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

By Abdulgafar Lateef, Kaitlyn Coley, Darcy Jones

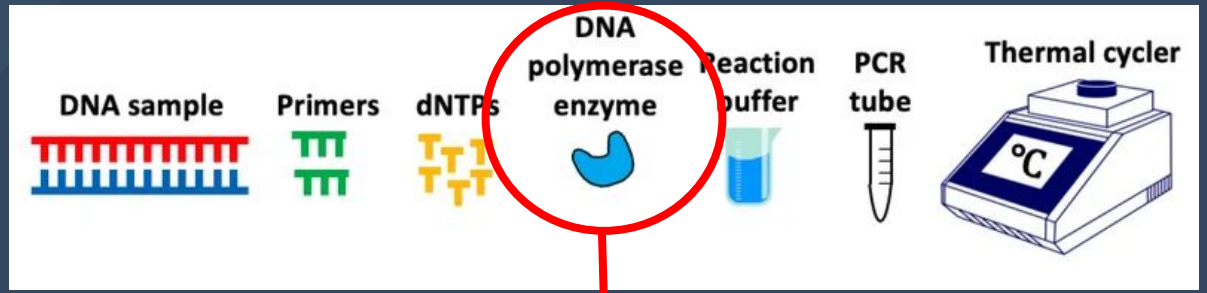
Background

- **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?

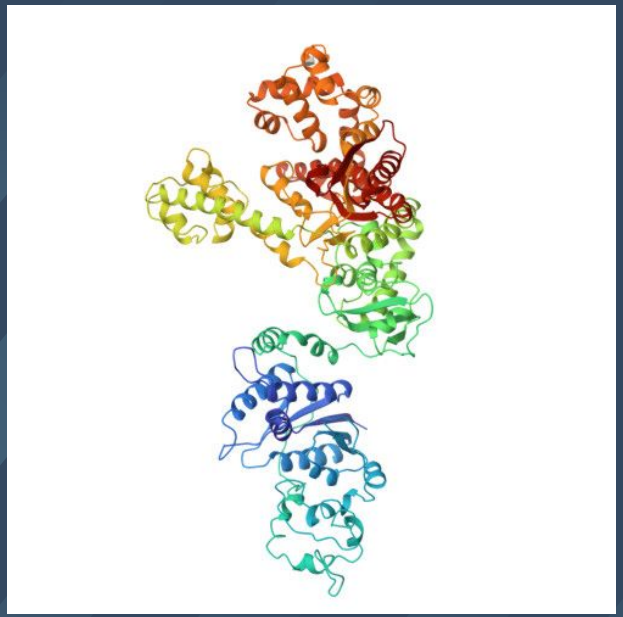
- “One of the most important scientific advances in molecular biology”
- Laboratory **nucleic acid** amplification technique
- Targets specific **DNA** (or **RNA**) fragments
- Three major phases of **amplification**:
  - Denaturation
  - Annealing
  - Extension
- 1 DNA molecule = **~1 billion copies**

Materials



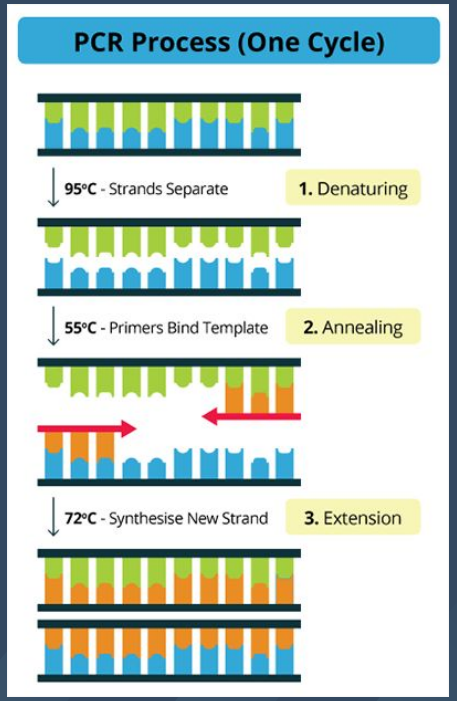
Taq Polymerase

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

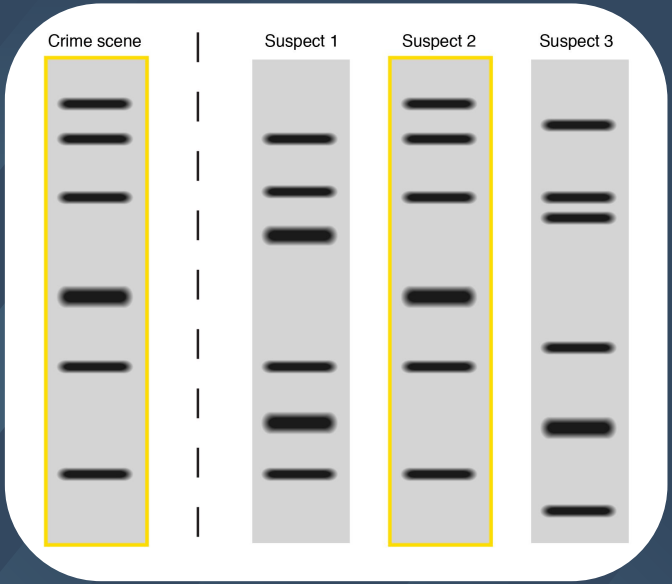
- Temperature= 75˚C to 80˚C
- **Taq** adds nucleotides
- New DNA strand synthesized

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

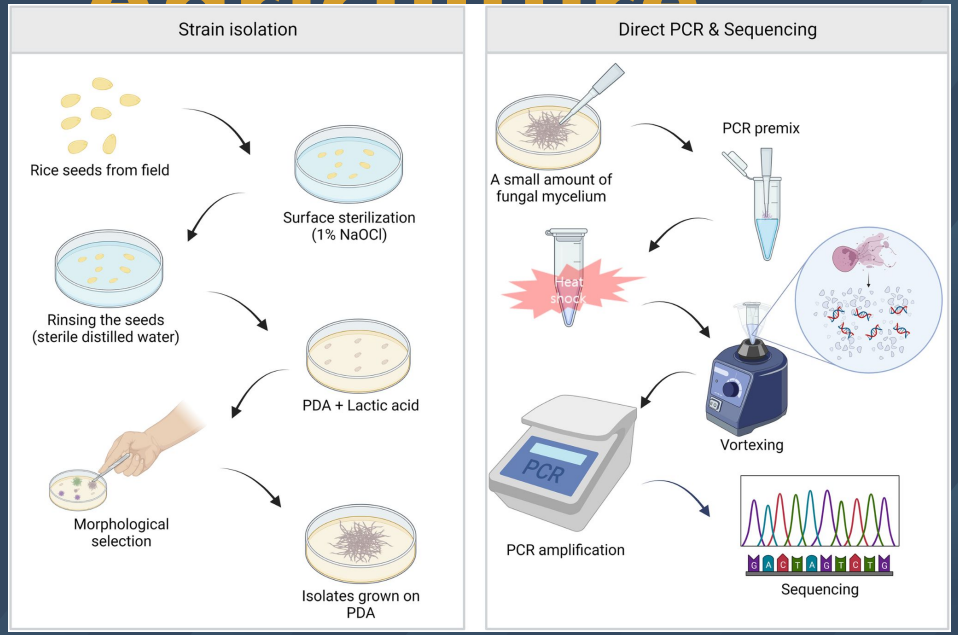
Forensic Fingerprinting



- 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

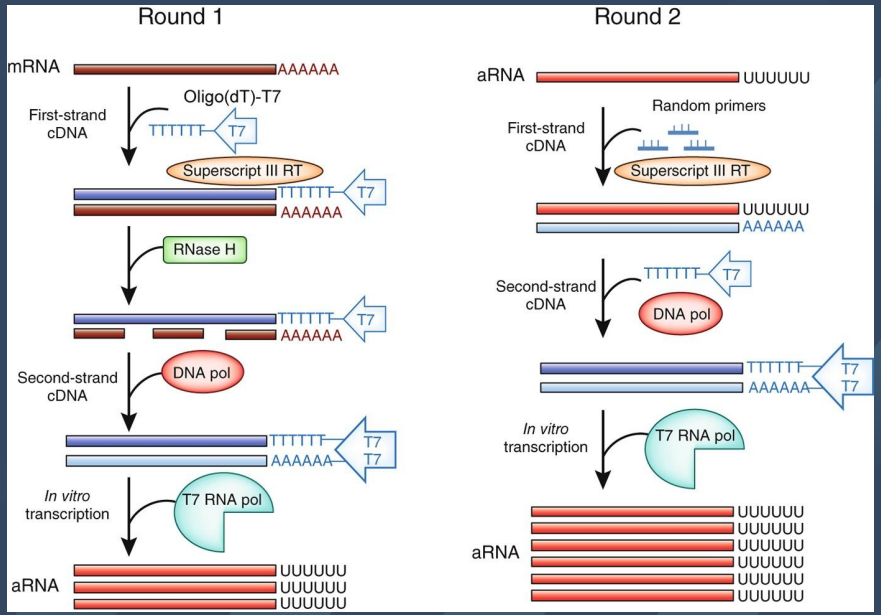


Advantages and Limitations

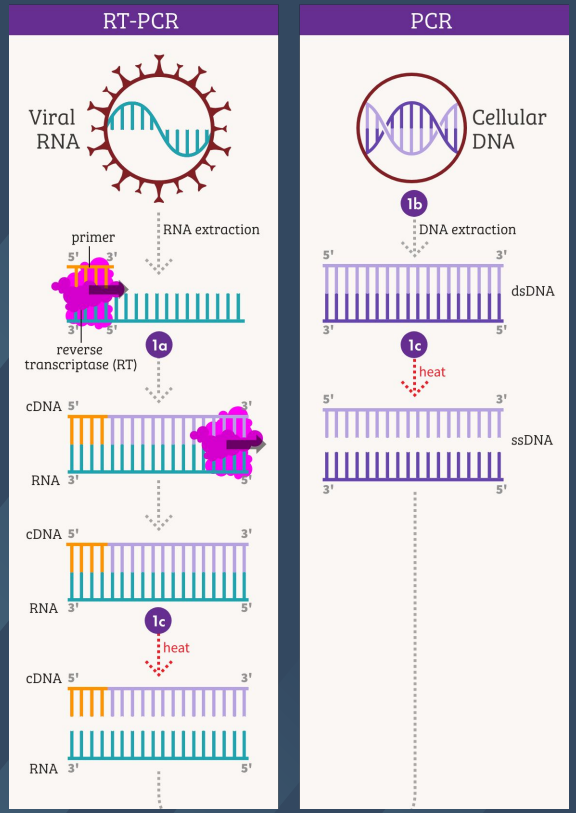
- Advantages:
  - Highly sensitive and specific
  - Can detect very small amounts of **DNA**
  - Fast and widely used
- Limitations:
  - Susceptible to contamination + mutations
    - False positives
  - Prior knowledge required (for **primer** design)
  - Cannot detect unknown pathogens
  - Positive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



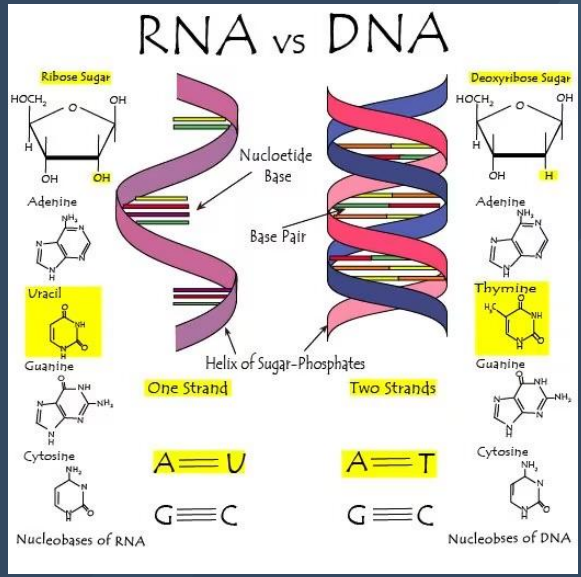
Why RT-PCR?



- 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**

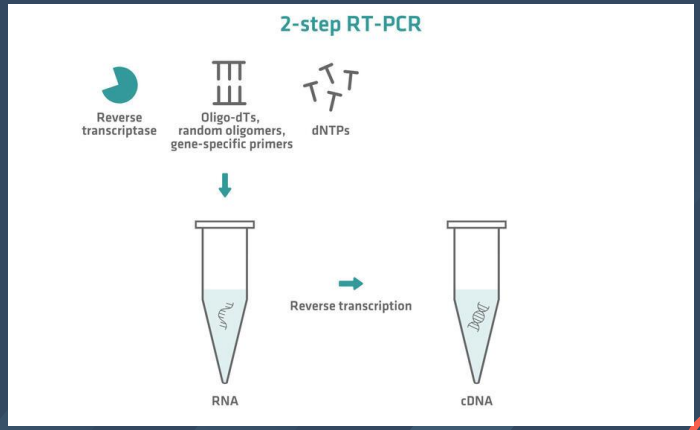
RNA vs DNA

- **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**

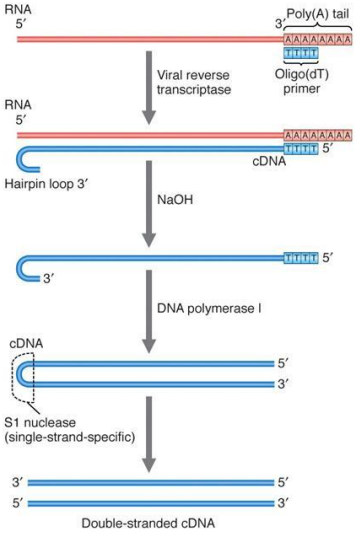


Key Enzyme - Reverse Transcriptase

- 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**



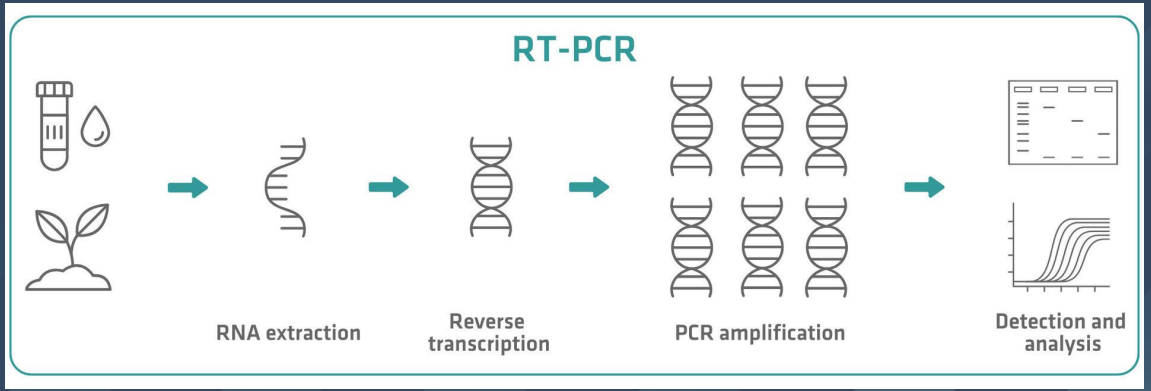
What is cDNA?



- **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

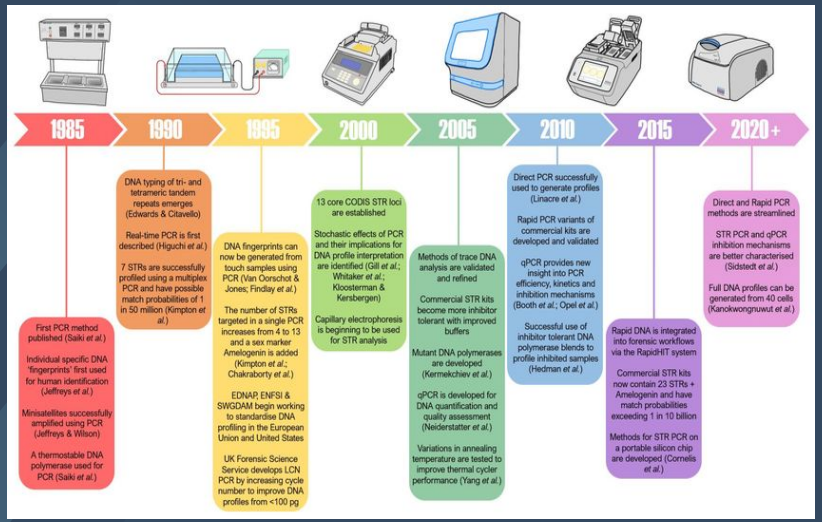
1. **RNA extraction** from sample
2. **Reverse transcription** → **cDNA** formation
3. Addition of **PCR components**
4. **Thermal cycling** (denaturation, annealing, extension)
5. Amplification of target DNA



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**

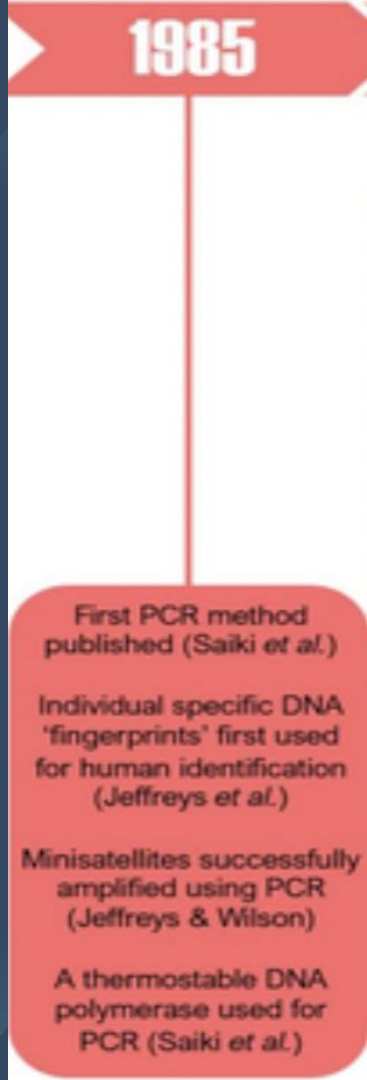
Evolution of PCR Techniques



- **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

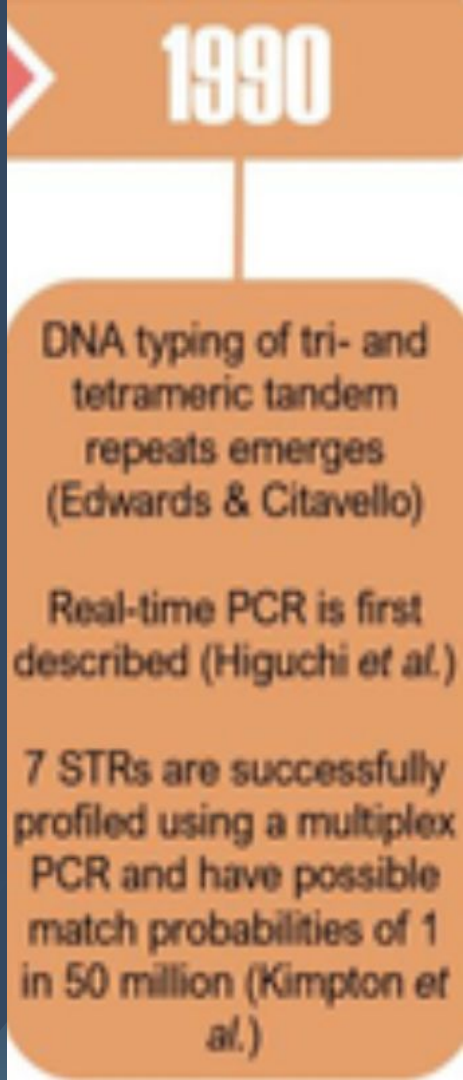
1985 : Birth of PCR

- 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



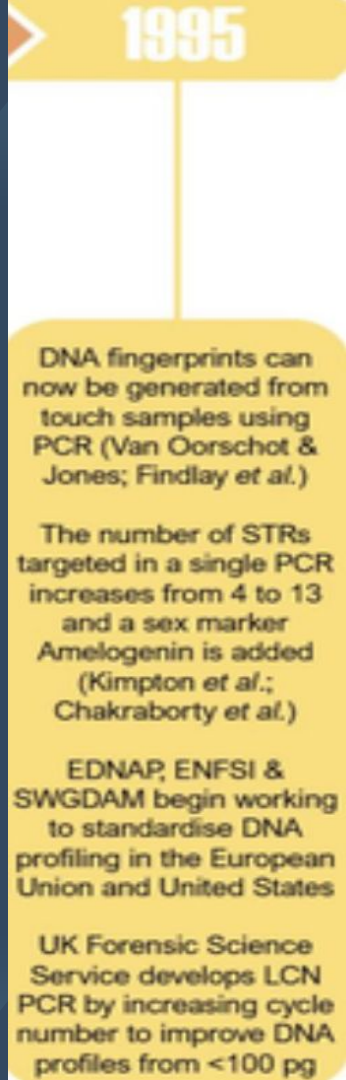
1990: Early Forensic Applications

- **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



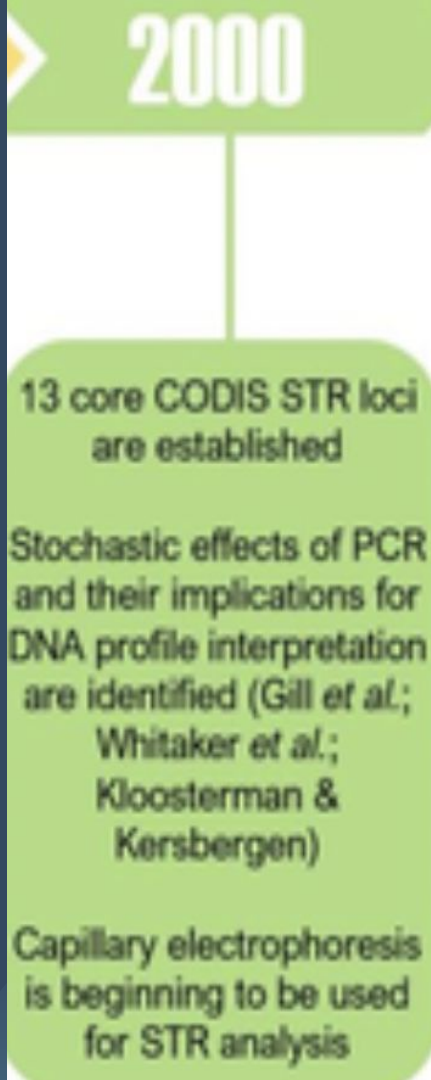
1995: Increased Sensitivity & STR Expansion

- **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)



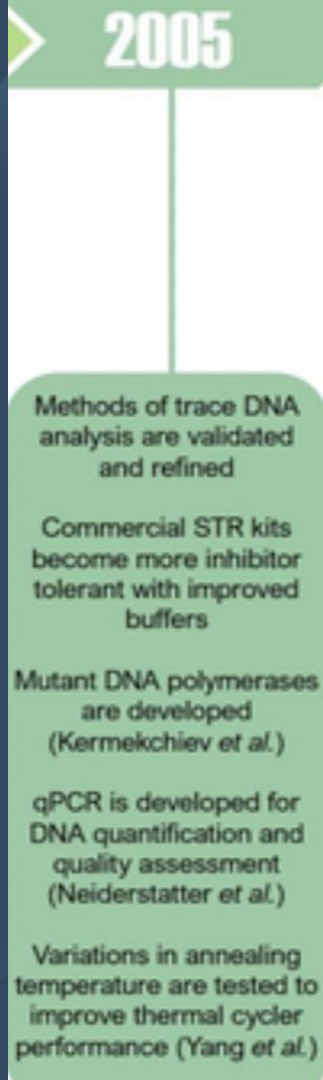
2000: Standardization & STR Databases

- **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**



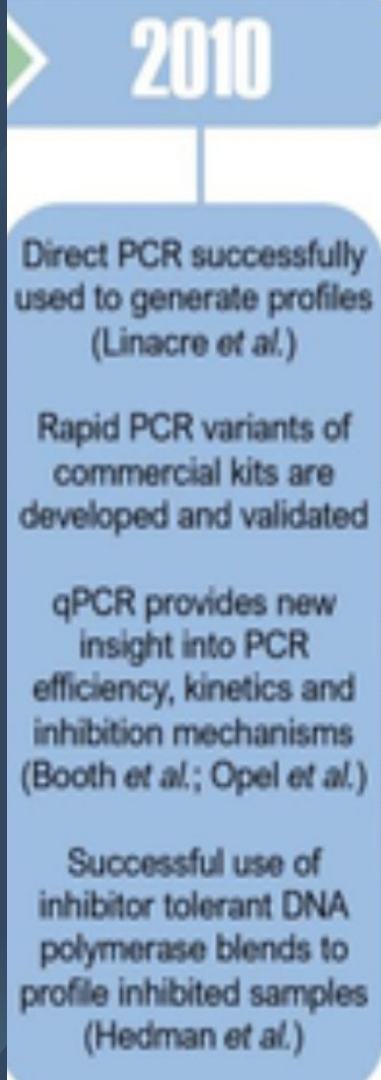
2005: Optimization & Quantification

- **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



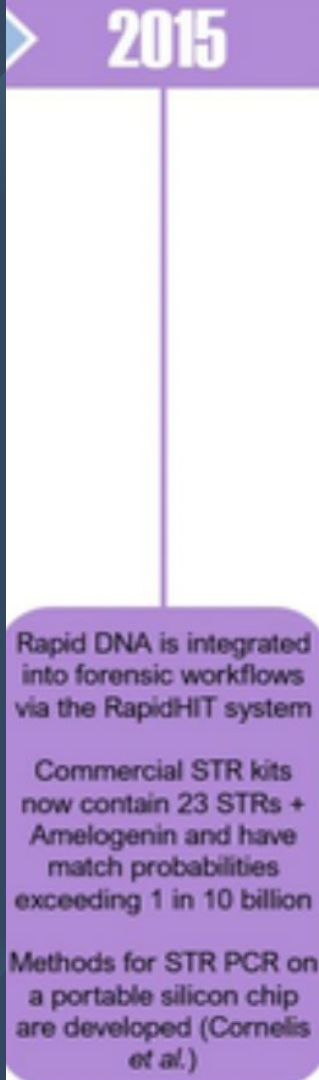
2010: Real-Time & Rapid PCR Expansion

- **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



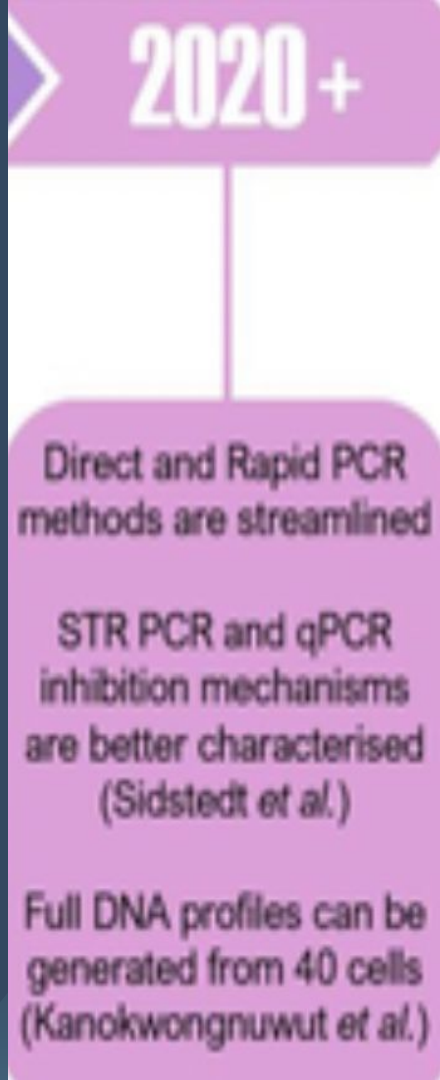
2015: Integration into Forensic Workflows

- 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**



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



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

References

Mullis, K. B., & Faloona, F. A. (1987).
Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods in Enzymology, 155, 335–350.

Nelson, D. L., & Cox, M. M. (2021).
Lehninger principles of biochemistry (8th ed.). W.H. Freeman and Company.

Watson, J. D., Baker, T. A., Bell, S. P., Gann, A., Levine, M., & Losick, R. (2014).
Molecular biology of the gene (7th ed.). Pearson.

Lodish, H., Berk, A., Kaiser, C. A., Krieger, M., Scott, M. P., Bretscher, A., Ploegh, H., & Matsudaira, P. (2016).
Molecular cell biology (8th ed.). W.H. Freeman and Company.

Bustin, S. A., & Nolan, T. (2004).
Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction. Journal of Biomolecular Techniques, 15(3), 155–166.

Centers for Disease Control and Prevention (CDC). (2020).
Real-time RT-PCR diagnostic panel for detection of viral RNA.

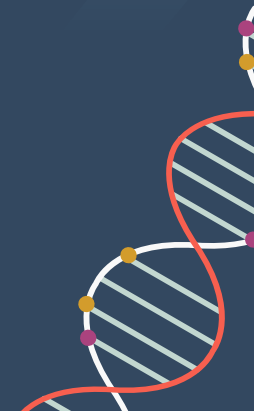
National Institutes of Health (NIH). (2021).
Polymerase chain reaction (PCR) fact sheet.

World Health Organization (WHO). (2020).
Laboratory testing for coronavirus disease (COVID-19) using RT-PCR.

Khan Academy. (2023).
Polymerase chain reaction (PCR). Khan Academy.

Thermo Fisher Scientific. (2022).
PCR and RT-PCR basics and applications. Thermo Fisher Scientific.

Nature Education. (2014).
Polymerase chain reaction (PCR) overview. Nature Education.



Thanks!