
Advanced Lab. Techniques
PCR Technique
Lec.5
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The Polymerase Chain Reaction (PCR)

PCR is a versatile and widely used technique in molecular biology. It enables scientists to amplify specific DNA sequences exponentially, generating millions of copies from a small starting sample.

What is PCR?

DNA Amplification

PCR is a powerful technique used to make millions of copies of a specific DNA sequence.

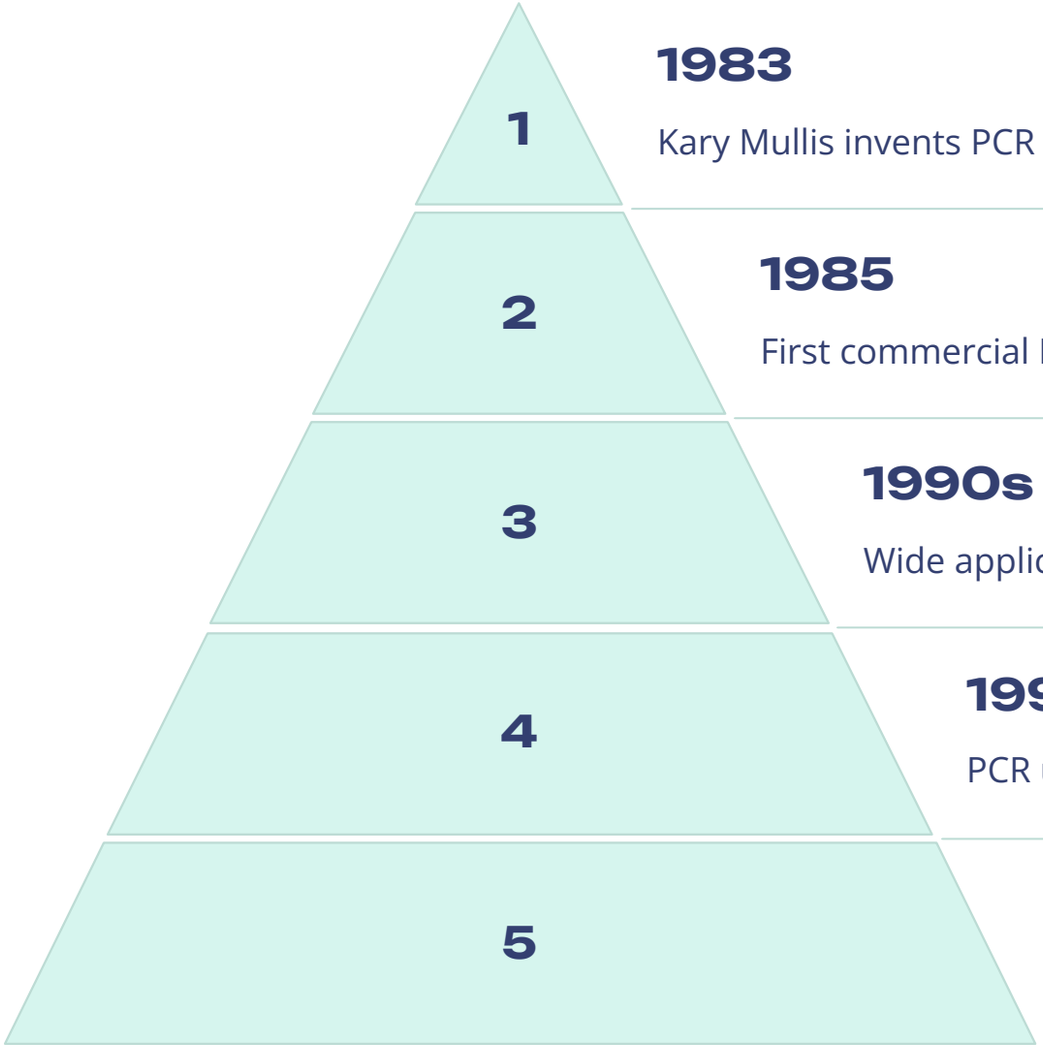
Versatile Tool

PCR is used in various fields like research, diagnostics, forensics, and genetic engineering.

Chain Reaction

The technique involves a cyclical process of heating and cooling, which replicates the DNA sequence exponentially.

History of PCR



1983

Kary Mullis invents PCR

1985

First commercial PCR kit

1990s

Wide applications in research and medicine

1993

PCR used in forensics

Present

Ongoing advancements and widespread use

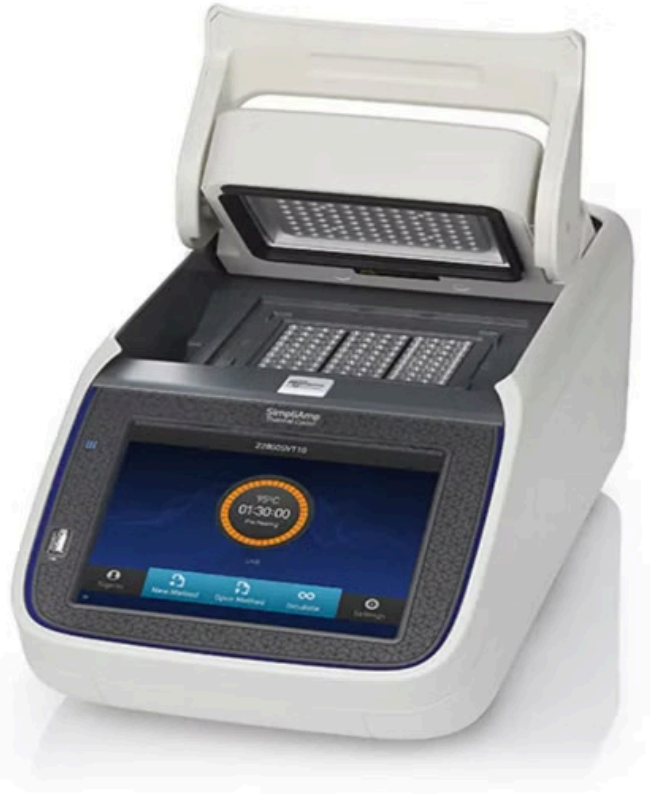
The invention of PCR revolutionized molecular biology. It enabled scientists to amplify specific DNA sequences exponentially. The technique has had a profound impact on various fields, including genetics, medicine, and forensics.

Thermal Cyclers

Thermal cyclers are essential instruments for PCR, controlling the temperature cycles that drive the DNA amplification process.

They precisely regulate temperature changes, allowing for the denaturation, annealing, and elongation steps of PCR.

Modern thermal cyclers offer advanced features like gradient temperature control, fast ramping speeds, and real-time monitoring.



PCR Reagents

DNA Template

The DNA to be amplified is the template. The template can be genomic DNA, cDNA, or plasmid DNA.

Primers

Short, single-stranded DNA sequences that bind to specific regions of the template DNA.

DNA Polymerase

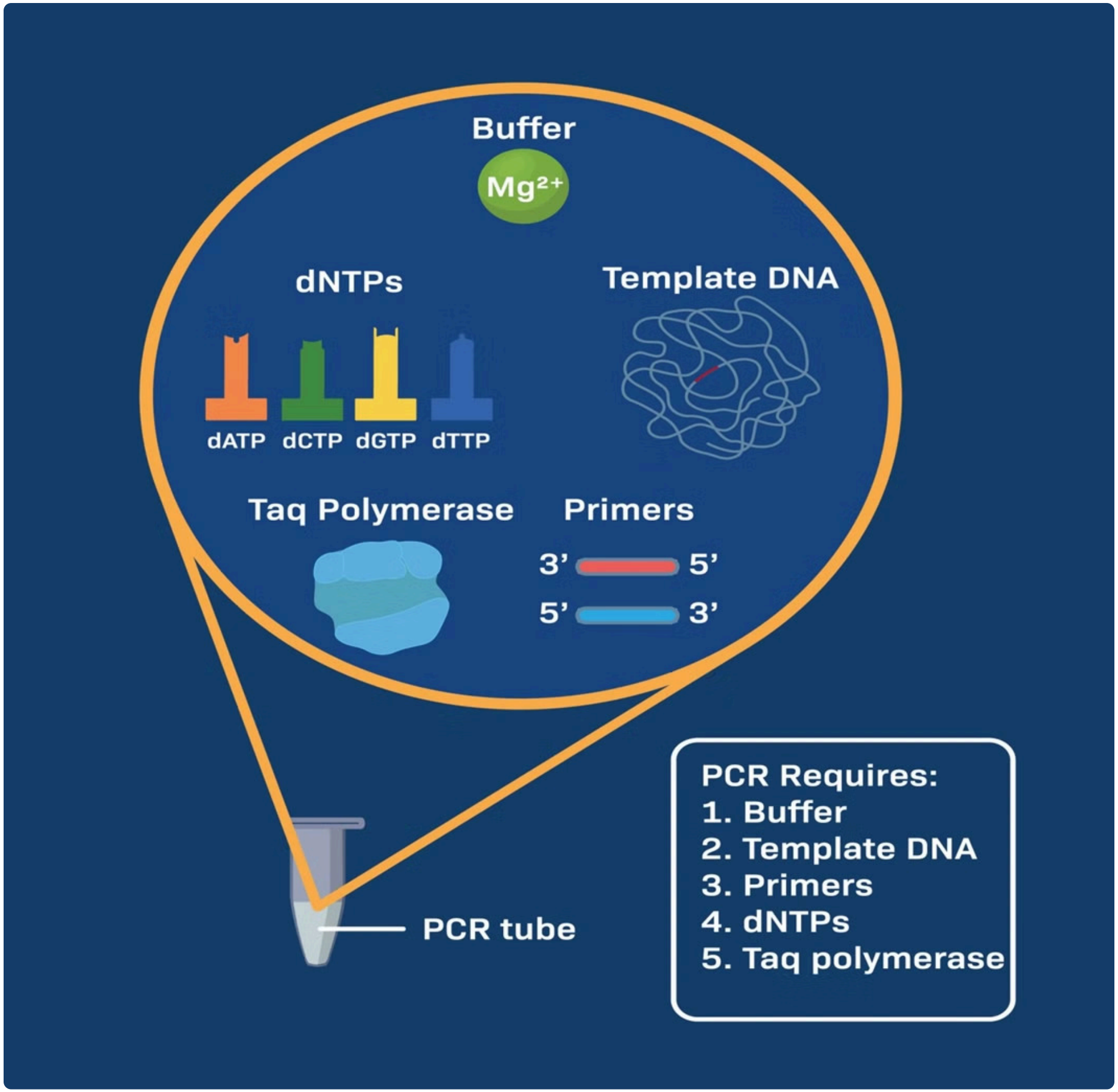
An enzyme that synthesizes new DNA strands, using the template DNA and primers as a guide.

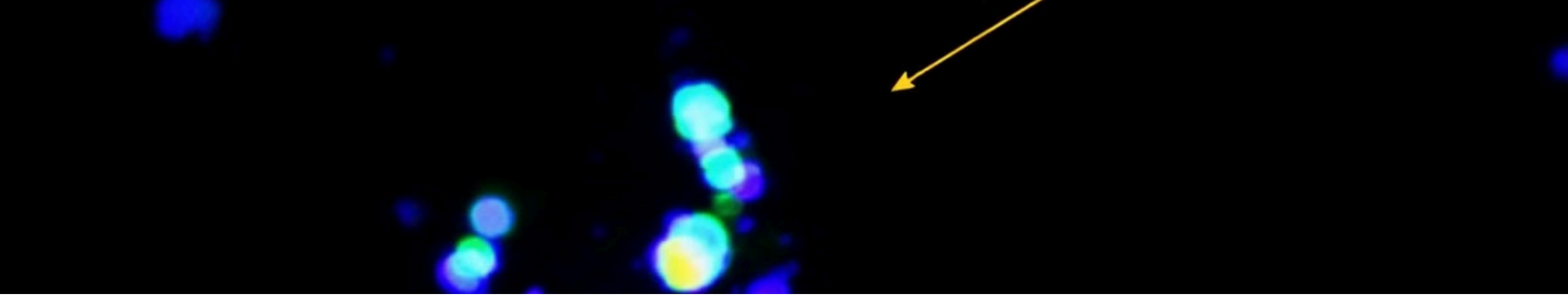
dNTPs

Building blocks of DNA: deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP).

Magnesium ions (Mg2+): Magnesium ions are cofactors for the DNA polymerase enzyme and are required for its activity.

Buffer: A buffer solution that provides the optimal pH and ionic conditions for the PCR reaction. The buffer also contains salts and stabilizers that help maintain the stability of the DNA polymerase


Buffer
Mg2+
dNTPs
dATP dCTP dGTP dTTP
Template DNA
Taq Polymerase
Primers
3’ 5’
5’ 3’
PCR tube
PCR Requires:
1. Buffer
2. Template DNA
3. Primers
4. dNTPs
5. Taq polymerase



Primer Design

1 Specificity

Primers must bind to a specific DNA sequence, avoiding unintended binding to other regions.

2 Length

Primer length typically ranges from 18 to 30 nucleotides, ensuring stable binding without excessive self-annealing.

3 Melting Temperature (Tm)

The Tm determines the optimal temperature for primer annealing, crucial for efficient amplification.

4 GC Content

The GC content should be balanced to avoid hairpin structures and primer-dimer formation.

PCR Amplification Cycles

1

Denaturation

The double-stranded DNA template is heated to 94-98°C, separating the strands.

2

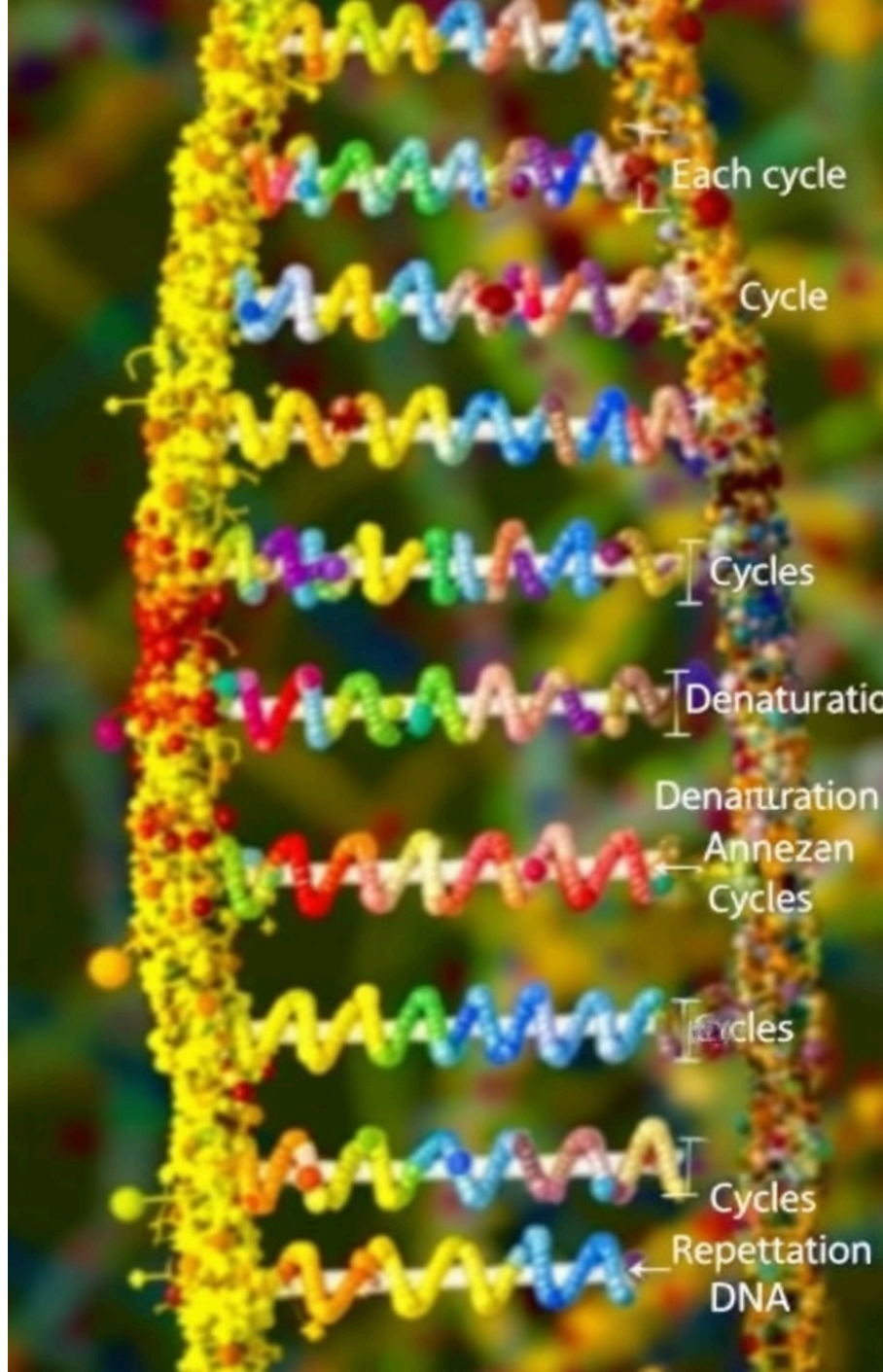
Annealing

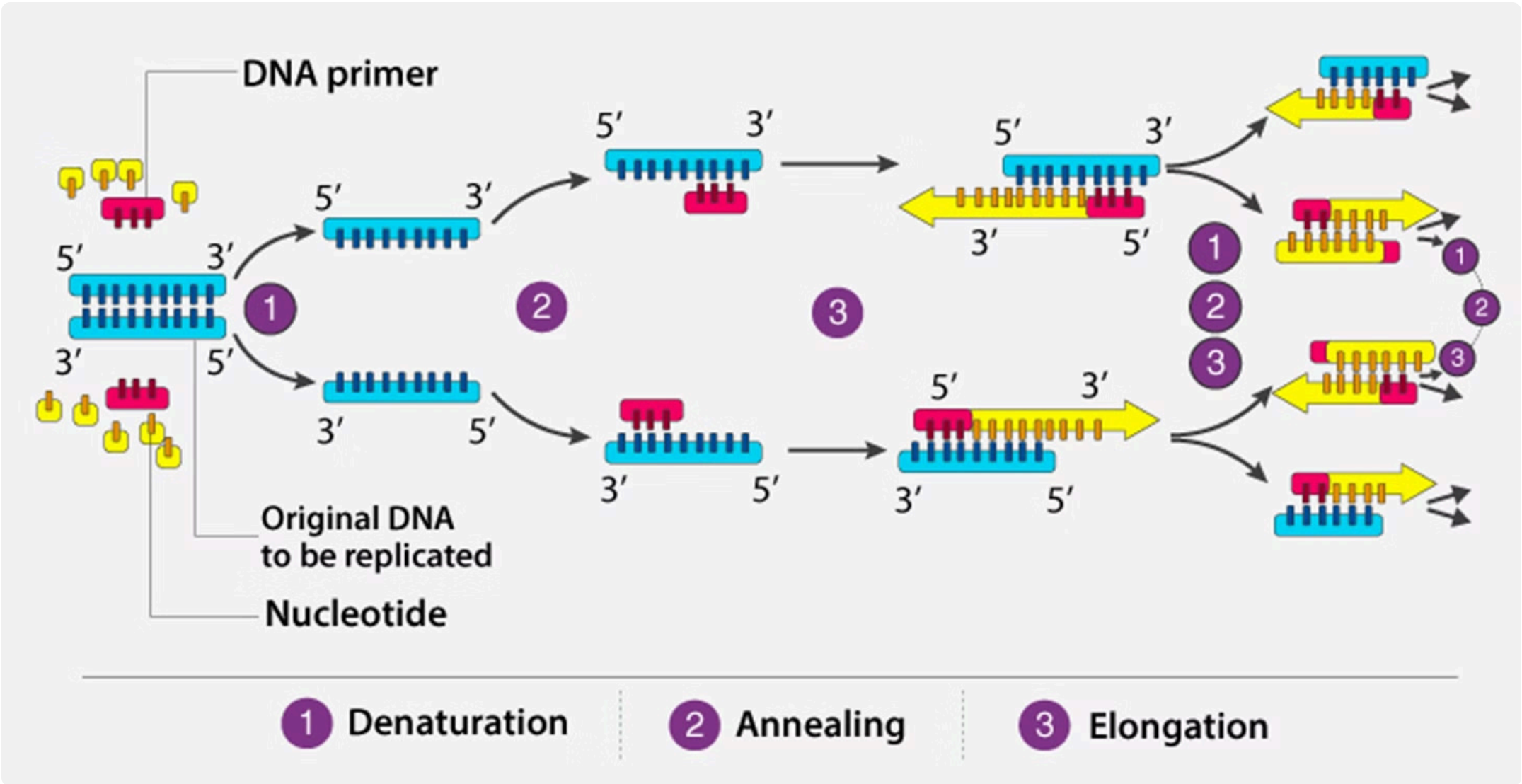
The temperature is lowered to 50-65°C, allowing the primers to bind to their complementary sequences on the single-stranded DNA.

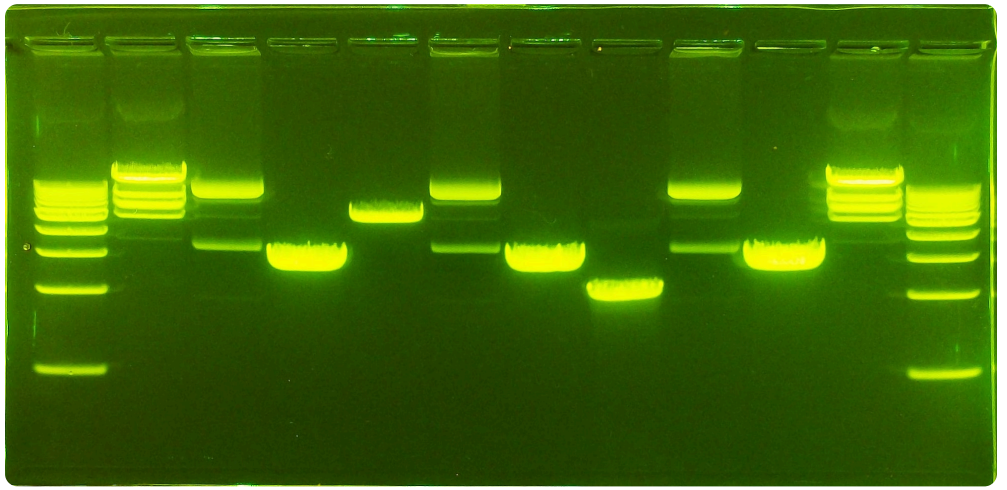
3

Extension

The temperature is raised to 72°C, the optimal temperature for DNA polymerase to synthesize new DNA strands, extending from the primers.



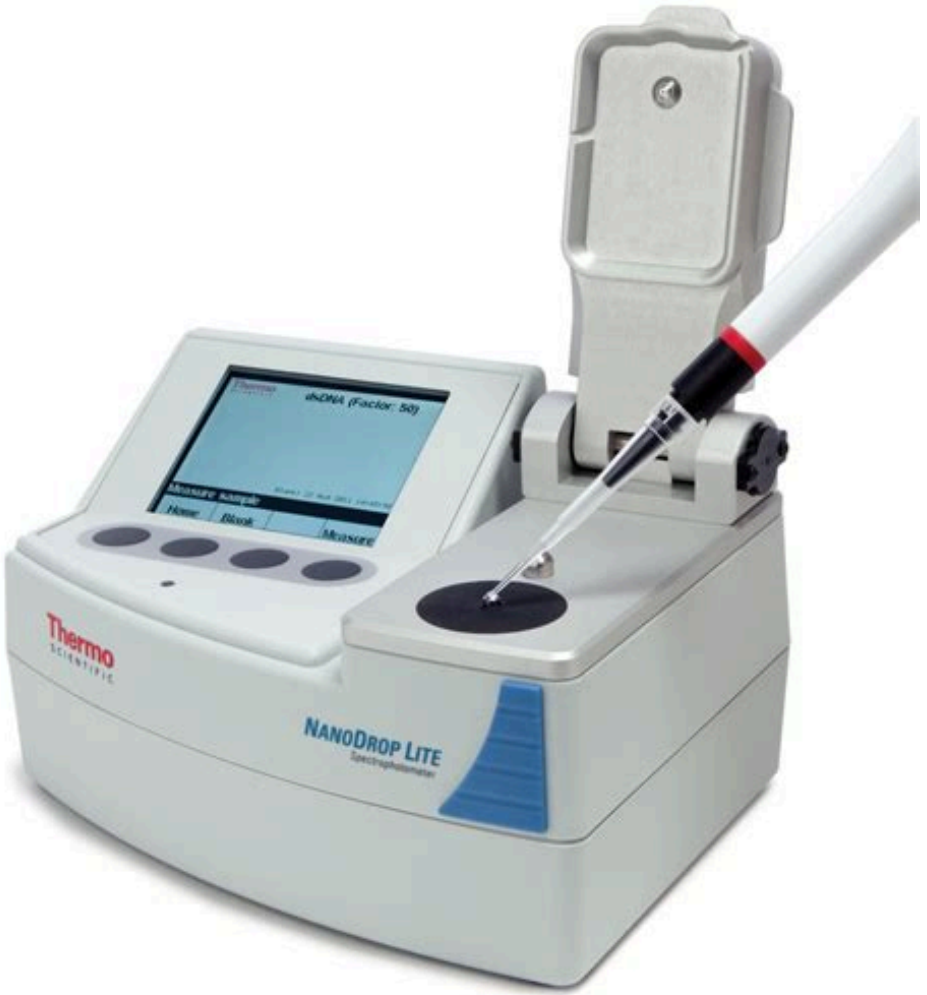

DNA primer
5′
3′
3′
5′
1
2
3
Original DNA
to be replicated
Nucleotide
1 Denaturation
2 Annealing
3 Elongation

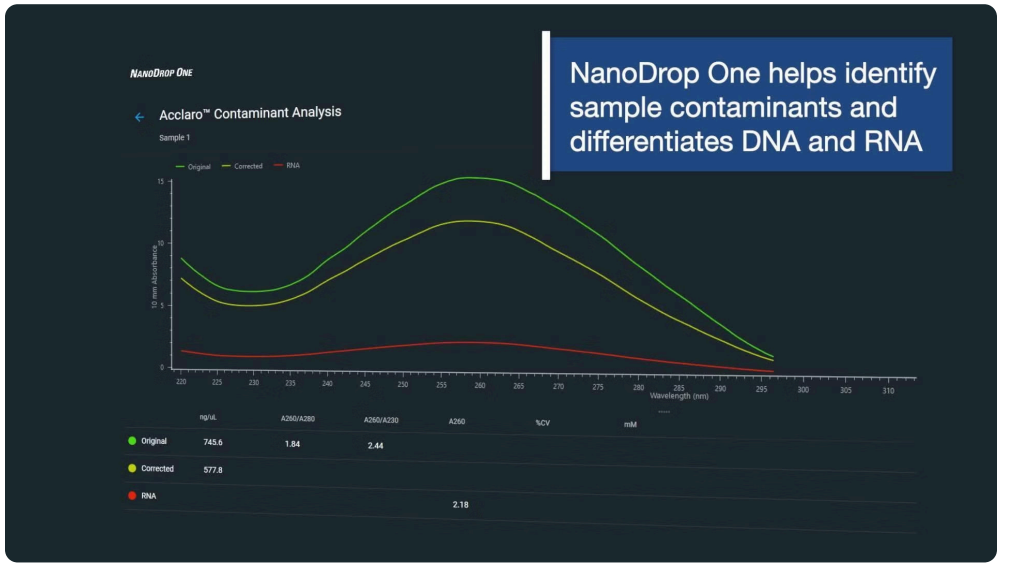


Bands of DNA under UV light

(Gel documentation device)

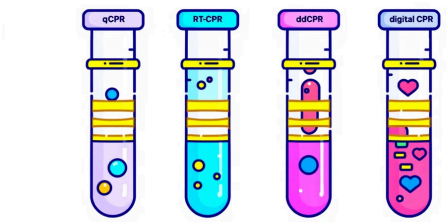
Loading of Samples





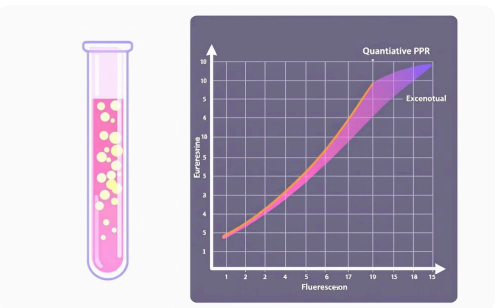
measurement of DNA content in a nanodrop device

Types of PCR



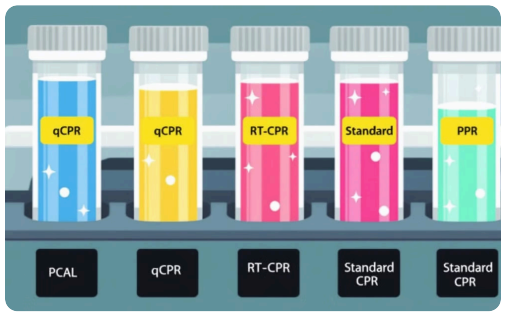
Conventional PCR

This basic PCR technique uses gel electrophoresis to visualize and analyze DNA products.



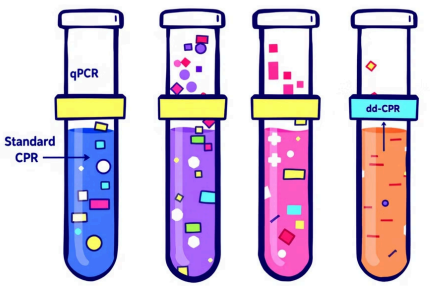
Quantitative PCR (qPCR)

qPCR measures the amount of DNA amplified in real-time using fluorescent probes.



Reverse Transcription PCR (RT-PCR)

RT-PCR is used to amplify RNA sequences, first converting them to cDNA.



Nested PCR

Nested PCR involves two rounds of PCR using different primer sets to increase specificity.

Quantitative PCR (qPCR)

Quantification

qPCR enables the quantification of specific DNA or RNA sequences.

It measures the amount of target nucleic acid present in a sample.

Real-Time Monitoring

The PCR reaction is monitored in real-time, using fluorescent probes.

This allows for the accurate quantification of the target nucleic acid.

Applications of PCR

Molecular Diagnostics

PCR is used to detect and diagnose a variety of diseases, including infectious diseases and genetic disorders.

Forensic Science

PCR is used to analyze DNA evidence from crime scenes, such as blood, hair, and saliva.

It can also be used to identify individuals and establish parentage.

Research

PCR is a crucial tool for research, as it allows scientists to study DNA and RNA in a variety of ways.

It is used to clone genes, sequence DNA, and analyze gene expression.

Advantages of PCR

Speed and Efficiency

PCR is a rapid technique, allowing for quick amplification of target DNA sequences within a few hours.

High Sensitivity

PCR is highly sensitive, capable of detecting even minute amounts of DNA, making it ideal for detecting low-abundance targets.

Versatility

PCR has numerous applications in various fields, including diagnostics, forensics, research, and biotechnology.

Cost-Effectiveness

PCR has become increasingly affordable, making it accessible for a wide range of applications.

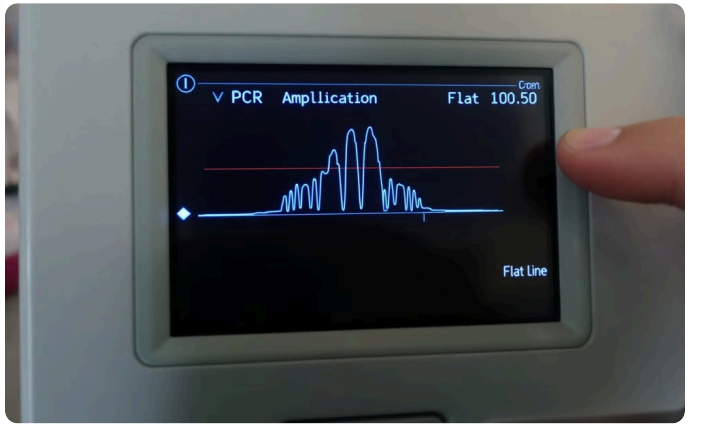
Limitations of PCR

Contamination

Contamination from other DNA can lead to false-positive results.

Sample Size

PCR requires a sufficient amount of DNA to be effective.



Sensitivity

PCR can sometimes miss low-abundance DNA targets.

Thanks