

# Polymerase Chain Reaction (PCR): History, Evolution, and Impact

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# Background



- **Kary B. Mullis** (1944-2019)
- American Biochemist
  - “Father of PCR”
- B.Sc., Ph.D
- Received many awards
  - Most notable: Nobel Prize (1993)
- Late-night drives + LSD → PCR
- *E. coli* = test template

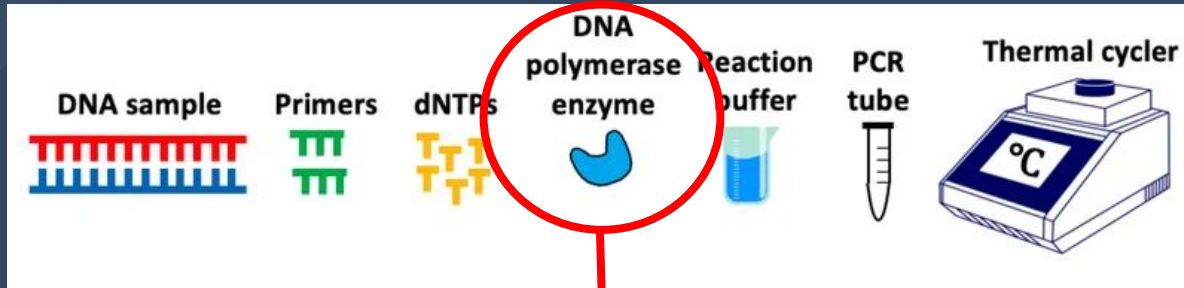


# What is PCR?

- “One of the most important scientific advances in molecular biology”
- Laboratory **nucleic acid** amplification technique
- Targets specific **DNA** (or **RNA**) fragments
- Three major phases of **amplification**:
  - Denaturation
  - Annealing
  - Extension
- 1 DNA molecule = **~1 billion copies**

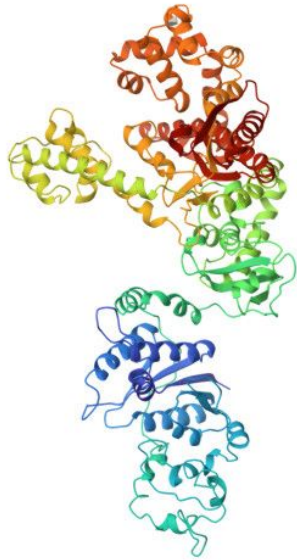


# Materials



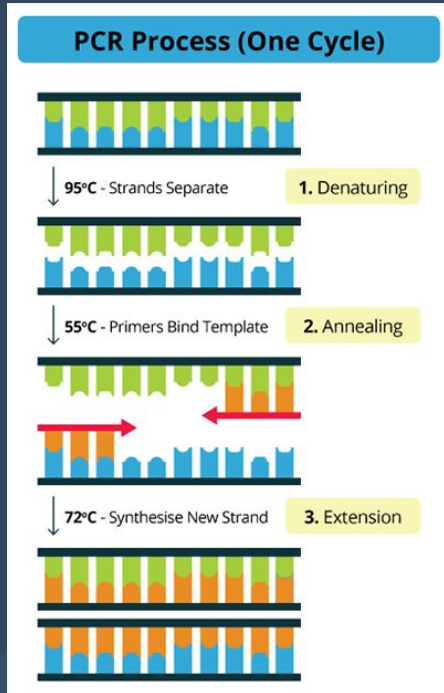
Taq Polymerase

# Key Enzyme- **Taq** **Polymerase**



- 94 kDa thermostable enzyme
- *Thermus aquaticus*
- “Right-hand” structure
  - Palm, finger, thumb domains
- Specialized for **high-temperatures**
- Synthesizes new **DNA** strands during **extension** phase

# Steps of PCR



1

## Denaturation

- Heat DNA to 95°C
- Disrupt **hydrogen bonds** between base pairs

2

## Annealing

- Cool denatured DNA
- 55°C to 72°C
- **Primers** bind **complementary strands**

3

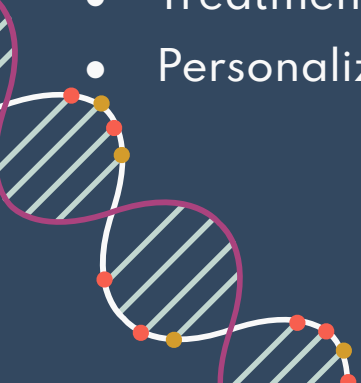
## Elongation

- Temperature = 75°C to 80°C
- **Taq** adds nucleotides
- New DNA strand synthesized

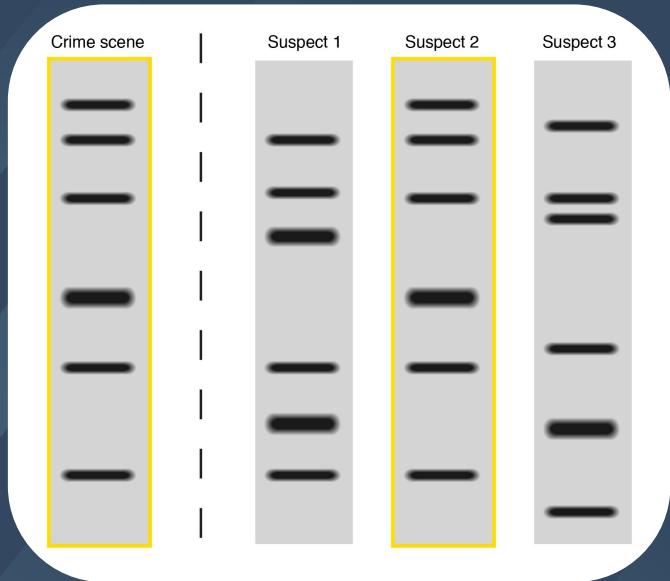
Repeat for **25-35 cycles**

# PCR in Modern Medicine

- Detection of **infectious** diseases
  - COVID-19
- Diagnosis of **genetic disorders**
- **Cancer mutation** detection
- Treatment effectiveness
- Personalized medicine



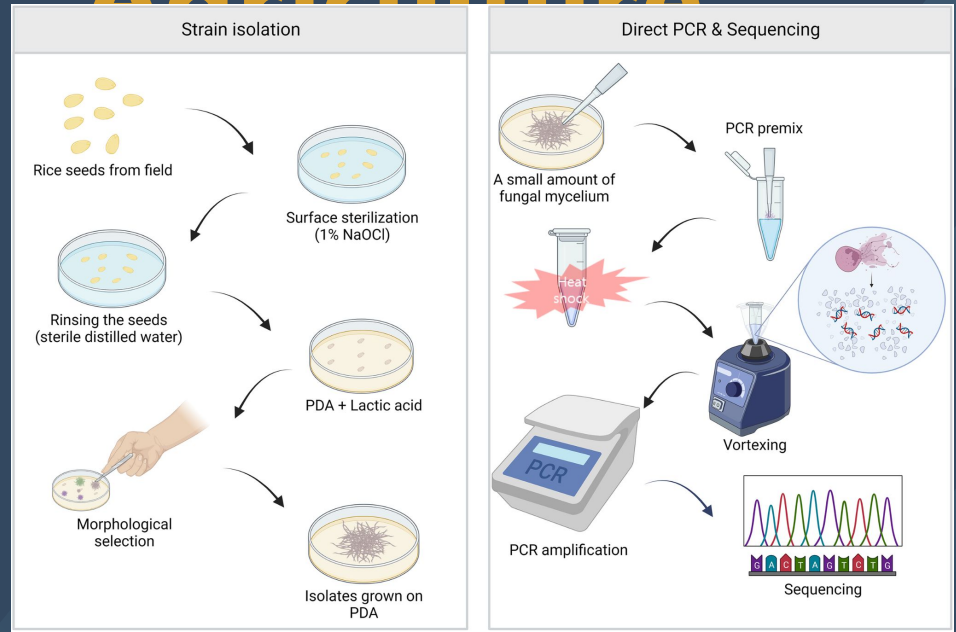
# Forensic Fingerprinting



- Accurate **DNA profiling**
- Paternity and kinship testing
- Suspect identification and elimination
- Analysis of degraded or limited samples
- Exoneration
- Common targets:
  - **Short Tandem Repeat (STR)**
  - **Mitochondrial DNA (mtDNA)**
  - **Sex determination (AMGX/AMGY)**

# Amplification of Agriculture

- Pathogen detection
- GMO analysis
- **Marker-Assisted Selection (MAS)**
- Verification of authenticity
- Genotype verification





# Advantages and Limitations

- Advantages:
  - Highly sensitive and specific
  - Can detect very small amounts of **DNA**
  - Fast and widely used
- Limitations:
  - Susceptible to contamination + mutations
    - False positives
  - Prior knowledge required (for **primer** design)
  - Cannot detect unknown pathogens
  - Positive result  $\neq$  **active infection**

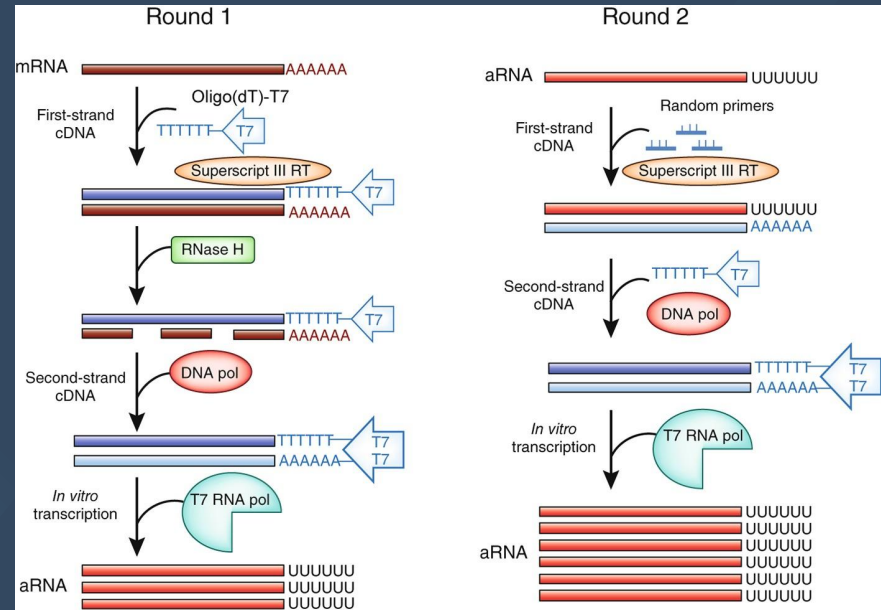


# RT-PCR

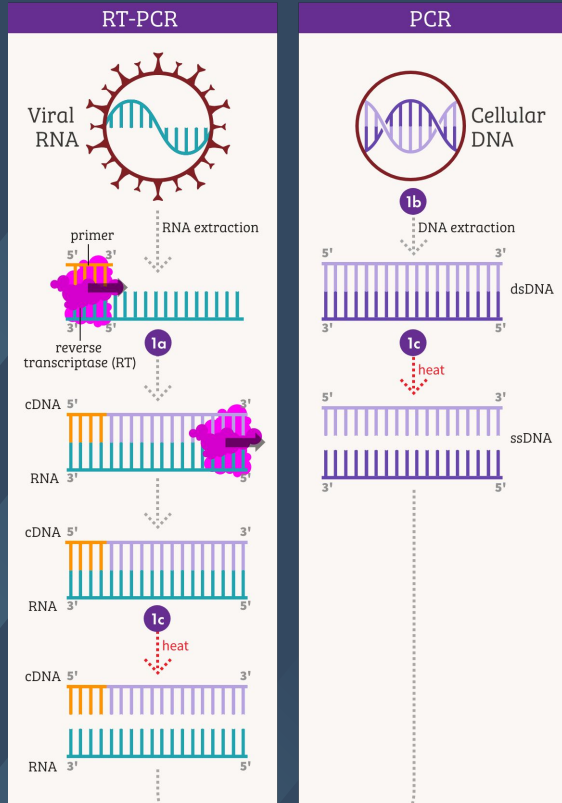
## • Reverse Transcription Polymerase Chain Reaction

(RT-PCR)

- Advanced variation of traditional PCR
- Designed for **RNA** analysis and detection
- Widely used in **research and diagnostics**



# Why RT-PCR?

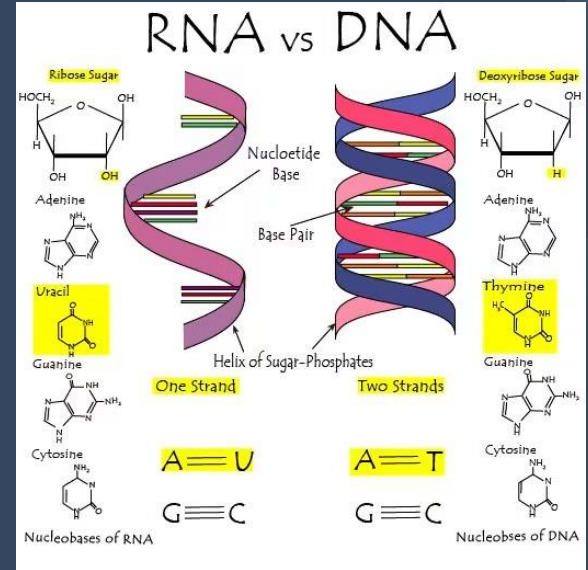


- Traditional **PCR** can only amplify **DNA**
- Many **viruses** and cells use **RNA** as genetic material
- **RNA cannot be directly amplified** by DNA polymerase
- Requires **conversion into DNA** before amplification
- **RT-PCR solves this limitation**



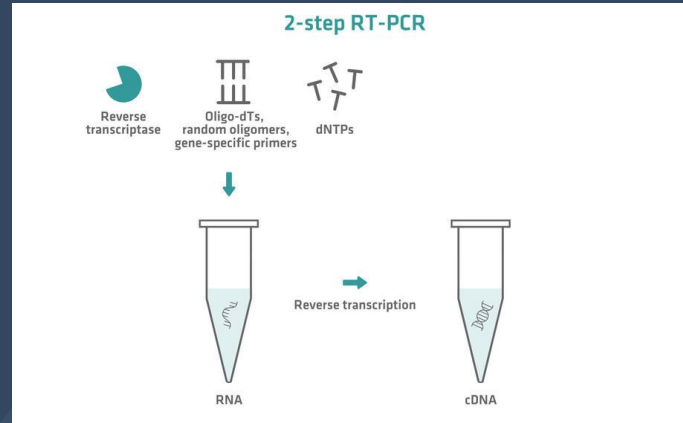
# RNA vs DNA

- **DNA:** double-stranded, **stable genetic material**
- **RNA:** single-stranded, **less stable**
- RNA found in viruses and **gene expression**
- RNA carries instructions from DNA to make proteins
- **RT-PCR allows study of RNA-based processes**

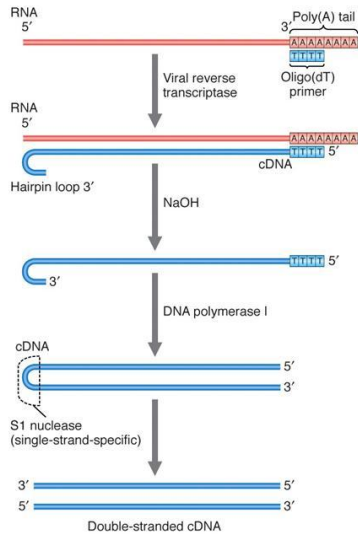


# Key Enzyme - Reverse Transcriptase

- Enzyme that **converts RNA into complementary DNA**
- Produces complementary DNA (cDNA)
- Originally discovered in **retroviruses**
- Essential **first step in RT-PCR**
- Enables **RNA analysis using PCR**



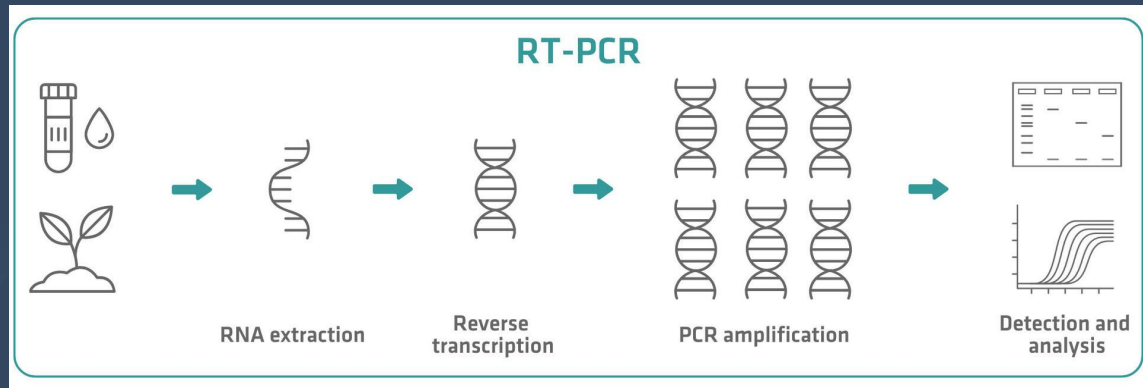
# What is cDNA?



- **Complementary DNA** made from RNA template
- Represents **expressed genes**
- **More stable than RNA**
- Used as **template in PCR amplification**
- Allows **indirect study of RNA**


# RT-PCR Process

1. **RNA extraction** from sample
2. **Reverse transcription** → cDNA formation
3. Addition of **PCR components**
4. **Thermal cycling** (denaturation, annealing, extension)
5. Amplification of target DNA

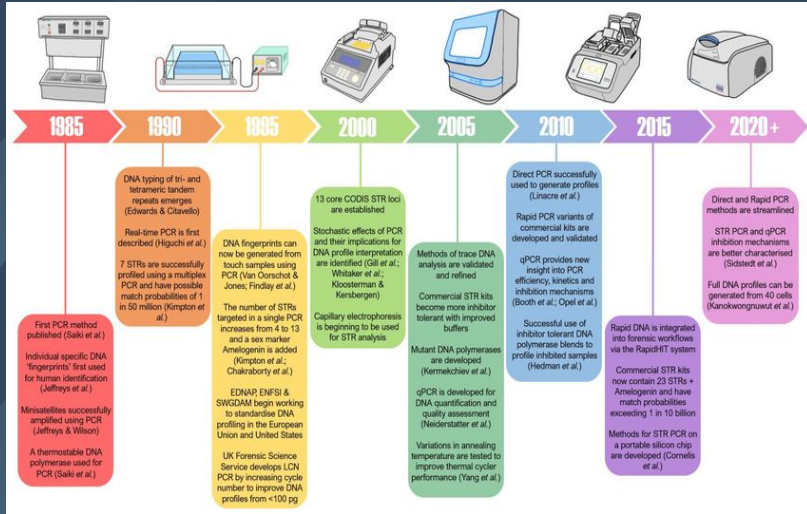




# Advantages and Limitations

- **Advantages:**
    - **Highly sensitive and specific**
    - **Can detect very small amounts of RNA**
    - **Fast and widely used**
  - **Limitations:**
    - **RNA is unstable** and easily degraded
    - **Requires careful handling**
    - **Risk of contamination**
- 

# Evolution of PCR Techniques



• **Conventional RT-PCR** → detects presence of RNA

• **Real-Time RT-PCR** → quantifies RNA levels

• **One-step RT-PCR** → combined reaction

• **Two-step RT-PCR** → separate steps for flexibility

• Each type used for different research purposes



1985

# 1985: Birth of PCR

- First PCR method published (Saiki et al.)
- **Thermostable DNA polymerase** introduced (taq)
- PCR enables exponential **DNA amplification**
- **Minisatellites** successfully amplified
- Early forensic **DNA fingerprinting** begins

First PCR method published (Saiki et al.)

Individual specific DNA 'fingerprints' first used for human identification (Jeffreys et al.)

Minisatellites successfully amplified using PCR (Jeffreys & Wilson)

A thermostable DNA polymerase used for PCR (Saiki et al.)

# 1990: Early Forensic Applications

- **DNA typing** of tri- & tetranucleotide repeats emerges
- **STR profiling** becomes possible
- **Real-time PCR** first described (Higuchi et al.)
- **Multiplex PCR** allows **multiple loci amplification**
- PCR enters **forensic genetics** workflows

1990

DNA typing of tri- and tetrameric tandem repeats emerges (Edwards & Citavello)

Real-time PCR is first described (Higuchi et al.)

7 STRs are successfully profiled using a multiplex PCR and have possible match probabilities of 1 in 50 million (Kimpton et al.)

1995

# 1995: Increased Sensitivity & STR Expansion

- **DNA profiles** generated from **touch DNA** (low template)
- Number of STR's in single PCR increased (**6 → 13 markers**)
- STR's established as **standard forensic markers**
- European forensic groups begin **standardization**
- **Low Copy Number (LCN) PCR** introduced (<100 pg DNA)

DNA fingerprints can now be generated from touch samples using PCR (Van Oorschot & Jones; Findlay et al.)

The number of STRs targeted in a single PCR increases from 4 to 13 and a sex marker Amelogenin is added (Kimpton et al.; Chakraborty et al.)

EDNAP, ENFSI & SWGDAM begin working to standardise DNA profiling in the European Union and United States

UK Forensic Science Service develops LCN PCR by increasing cycle number to improve DNA profiles from <100 pg

# 2000: Standardization & STR Databases

- 13 core CODIS STR loci established
- Capillary electrophoresis adopted for STR analysis
- Stochastic effects of PCR recognized (allelic dropout, imbalance)
- DNA profile interpretation frameworks developed
- Foundation for national DNA databases

2000

13 core CODIS STR loci are established

Stochastic effects of PCR and their implications for DNA profile interpretation are identified (Gill *et al.*; Whitaker *et al.*; Kloosterman & Kersbergen)

Capillary electrophoresis is beginning to be used for STR analysis

# 2005: Optimization & Quantification

- Trace DNA analysis methods refined
- Commercial PCR kits improved (buffers, sensitivity)
- Mutant DNA polymerases developed (higher efficiency)
- qPCR introduced for DNA quantification & quality control
- Thermal cycling conditions optimized

Methods of trace DNA analysis are validated and refined

Commercial STR kits become more inhibitor tolerant with improved buffers

Mutant DNA polymerases are developed (Kermekchiev *et al.*)

qPCR is developed for DNA quantification and quality assessment (Neiderstatter *et al.*)

Variations in annealing temperature are tested to improve thermal cycler performance (Yang *et al.*)

# 2010: Real-Time & Rapid PCR Expansion

- **Direct PCR** used without DNA extraction
- **Rapid PCR** system developed & validated
- **qPCR** provides insight into:
  - **Amplification kinetics**
  - **PCR inhibition**
- **Polymerase blends** improve profiling of degraded DNA

Direct PCR successfully used to generate profiles (Linacre *et al.*)

Rapid PCR variants of commercial kits are developed and validated

qPCR provides new insight into PCR efficiency, kinetics and inhibition mechanisms (Booth *et al.*; Opel *et al.*)

Successful use of inhibitor tolerant DNA polymerase blends to profile inhibited samples (Hedman *et al.*)

# 2015: Integration into Forensic Workflows

- Rapid DNA integration into **forensic pipelines**
- **Automated systems** (e.g., RapidHIT) introduced
- **Commercial STR kits** expanded:
  - **23 STR loci**
  - **Amelogenin + Y markers**
- Match probabilities reach **~1 in 10 billion**

Rapid DNA is integrated into forensic workflows via the RapidHIT system

Commercial STR kits now contain 23 STRs + Amelogenin and have match probabilities exceeding 1 in 10 billion

Methods for STR PCR on a portable silicon chip are developed (Cornelis *et al.*)

# 2020+ : Advanced & Portable PCR

- **Direct & rapid PCR** methods streamlined
- **STR PCR + qPCR inhibition** better characterized
- Full DNA profiles from **~40 cells** possible
- **Portable PCR** devices (lab-on-chip systems) developed
- Increased **field-based** and **real-time** applications

2020+

Direct and Rapid PCR methods are streamlined

STR PCR and qPCR inhibition mechanisms are better characterised (Sidstedt *et al.*)

Full DNA profiles can be generated from 40 cells (Kanokwongnuwut *et al.*)

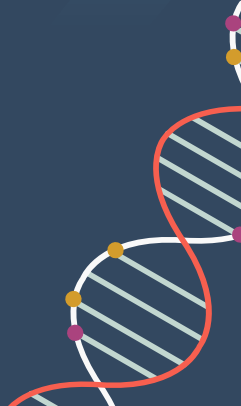
# Overall Trends

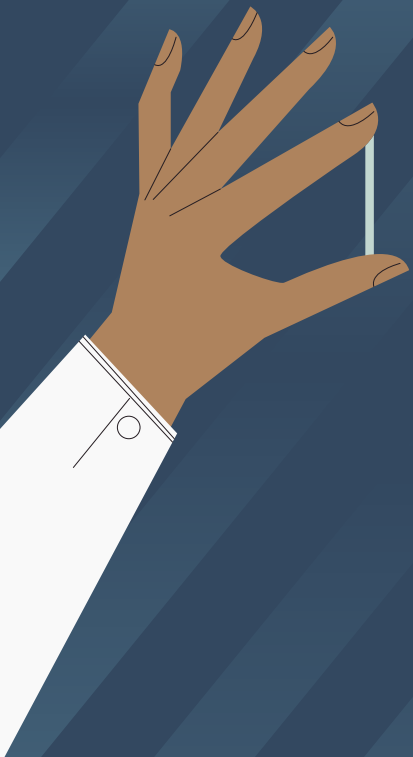
- Shift from **basic amplification** → **high sensitivity & speed**
- Movement toward **automation & portability**
- Increasing forensic precision (**STR expansion**)
- Integration of quantification (**qPCR**)
- PCR remains central to:
  - **Forensics**
  - **Diagnostics**
  - **Gene expression studies**



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**Thanks!**