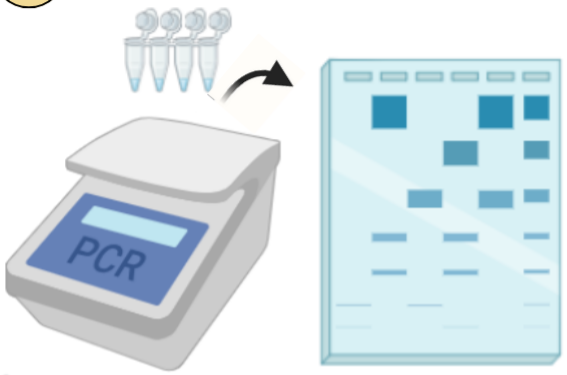




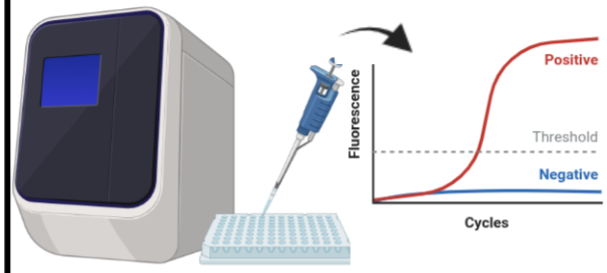
Types (Variants) of PCR

Overview of Various Types of Polymerase Chain Reaction (PCR)

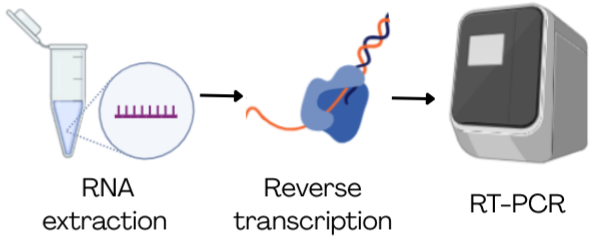
1 Conventional PCR



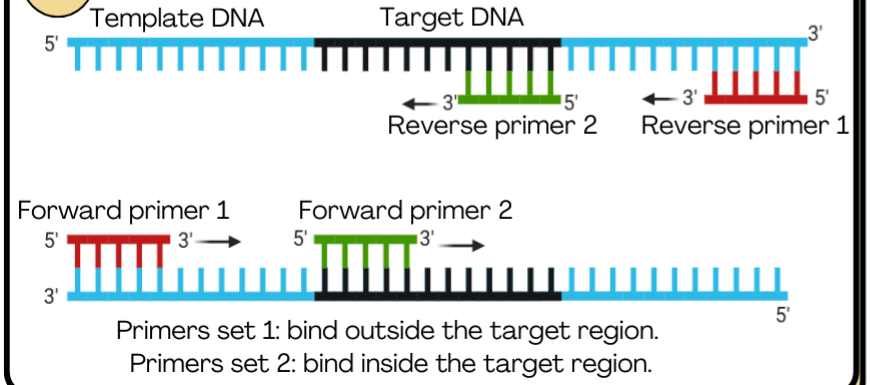
2 Quantitative Real Time PCR



3 Reverse Transcriptase PCR



4 Nested PCR



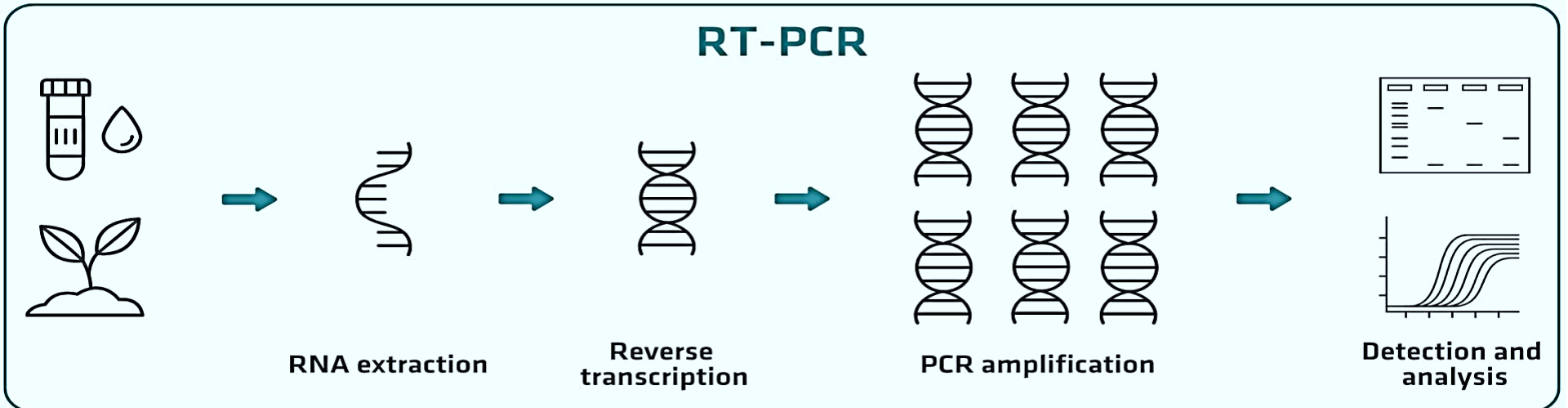
5 Some Other Types of PCR

- Gradient PCR
- Touchdown PCR
- Hot Start PCR
- Overlapping PCR
- Colony PCR
- MAMA PCR
- DADA PCR
- And others...

1. RT-PCR (Reverse Transcription Polymerase Chain Reaction)

- It is a laboratory technique used to detect and measure RNA.
- RNA template is first converted into a complementary DNA (cDNA). The cDNA is then used as a template for amplification using PCR

1. **RT = Reverse Transcription** → converts RNA into complementary DNA (cDNA) using reverse transcriptase enzyme.
2. **PCR = Polymerase Chain Reaction** → amplifies that DNA



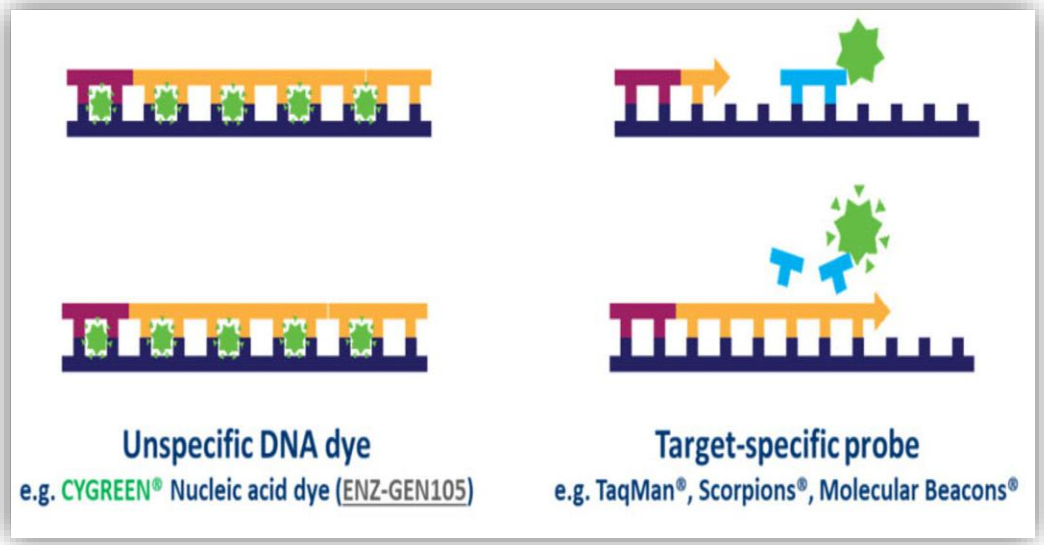
2. Quantitative PCR (qPCR) =Real-Time PCR

- It monitors the amplification of a targeted DNA molecule during the PCR, i.e. in real-time, and not at its end, as in conventional PCR.
- More accurate and sensitive method as it used to amplify and **simultaneously** quantify (measure the specific amount) target DNA in a sample during the PCR process

2. Quantitative PCR (qPCR) =Real-Time PCR

- It uses fluorescent dyes or probes. Fluorescent signals track amplification of DNA in real time after each cycle.

- widely used in:
  - gene expression analysis,
  - pathogen detection,
  - genetic mutation studies.



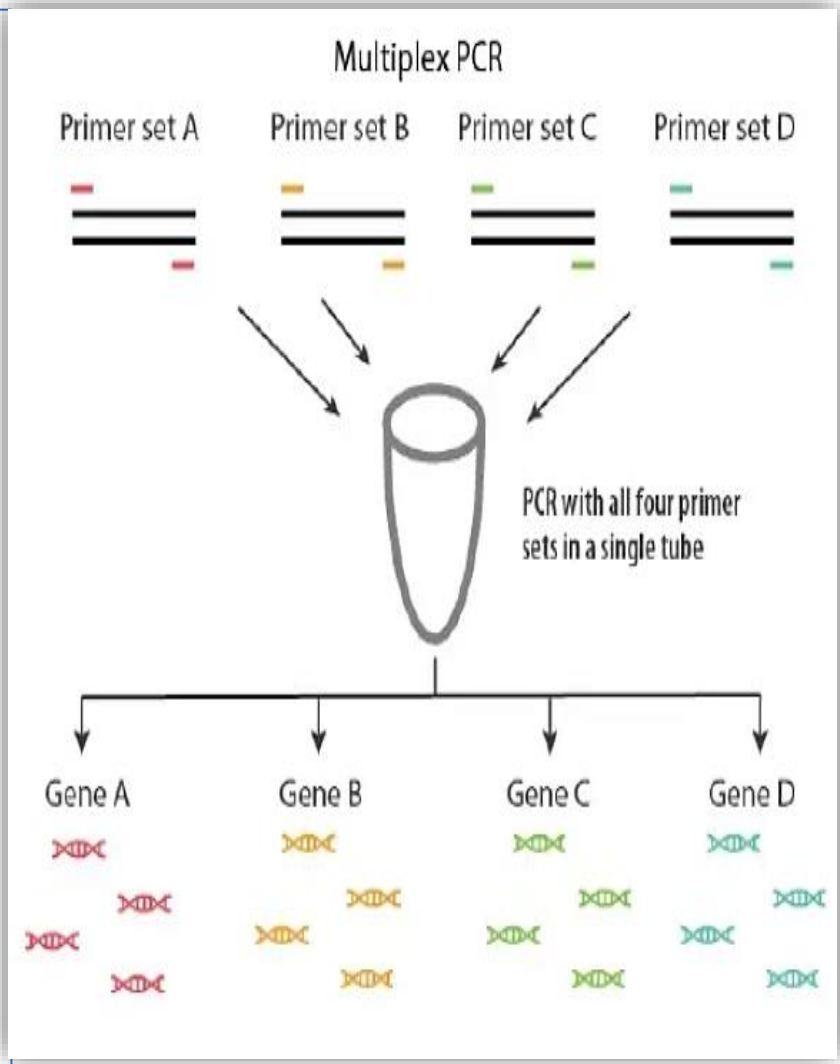
- When RNA is analyzed, the method called **RT-qPCR** after converting RNA into complementary DNA (cDNA).

3. Multiplex PCR

- It is a variation of the standard PCR technique in Molecular Biology that allows **simultaneous amplification of multiple DNA targets in a single reaction**.

- **What it means?**

-In standard PCR, you amplify **one specific DNA sequence** using one pair of primers.
-In multiplex PCR, you use **multiple primer pairs** in the same tube to amplify **several DNA regions at once**.



3. Multiplex PCR

- The results are usually visualized as **multiple bands on a gel**

Advantages

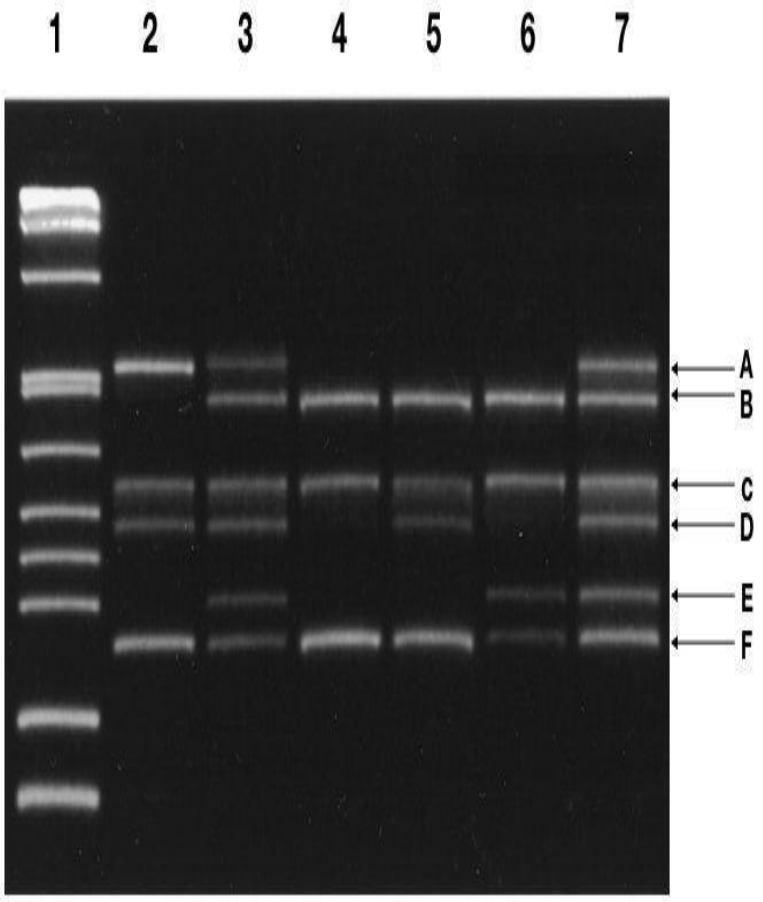
- Saves time and reagents

Limitations

- More complex to design and optimize

Applications

- Detection of multiple pathogens in one test
- Genetic disease screening
- Cancer mutation analysis

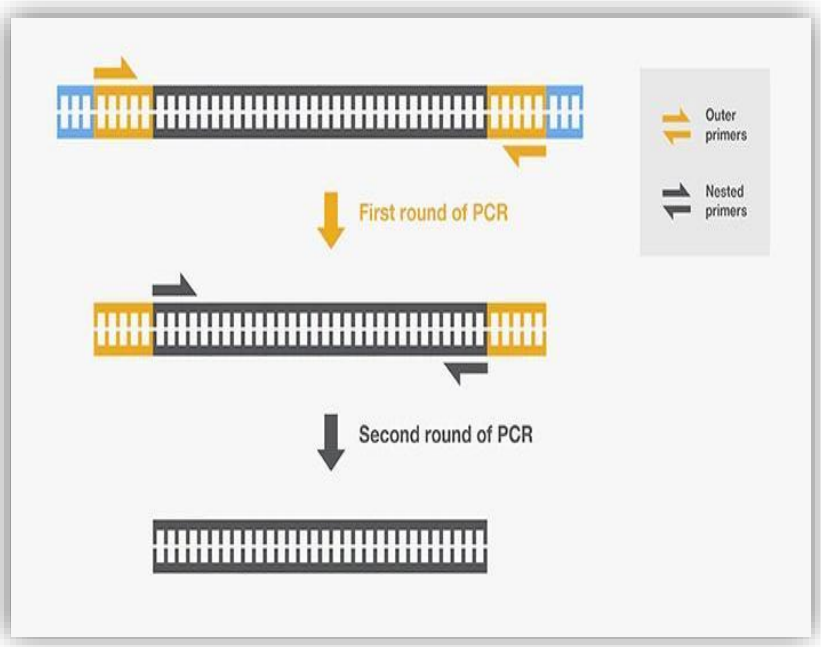


Multiplex PCR results

4. Nested PCR

- **Nested PCR** is a modification of standard PCR used to **increase specificity and sensitivity** when amplifying DNA.
- Nested PCR uses **two sets of primers** and **two rounds of PCR**:

- **First PCR (outer primers)**
  - Outer primers bind to the target DNA
  - Amplifies a larger region of DNA
- **Second PCR (inner/nested primers)**
  - Inner primers (nested inside the first region)
  - Uses the product from the first PCR
  - Amplifies a smaller, more specific region within the first product



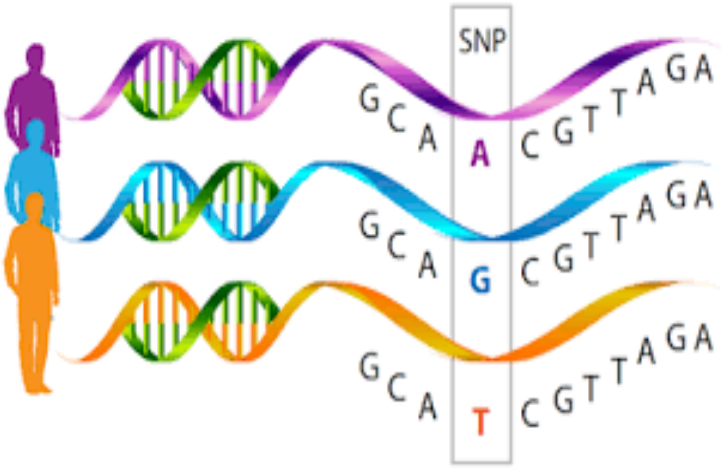
Applications of PCR

1. **Detection of infectious agents**, especially bacteria and viruses as HBV,HCV and COVID19
2. **Forensic medicine:**

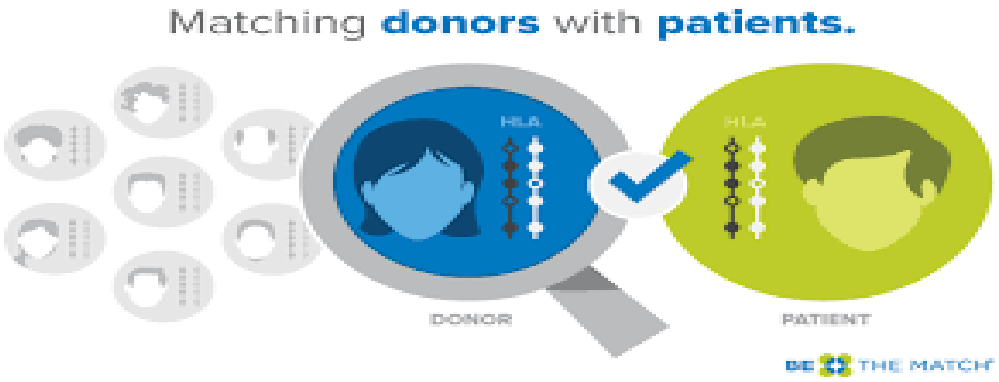
- Genetic fingerprinting at crime scenes (fingerprinting allow amplification & analysis of the DNA in a single cell as blood spot & hair follicle)
- Paternity testing

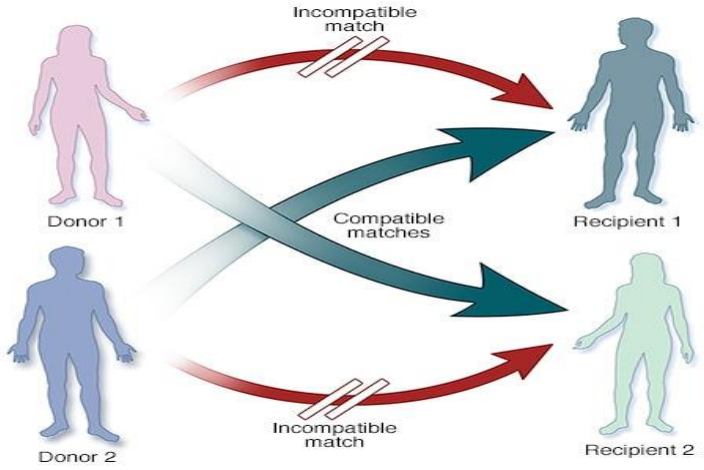
3. **Prenatal genetic diagnosis of diseases** using chorionic villous samples or cells from amniocentesis.

4. Detection of genetic polymorphism.



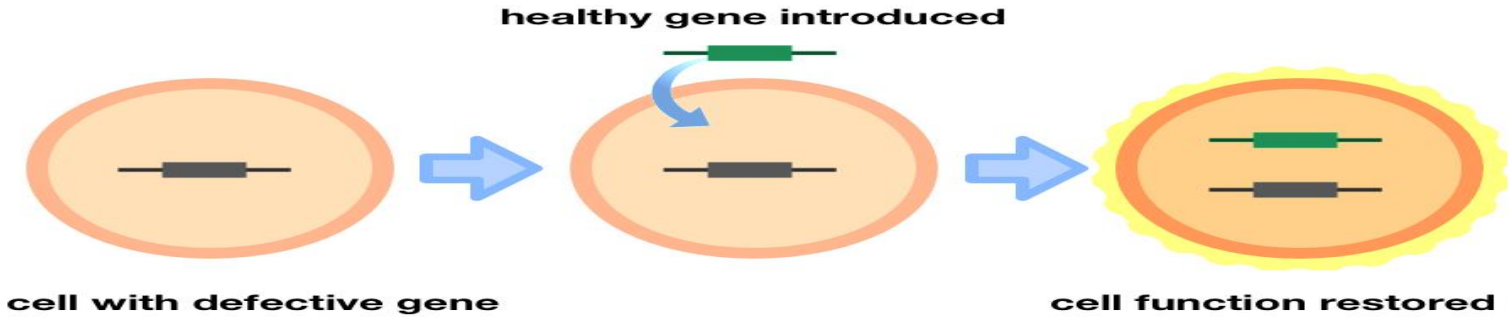
5. Tissue typing: before organ transplant to test for compatibility





Applications of PCR

6. Gene therapy: It is the introduction of normal genes into individuals who have defective genes



Eg:

- Insulin: to treat D.M.
- Human growth hormone: to treat dwarfism

7. Study of evolution

Using DNA from archaeological sample.

PCR Virtual Lab

begin

https://learn.genetics.utah.edu/content/labs/pcr/

THANK YOU