Enzyme-based molecular techniques (part II)

Polymerase chain reaction (PCR)

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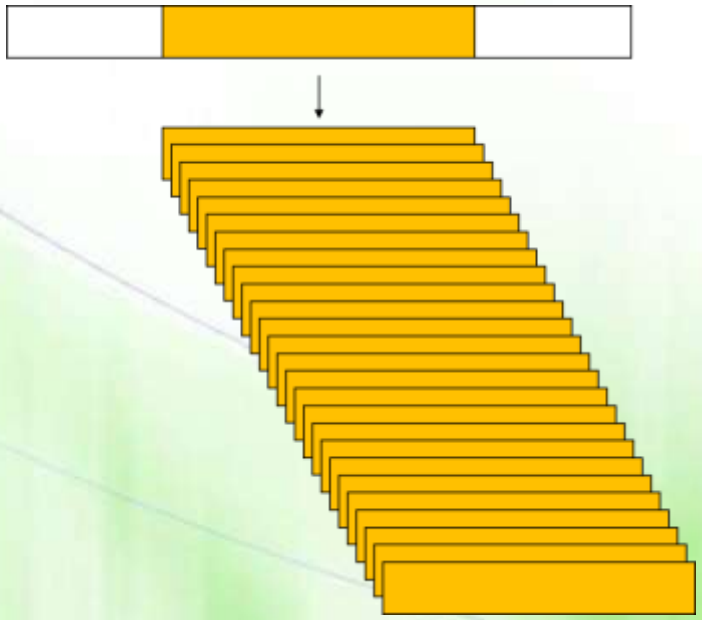
Challenges in research and medicine

- Genetic variation
  - STR, VNTR, SNPs, and mutations
- Minute amounts of genetic material
  - Dinosaurs and early humans
- Identification of organisms (e.g. infectious agents)

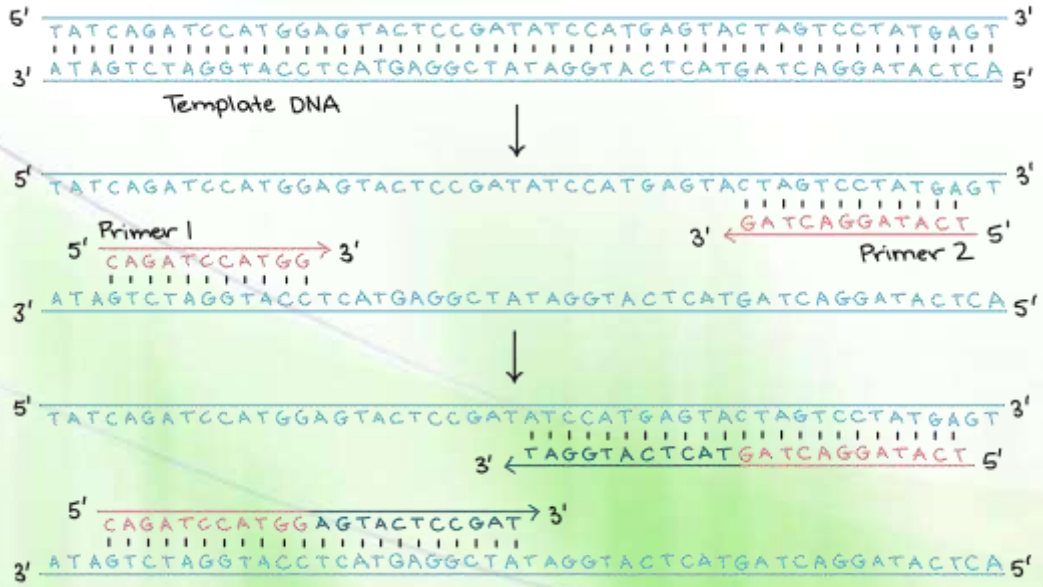
Polymerase Chain Reaction

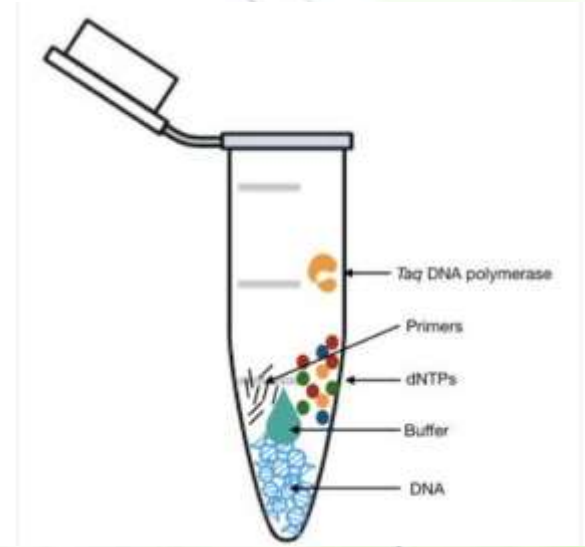
- Polymerase chain reaction (PCR) allows the DNA from a selected region of a genome to be amplified a billionfold, effectively "purifying" this DNA away from the remainder of the genome.
- It is extremely sensitive; it can detect a single DNA molecule in a sample.



Components of PCR reaction

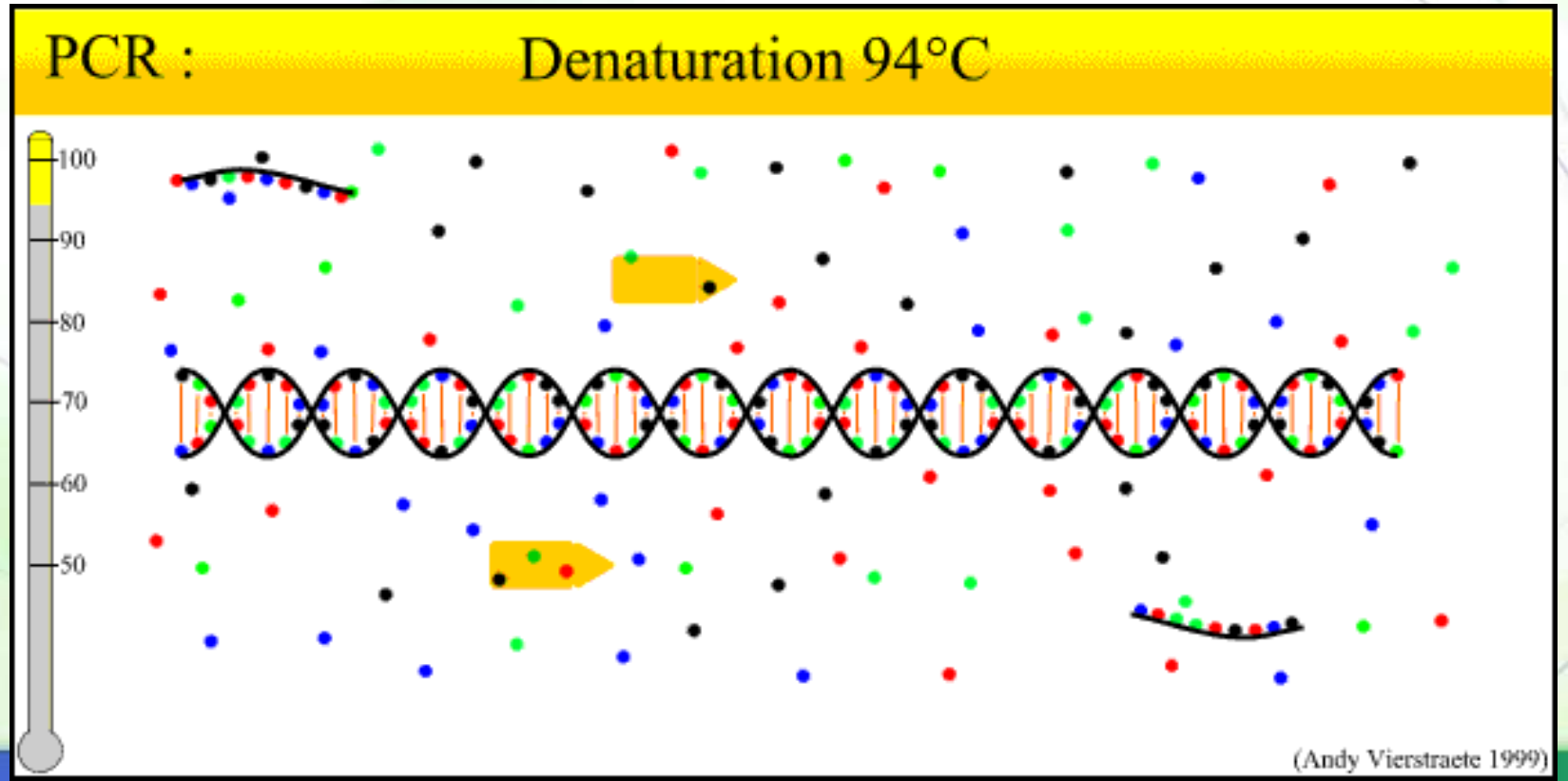
- The DNA template
- A pair of DNA primers
  - The 15-25 nucleotides-long primers should surround the target sequence.
- All four deoxyribonucleoside triphosphates
- A heat-stable DNA polymerase





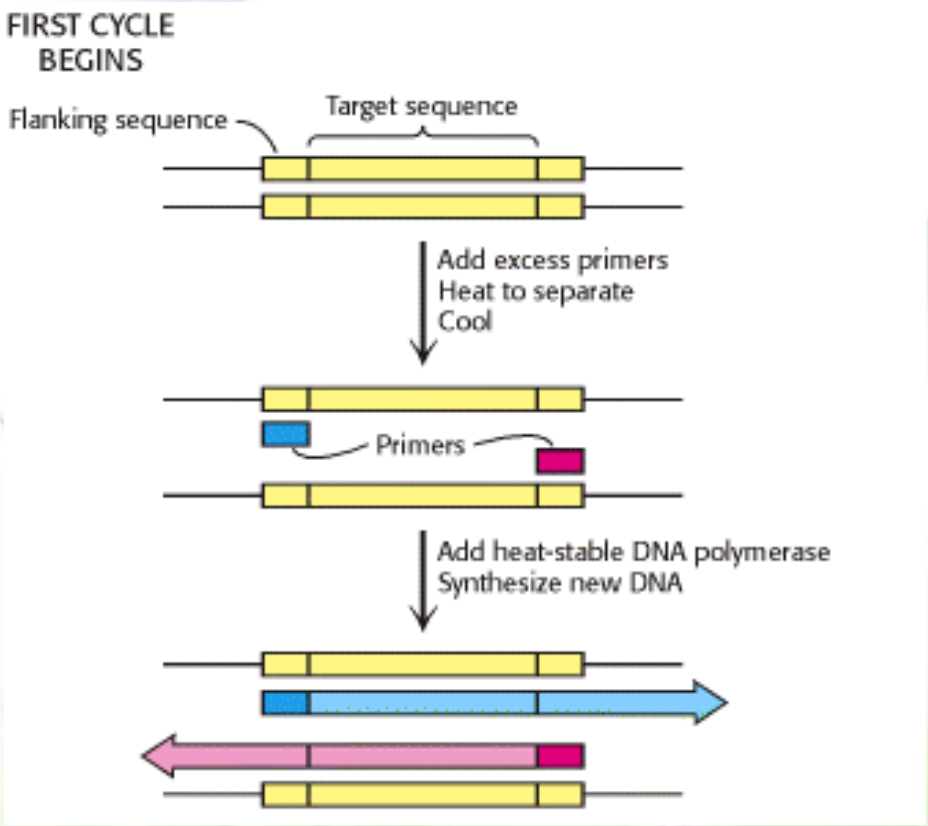
The PCR cycles

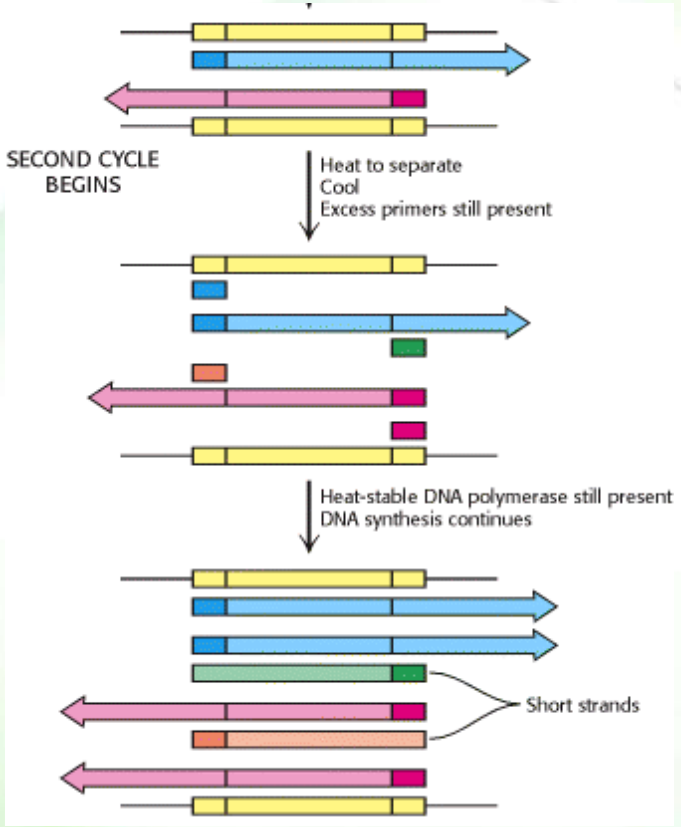
- Denaturation (at 95°C): DNA is denatured into single-stranded molecules.
- Reannealing (50°C to 70°C): the primers anneal to the DNA.
- DNA synthesis (at 72°C): optimal for the polymerase.

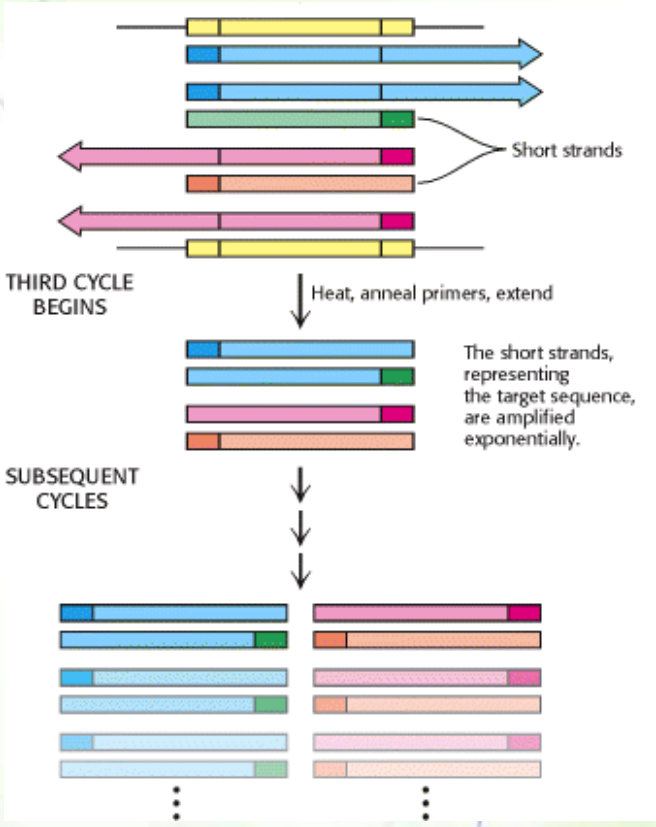


The DNA polymerase

- Suitably heat-stable DNA polymerases have been obtained from microorganisms whose natural habitat is hot springs.
- For example, the widely used Taq DNA polymerase is obtained from a thermophilic bacterium, Thermus aquaticus, and is thermostable up to 95°C.


FIRST CYCLE BEGINS
Flanking sequence
Target sequence
Add excess primers
Heat to separate
Cool
Primers
Add heat-stable DNA polymerase
Synthesize new DNA


SECOND CYCLE BEGINS
Heat to separate
Cool
Excess primers still present
Heat-stable DNA polymerase still present
DNA synthesis continues
Short strands

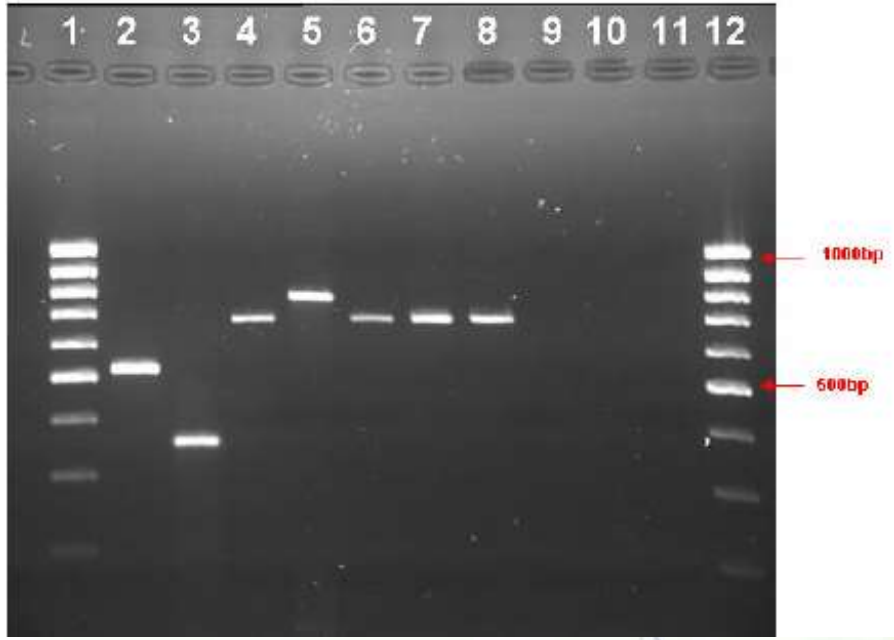

Short strands
THIRD CYCLE BEGINS
Heat, anneal primers, extend
The short strands, representing the target sequence, are amplified exponentially.
SUBSEQUENT CYCLES

PCR cycles

- 20-30 cycles of reaction are required for DNA amplification.
  - the products of each cycle serving as the DNA templates for the next-hence the term polymerase "chain reaction“.
- Every cycle doubles the amount of DNA.
- After 30 cycles, there will be over 250 million short products derived from each starting molecule.

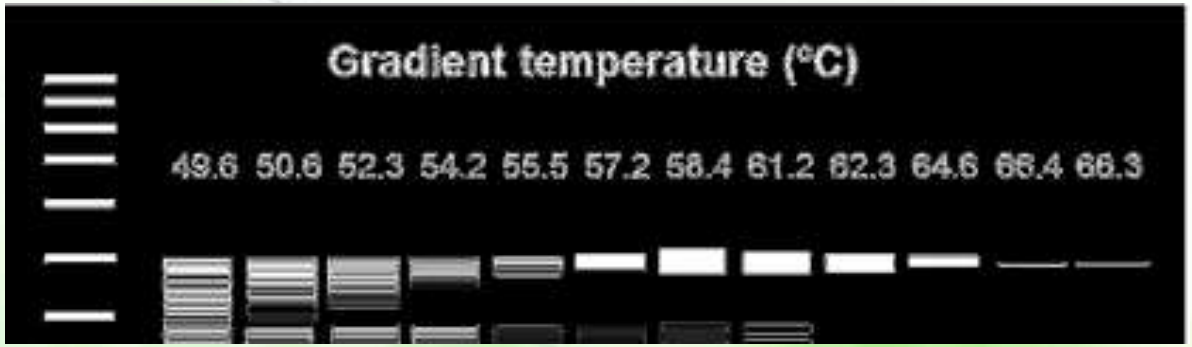
Detection of DNA fragments

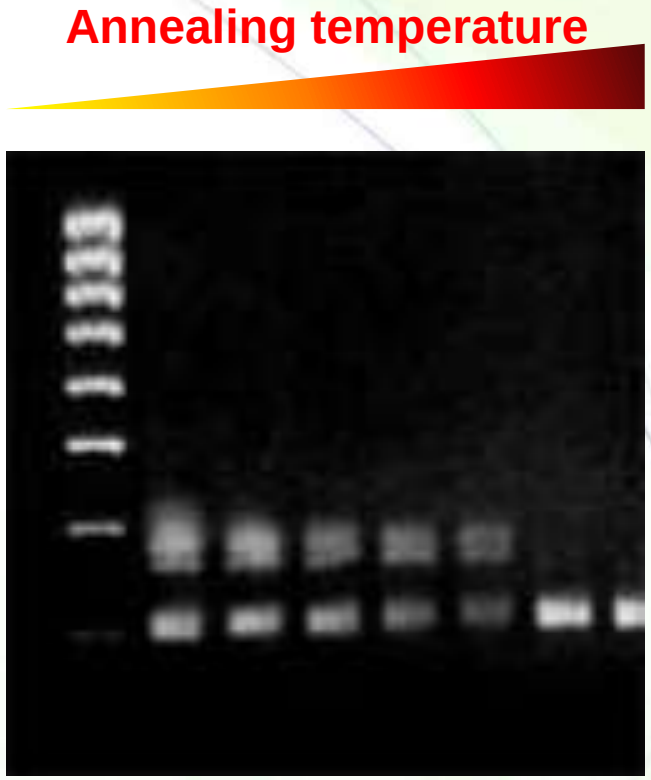
- This DNA fragment can be easily visualized as a discrete band of a specific size by agarose gel electrophoresis.



Importance of primers

- The specificity of amplification depends on the specificity of the primers to not recognize and bind to sequences other than the intended target DNA sequences.
- How can you prevent it?
- How can you take advantage of it?



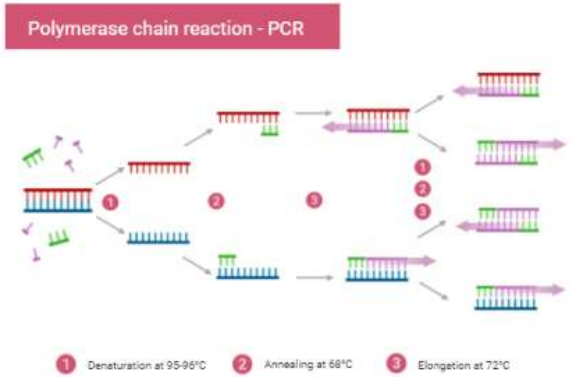


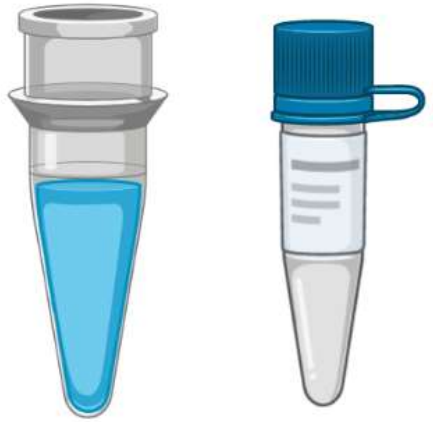
Polymerase Chain Reaction

Types of PCR with definition and uses

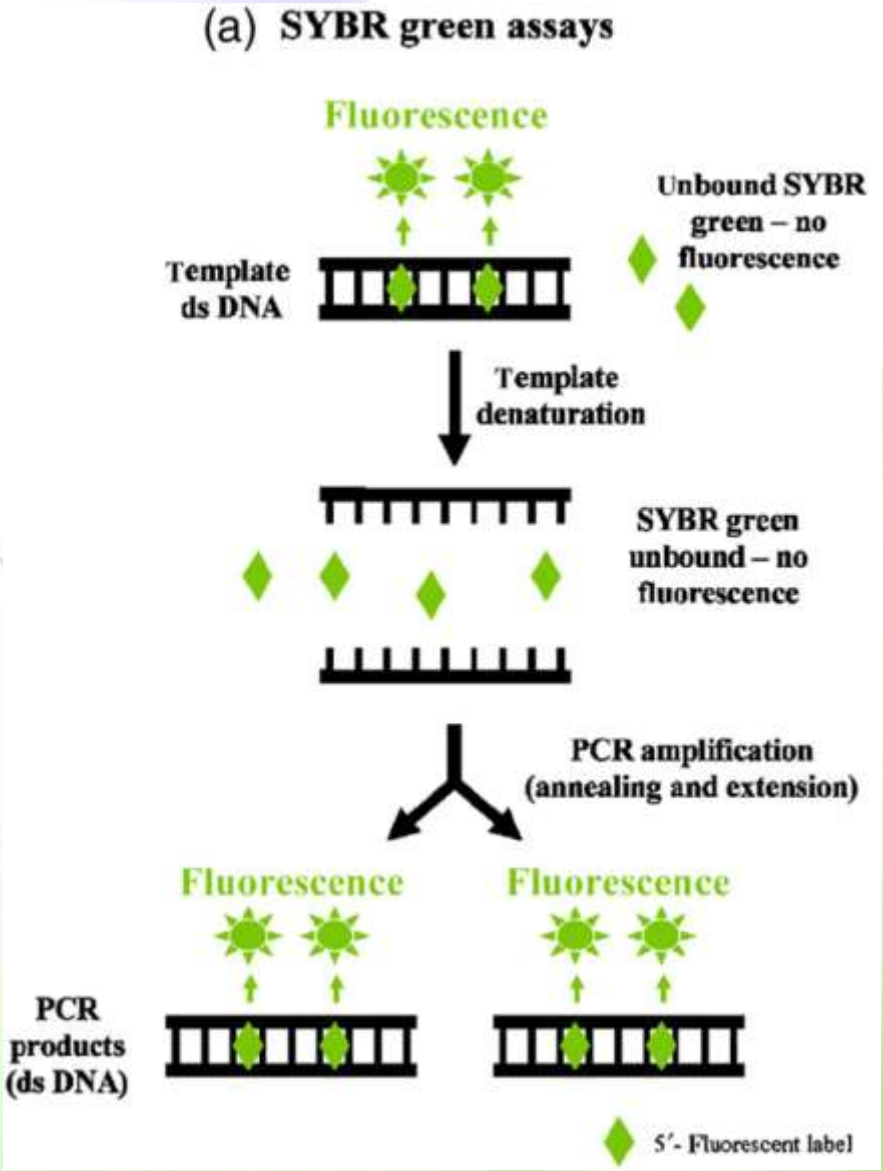
1. AFLP PCR
2. Allele-specific PCR
3. Alu PCR
4. Assembly PCR
5. Asymmetric PCR
6. COLD PCR
7. Colony PCR
8. Conventional PCR
9. Digital PCR (dPCR)
10. Fast-cycling PCR
11. High-fidelity PCR
12. Hot-start PCR
13. In situ PCR
14. Intersequence-specific (ISSR) PCR
15. Inverse PCR
16. LATE (linear after the exponential) PCR
17. Ligation-mediated PCR
18. Long-range PCR

19. Methylation-specific PCR (MSP)
20. Miniprimer PCR
21. Multiplex-PCR
22. Nanoparticle-Assisted PCR (nanoPCR)
23. Nested PCR
24. Overlap extension PCR
25. Real-Time PCR (quantitative PCR or qPCR)
26. Repetitive sequence-based PCR
27. Reverse-Transcriptase (RT-PCR)
28. Reverse-Transcriptase Real-Time PCR (RT-qPCR)
29. RNase H-dependent PCR (rhPCR)
30. Single cell PCR
31. Single Specific Primer-PCR (SSP-PCR)
32. Solid phase PCR
33. Suicide PCR
34. Thermal asymmetric interlaced PCR (TAIL-PCR)
35. Touch down (TD) PCR
36. Variable Number of Tandem Repeats (VNTR) PCR

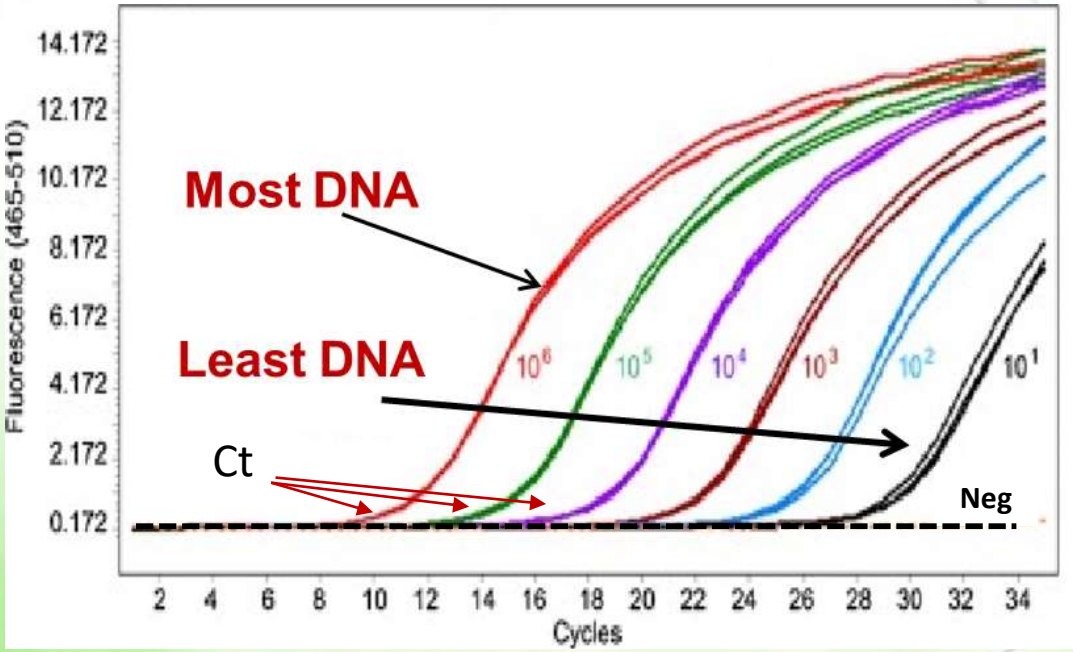




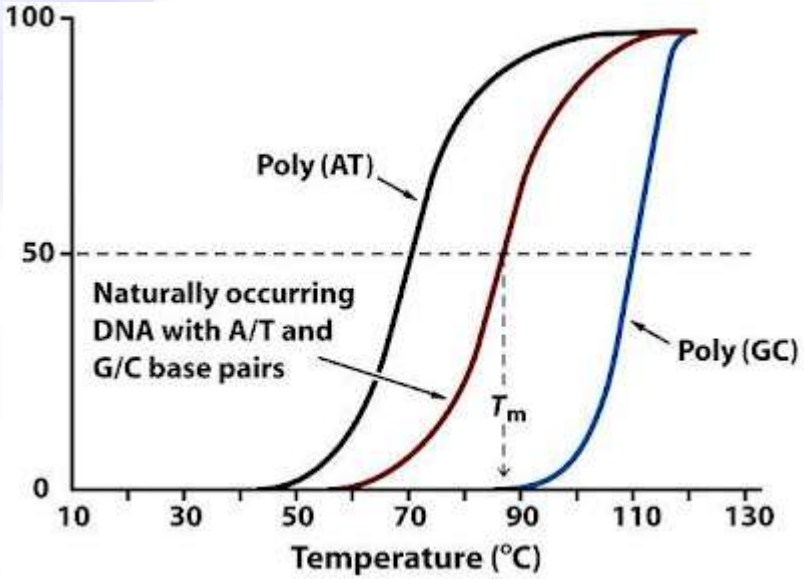
Real-time quantitative PCR (qPCR)

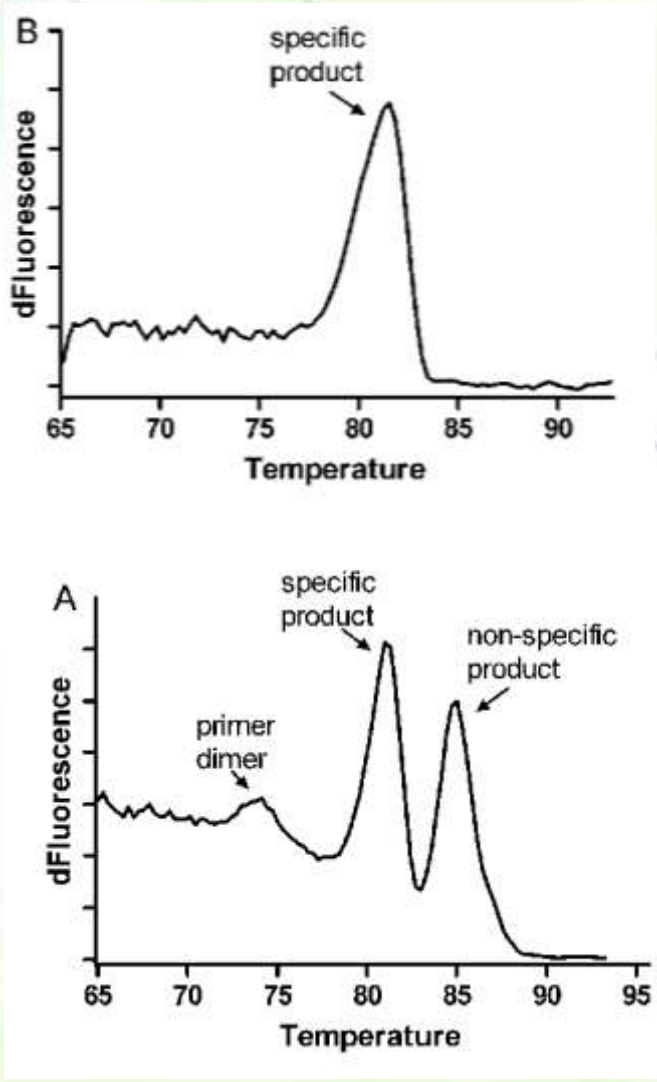


- SYBR green binds to double-stranded DNA and fluoresces only when bound.
- A way of relative quantitation of amount of DNA in a sample is by amplifying it in the presence of SYBR green.
- The higher the amount of DNA, the sooner it is detected.
- Threshold cycle (Ct) tells us at which cycle the signal is detected and is a measure of starting amount of DNA.



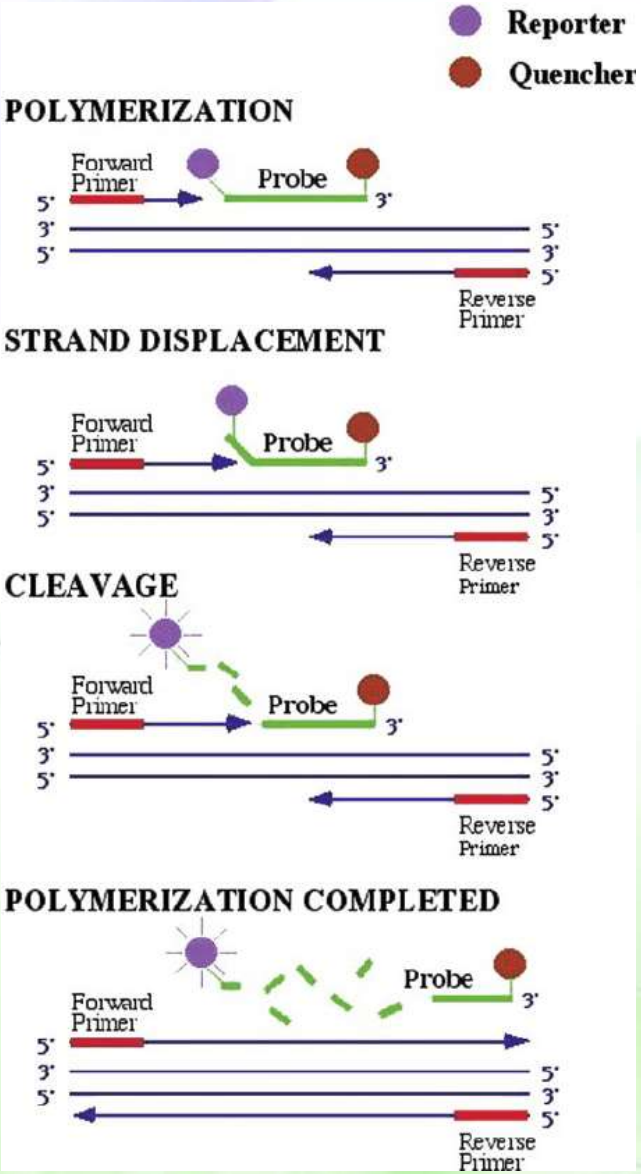
Melting curve analysis of qPCR





- A melting curve charts the change in fluorescence observed when double-stranded DNA (dsDNA) with incorporated dye molecules dissociates, or “melts” into single-stranded DNA (ssDNA) as the temperature of the reaction is raised.

Taqman qPCR



- Advantages (versus SYBR chemistry)
  - More specific
  - More sensitive
  - More reproducible
  - Multiplexing