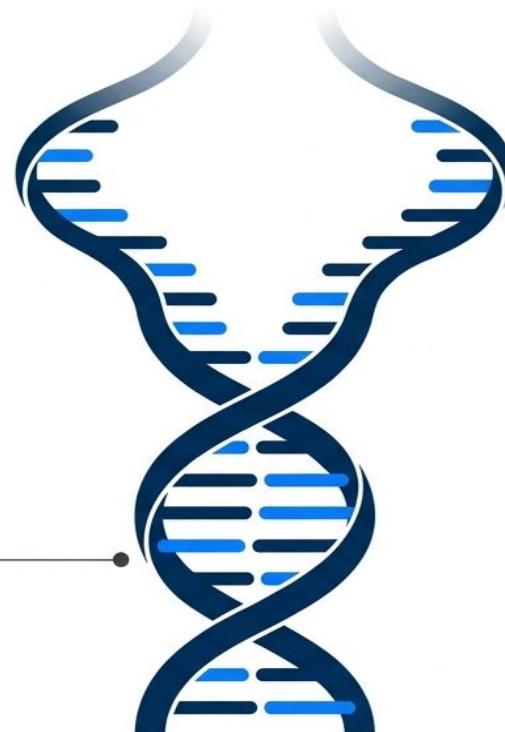

Polymerase Chain Reaction (PCR)

Dr. Luu Phuc Loi, PhD.
Presenter: Hoang Kim

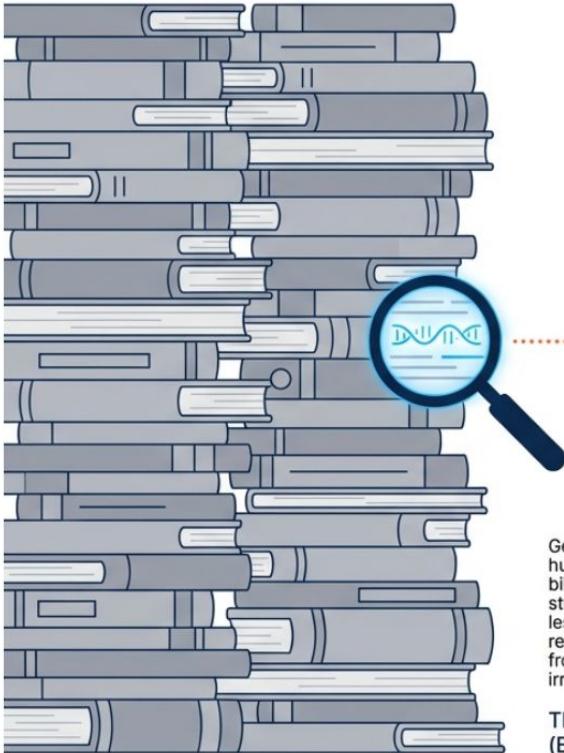
Polymerase Chain Reaction (PCR)

The Mechanics of Molecular Photocopying

PCR is an in vitro technique used to amplify specific DNA sequences. It allows researchers to take a trace amount of genomic material and generate billions of identical copies in a matter of hours, revolutionizing genetics, forensics, and diagnostics.



The Challenge: Finding the Needle in the Genomic Haystack



Genomes are massive. The human genome contains billions of nucleotide pairs. To study a specific gene (often less than 2,000 base pairs), researchers must isolate it from the surrounding mass of irrelevant genetic data.

The Haystack
(Billions of Base Pairs)

Imagine trying to find and read a single specific page in a library containing millions of books. You cannot read the whole library; you need a way to locate that specific page and photocopy it billions of times.

The Needle
(Target Sequence)



Search NCBI ...

Log in

NCBI Datasets Taxonomy Genome Gene Command-line tools Documentation

Genome

Search by taxonomic name or ID, Assembly name, BioProject, BioSample, WGS or Nucleotide accession

Search term

Search

Try examples: [Homo sapiens](#) [GCF_000001405.40](#) [PRJNA489243](#) [SAMN15960293](#) [WFKY01](#) [GRCh38.p14](#) [NC_000913.3](#)

Genomic data available from NCBI Datasets

Click below to learn more about the genomic data available from NCBI Datasets.



Eukaryota



Archaea



Bacteria



Viruses

All Genomes

3.27M

Total

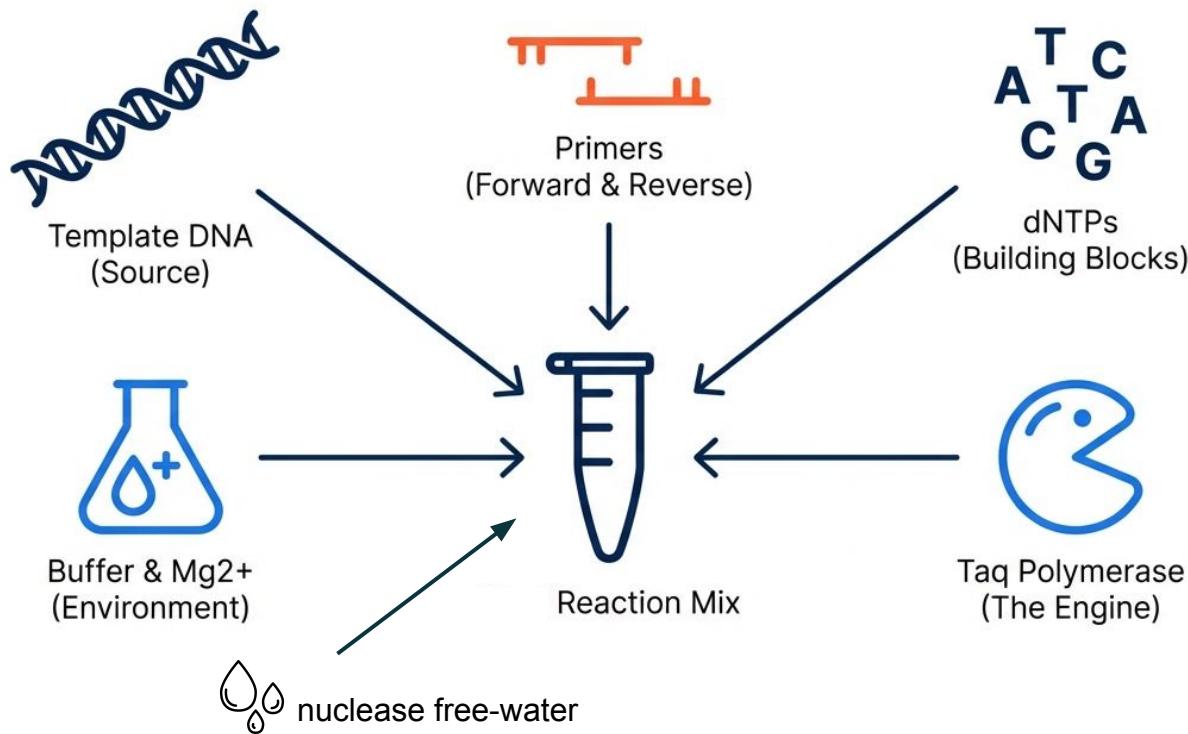
43.45K

Reference

2.63M

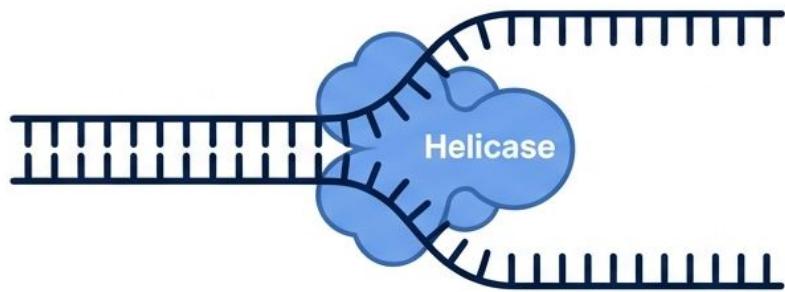
Annotated

The Master Mix: Ingredients for Amplification

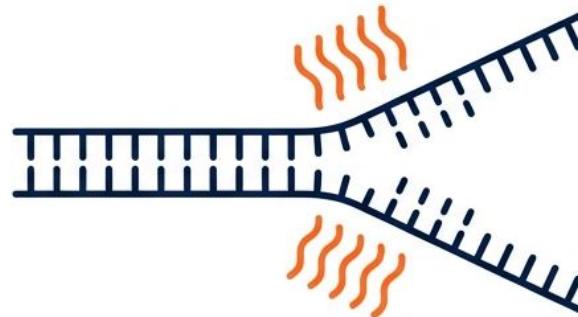


Nature vs. The Machine: Helicase vs. Heat

In Vivo (The Cell)



In Vitro (The PCR Tube)



Mechanism: Enzyme (Helicase)

Temperature: 37°C (Body Temp)

Speed: Moderate

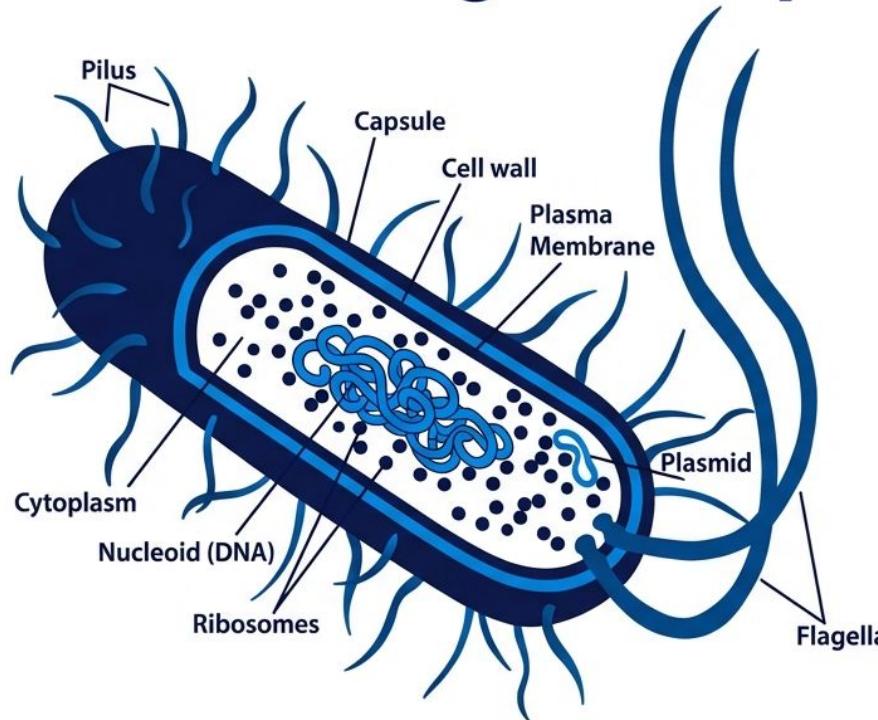
Mechanism: Thermal Energy (Heat)

Temperature: 94–98°C (Near Boiling)

Speed: Rapid

“You don't have any helicase in your PCR reactions... the answer is heat. Heat serves as the brute-force opener of the double helix.”

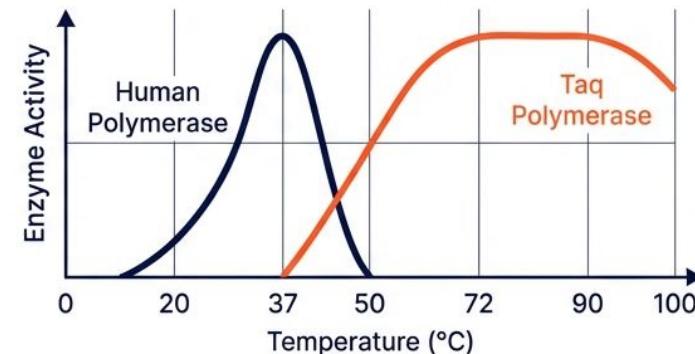
The Engine: Taq DNA Polymerase



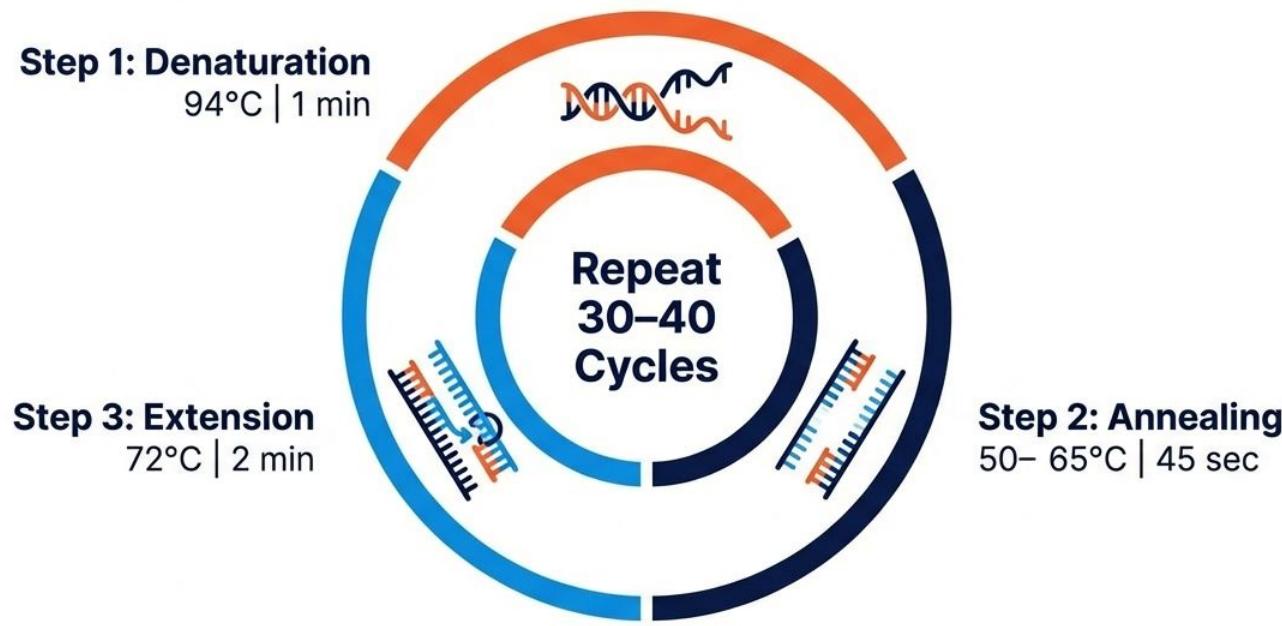
Origin: *Thermus aquaticus*, a thermophilic bacterium found in Yellowstone hot springs.

The Superpower: Thermostability.

Why it matters: Normal human enzymes denature (cook) at 90°C. Taq polymerase survives the boiling temperatures required for PCR and functions optimally at 72°C.



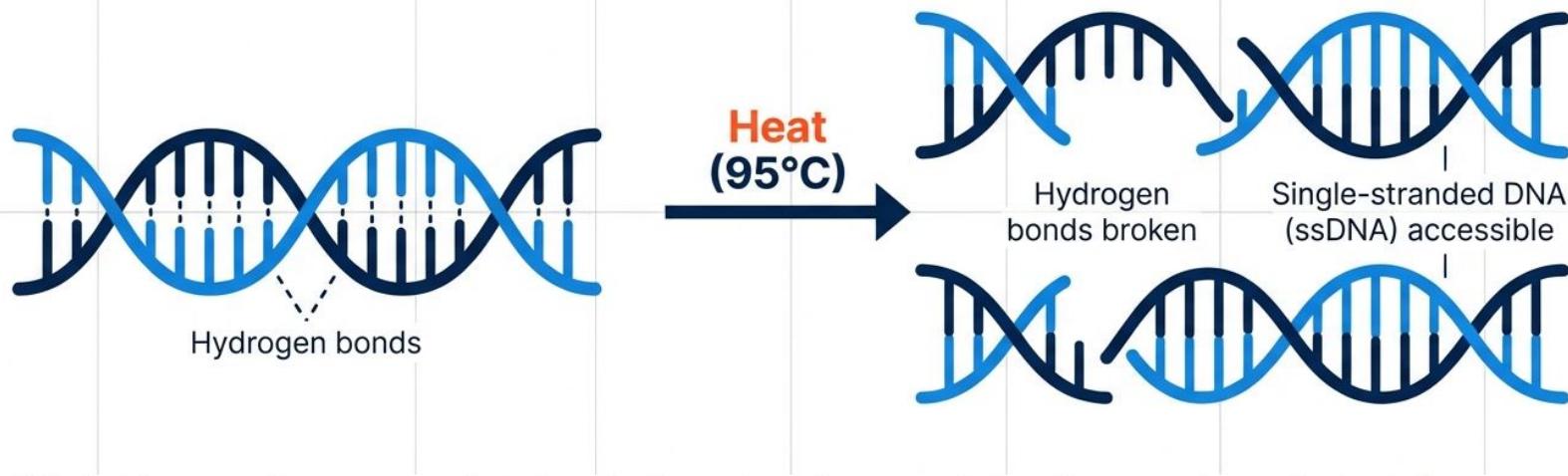
The Thermal Cycle



Process controlled by a device called a Thermocycler.

Step 1: Denaturation (The Melt)

Temperature: 94–98°C



High thermal energy physically breaks the weak hydrogen bonds holding the base pairs together. Complete denaturation is critical; if strands remain partially bonded, primers cannot bind.

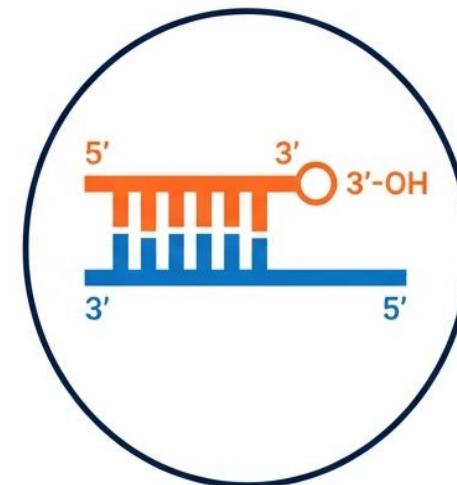
Step 2: Annealing (The Target)

Temperature: 50–65°C



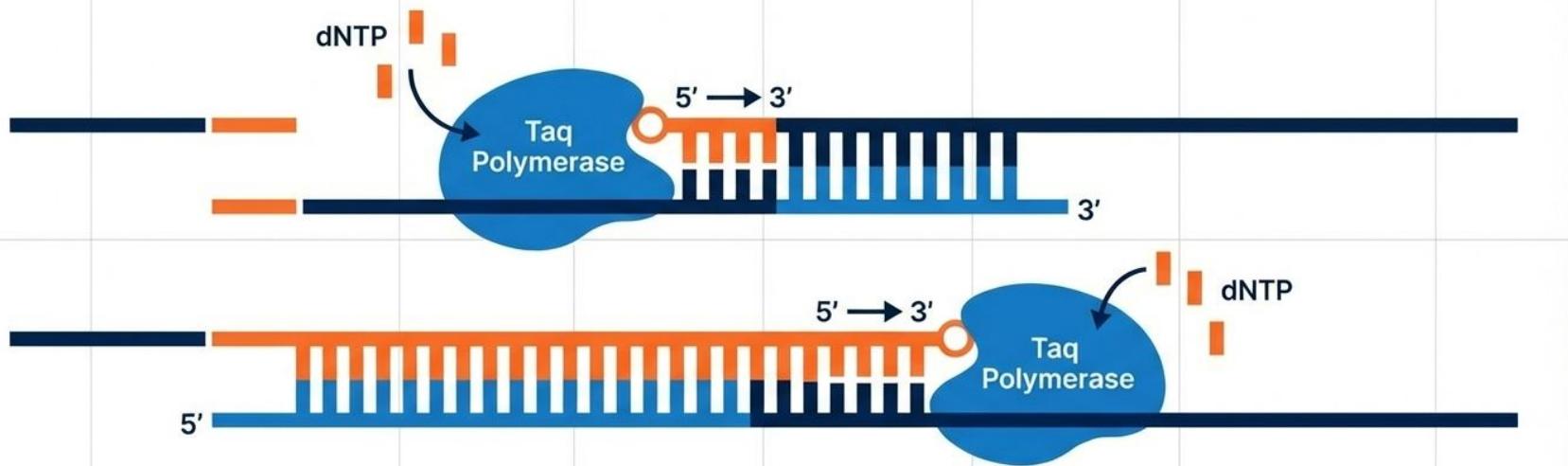
$$T_m \approx 4(G-C) + 2(A-T)$$

Temperature drops to allow Primers to hydrogen-bond to complementary sequences. Primers define the boundaries of the amplification. The polymerase requires the free 3'-OH group of the primer to start.



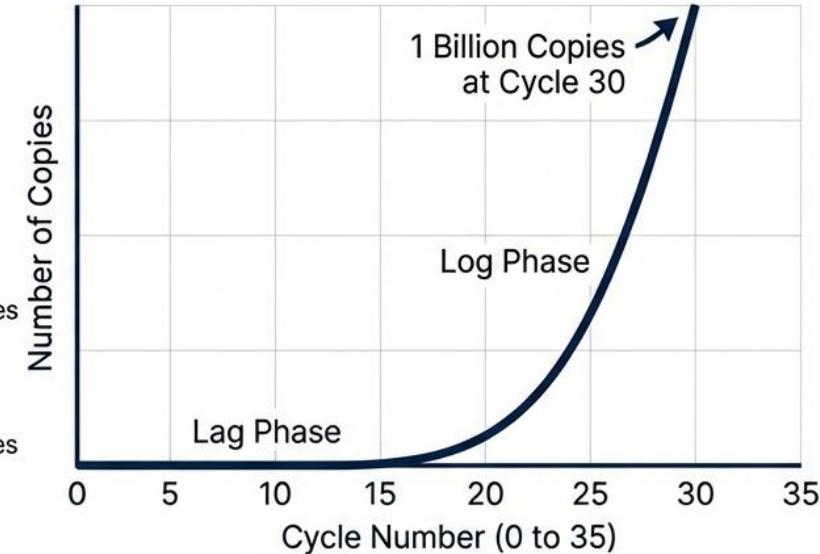
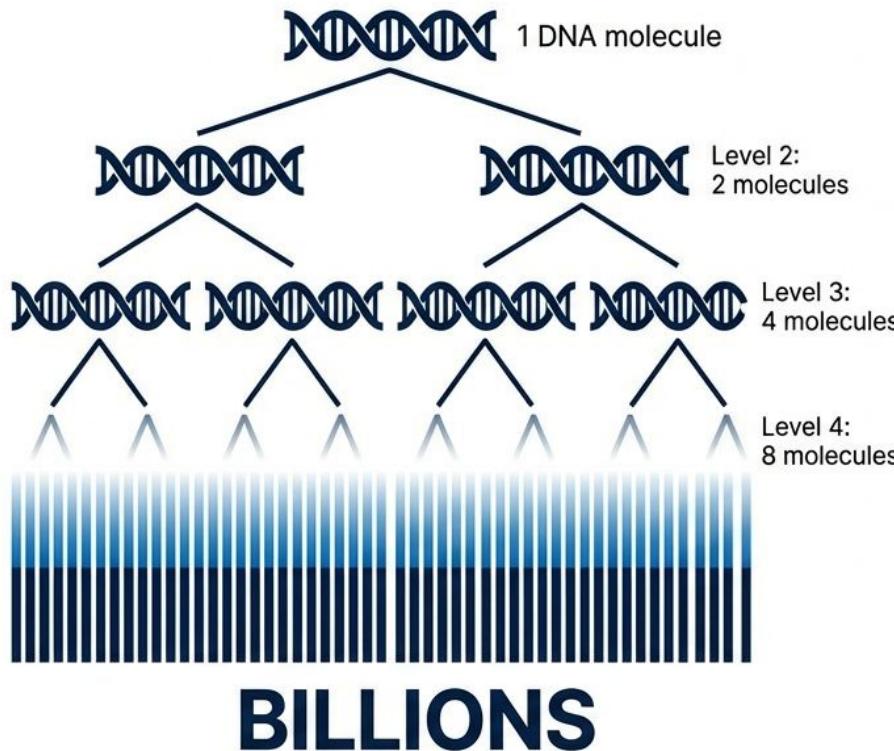
Step 3: Extension (The Copy)

Temperature: 72°C



Temperature is raised to the optimum for Taq. The enzyme binds to the primer-template junction and synthesizes the new complementary strand ($5' \rightarrow 3'$) by adding free dNTPs. Rate: ~1,000 base pairs per minute.

The Power of Exponential Amplification

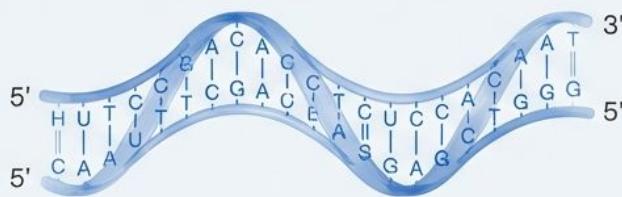


The reaction is exponential (2^n), not additive. Cycle 1 = 2 copies. Cycle 30 = ~1 Billion copies. This allows detection of a single drop of blood or a few viral particles.

Designing the Keys to the Code

Primer Design Rules

A. Ideal Primer



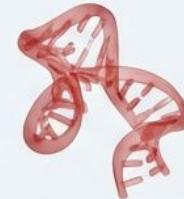
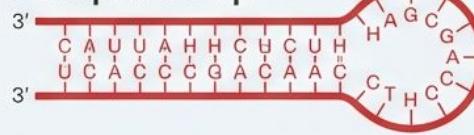
Length: 17–30 nucleotides.

GC Content: ~50%

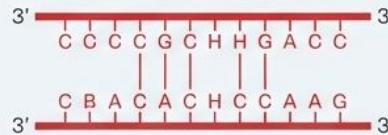
Melting Temp (Tm): Matched pair.

B. Common Pitfalls

Hairpin Loop



Primer Dimers



Specificity depends entirely on the primers. Poor design leads to no reaction or false positives.

Why PCR Took Over the World



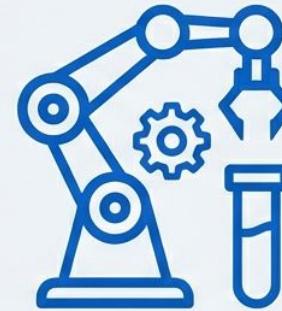
Sensitivity

Can detect as little as 1–10 copies of a target sequence.
Works on crude extracts (blood, hair).



Speed

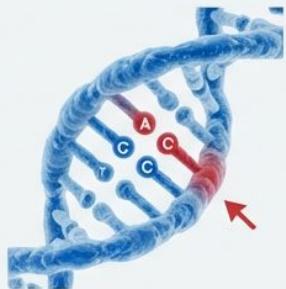
Replaces weeks of cloning with hours of amplification.
Fast turnaround for diagnostics.



Automation

Modern thermocyclers automate temperature changes. “Set and forget” workflow.

The Limits of the Technology



1. Taq Error Rate

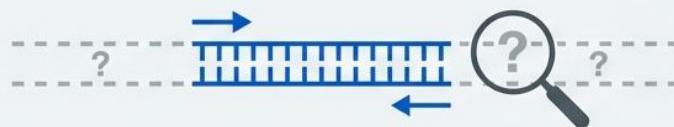
Lacks 3' → 5' proofreading exonuclease. Makes a mistake approx. 1 in every 10^4 nucleotides.



2. Contamination Risk

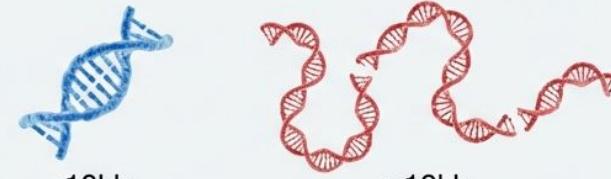
Extreme sensitivity means a single speck of dust containing DNA can cause false positives.

3. Prior Knowledge Required



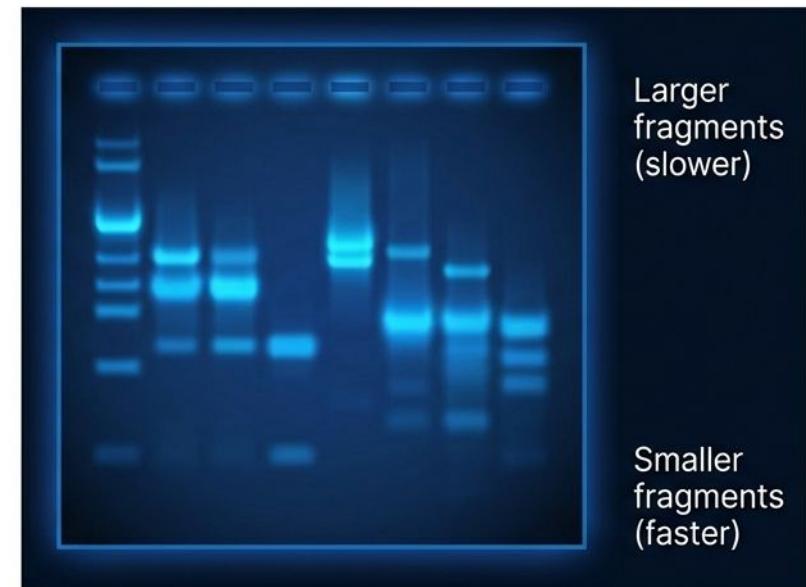
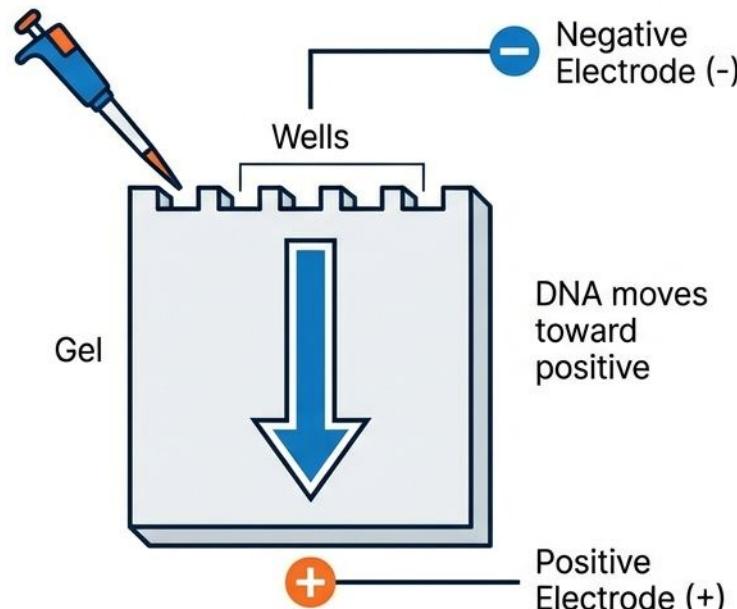
Must know flanking sequences to design primers.
Cannot amplify completely unknown DNA.

4. Size Limit



Typically effective for fragments $<10\text{kb}$.

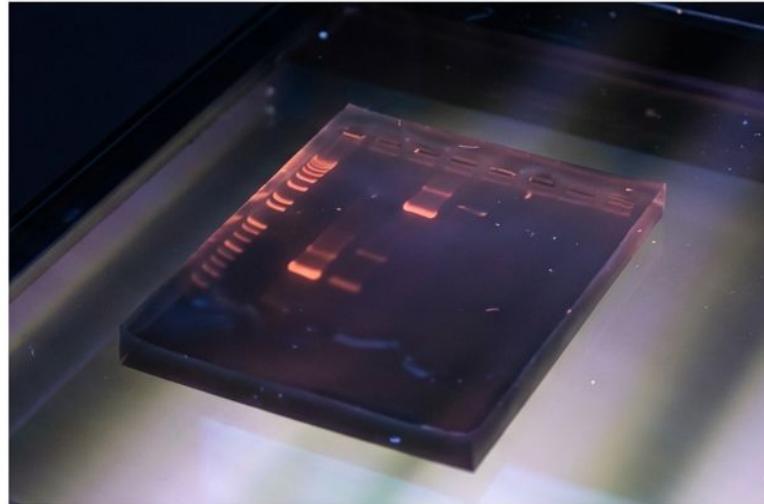
Visualizing the Result: Gel Electrophoresis



DNA is negatively charged and moves through the gel matrix. The amplified PCR product appears as a distinct band, sorted by size.

Agarose Gel Electrophoresis

Principles, Procedure, and Analysis of Macromolecule Separation



Standard Operating
Protocol Guide

Separation and Analysis of Macromolecules

Definition

A method for separation and analysis of macromolecules (DNA, RNA, and proteins) and their fragments, based on their size and charge.

Applications



Clinical Chemistry

Used to separate proteins by charge and/or size.



Biochemistry & Molecular Biology

Used to separate DNA and RNA fragments by length (e.g., post-PCR analysis) or proteins by charge.

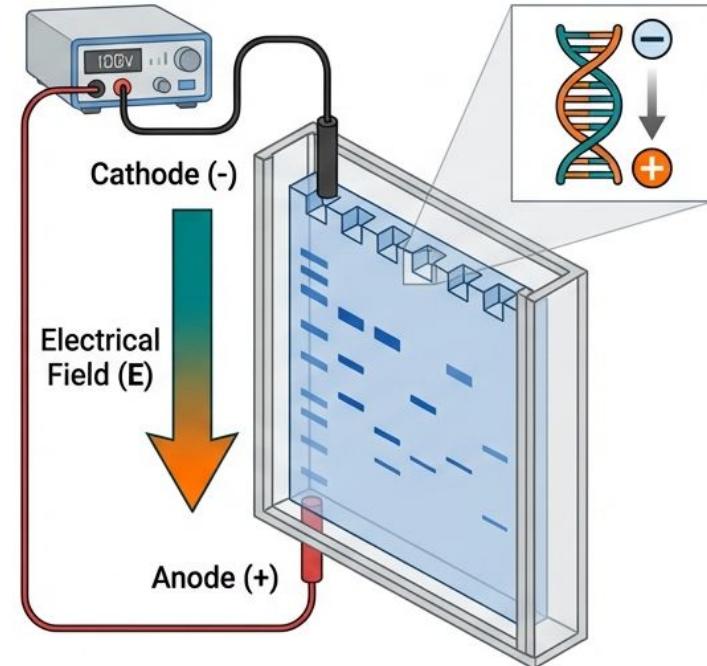
The Driving Force: Charge Migration

The Circuit: Gel is placed in an electrophoresis chamber connected to a DC power source.

The Field: Establishes a negative end (Cathode, Black) and a positive end (Anode, Red).

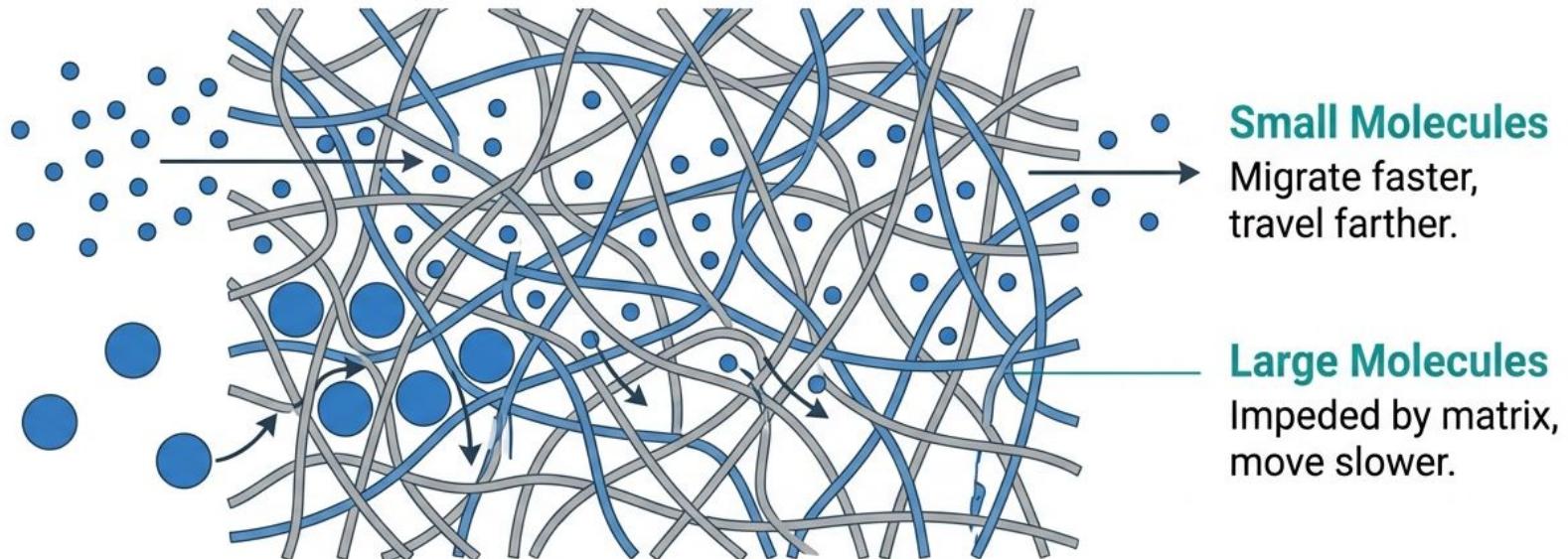
Migration Rule:

- DNA/RNA are Anions (Negatively charged).
- They migrate towards the Anode (Positive).
- Mnemonic: "Run to Red".



The Matrix: Molecular Sieving

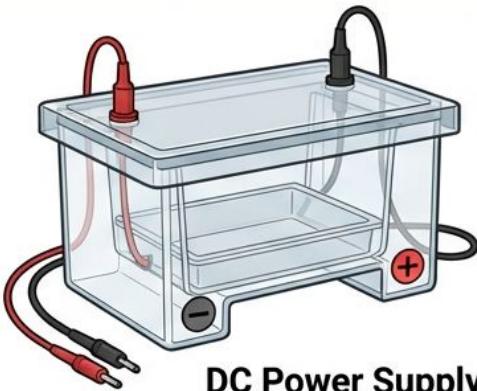
Agarose gel acts as a porous lattice to filter molecules.



Outcome: Molecules sort into distinct bands based on size.

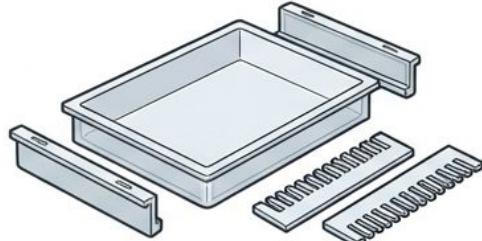
Essential Materials and Equipment

Hardware



DC Power Supply
(80-120V)

Electrophoresis
Chamber (Tank)



Gel Casting Tray
& Combs

Reagents



Agarose
Powder



Running Buffer
(TBE pH 8.0)
Conducts current,
maintains pH

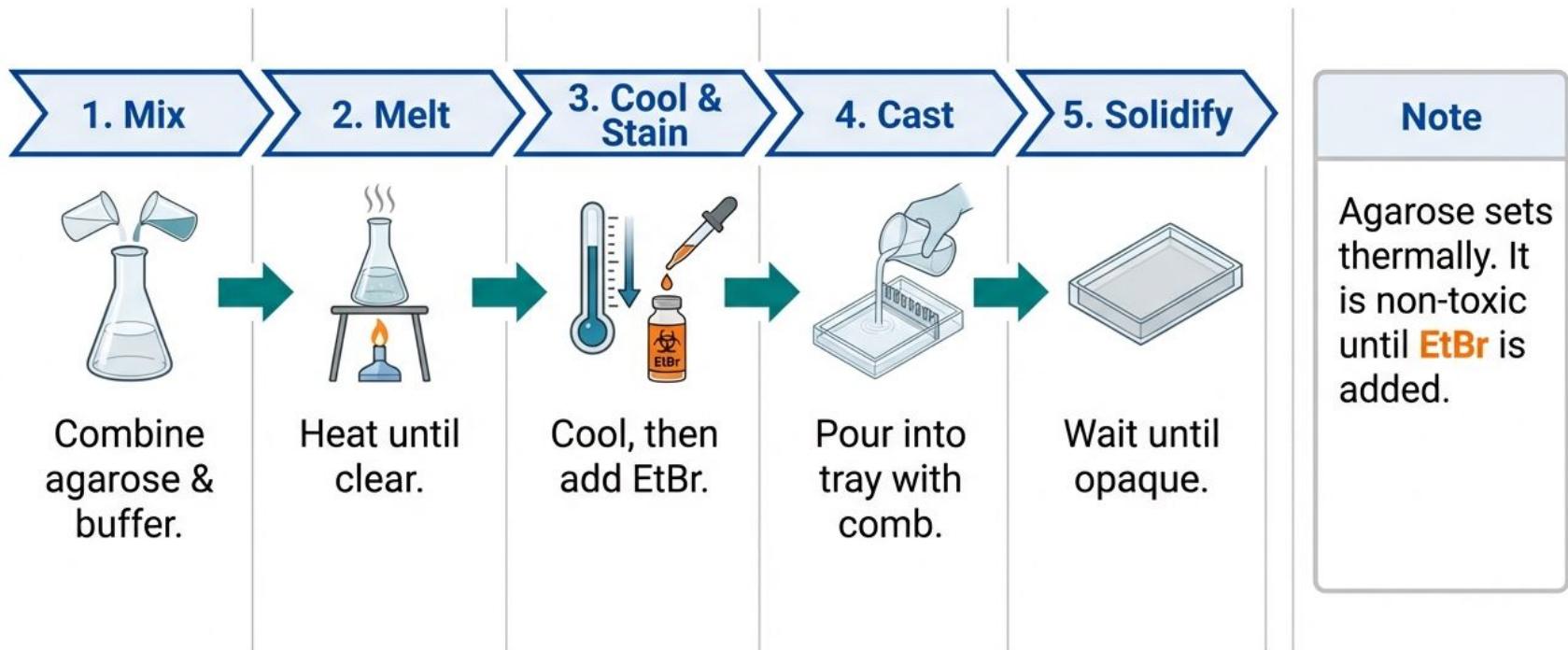


Loading Dye
Weighs down
sample, tracks
progress



Biohazard
Orange
(FF6F00)
Staining Agent

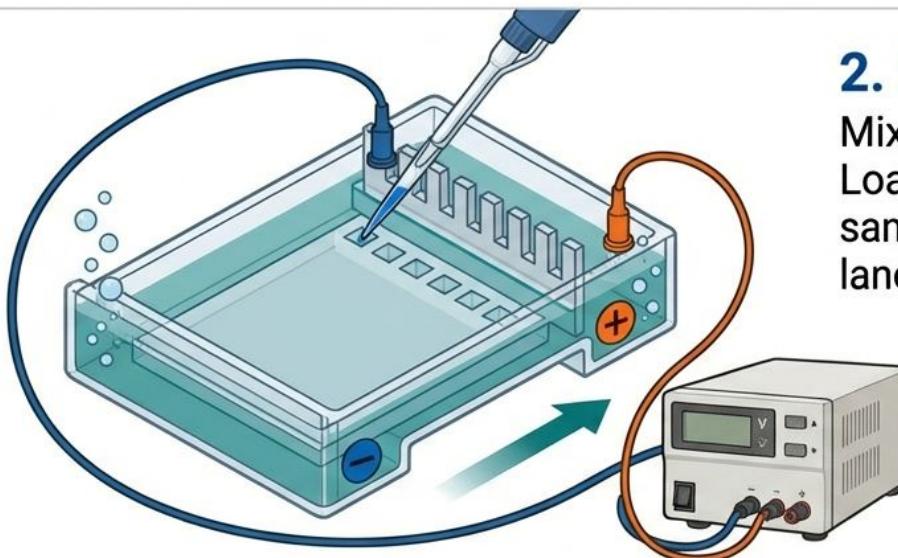
Protocol: Casting the Gel



Protocol: Loading and Running

1. Submerge

Place gel in tank.
Cover completely
with buffer.



2. Load

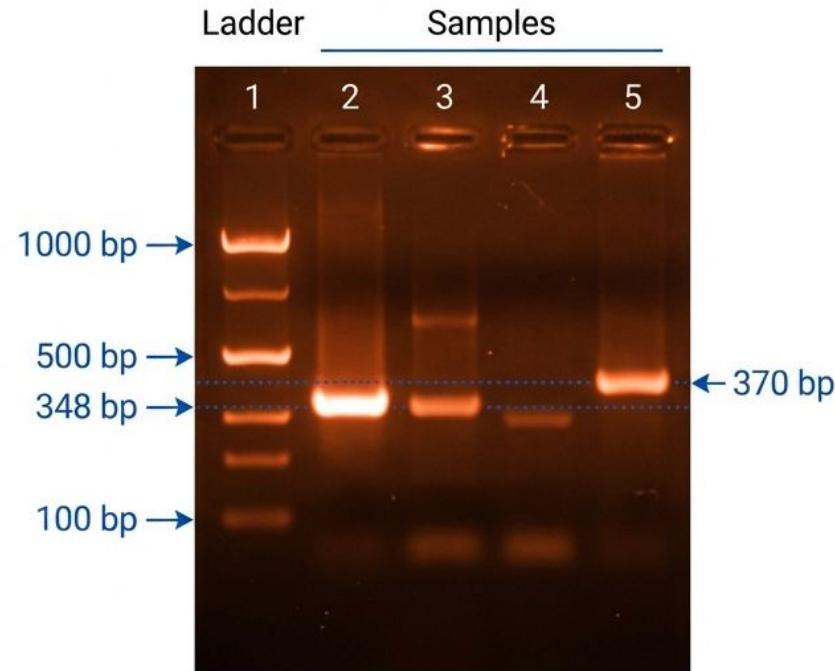
Mix sample with dye.
Load Ladder in Lane 1,
samples in subsequent
lanes.

3. Run

Connect power (80–120V). Run 30–45 mins.
Look for bubbles (electrolysis) to confirm current.

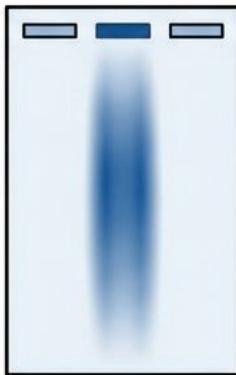
Interpreting Results: Reading the Gel

- **The Ladder (Marker):** A mix of fragments with known sizes.
- **Sizing:** Compare sample band position to the ladder.
- **Logarithmic Scale:** Distance migrated is inversely proportional to $\log(\text{molecular weight})$.
- **Quantity:** Brighter band = More DNA.



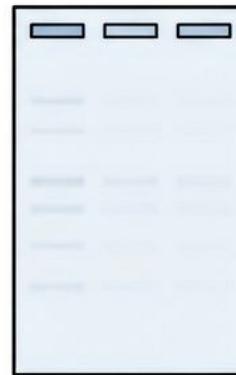
Troubleshooting: Band Appearance

Smeared Bands



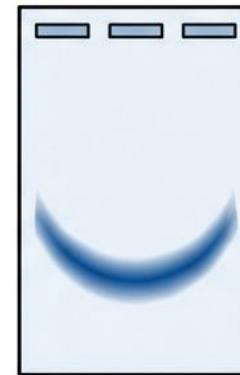
Cause: DNA overload, nucleases, >30 cycles.
Fix: Reduce template, use fresh buffer.

Faint/Weak Bands



Cause: Low DNA, short staining.
Fix: Check PCR cycles, increase stain time.

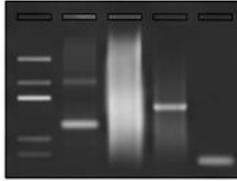
Smiling Bands



Cause: Overheating/High Voltage.
Fix: Run at lower voltage.

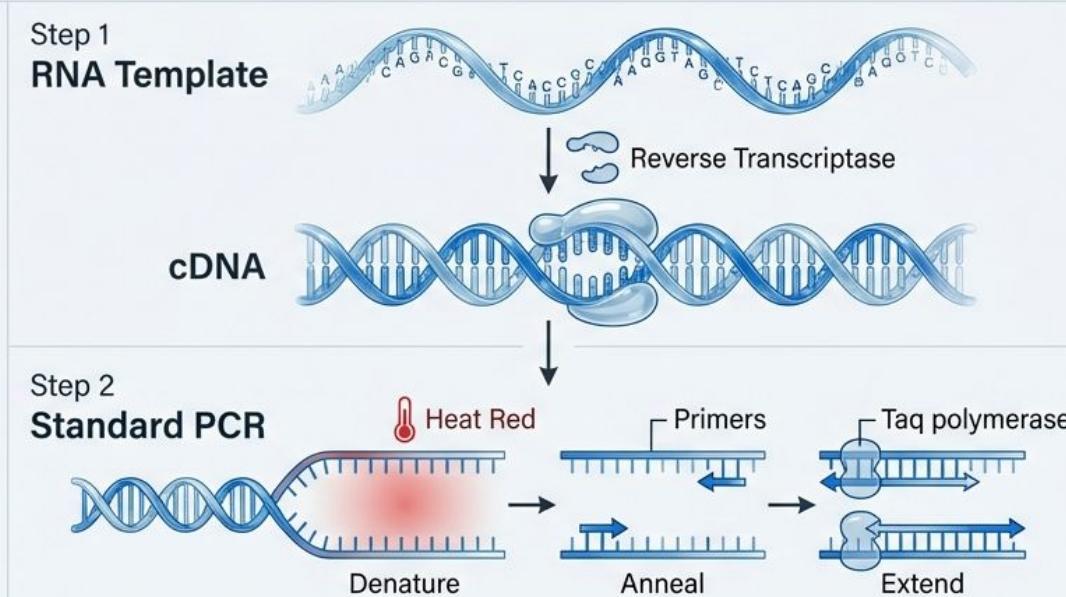
Troubleshooting: Reading the Gel

Systematic approach to analyzing and correcting PCR issues.

Visual	Causes	Fixes
Weak / Faint Bands 	Too few cycles, Extension too short, Annealing Temp too high.	Lower Annealing Temp , Increase Cycles (max 35), Increase Template .
Smearing / Non-Specific Bands 	Annealing Temp too low, Mg2+ too high, Contamination.	Raise Annealing Temp , Reduce Mg2+, Use Hot Start .
No Product (Empty) 	Check Master Mix, Ensure Taq added, Check for Inhibitors.	Check Master Mix , Ensure Taq added, Check for Inhibitors .

Adapting for RNA: RT-PCR

Crucial for RNA Viruses (e.g., HIV, SARS-CoV-2)



Taq polymerase only works on DNA. Reverse Transcription (RT) bridges the gap by converting RNA genomes or mRNA into DNA before amplification.