



Polymerase chain reaction (PCR)

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School of Medicine

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Challenges in research and medicine



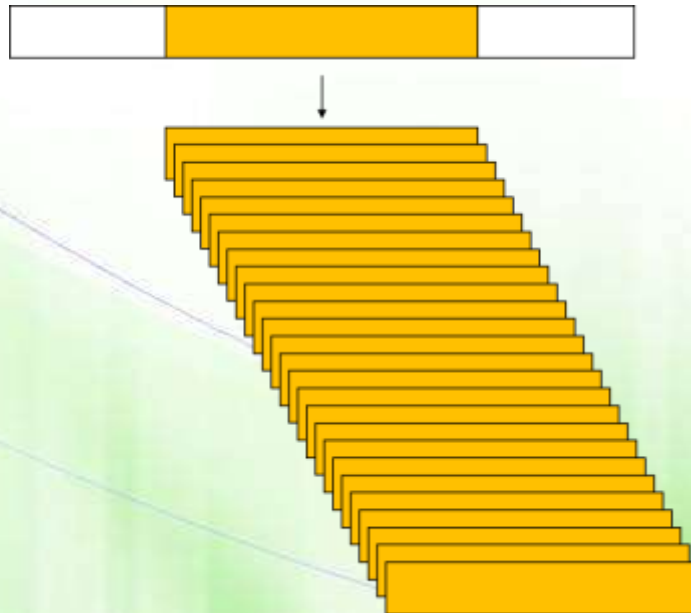
- Genetic variation
 - STR, VNTR, SNPs, and mutations
- Minute amounts of genetic material
 - Dinosaurs and early humans
- Identification of organisms (e.g. infectious agents)



Polymerase Chain Reaction



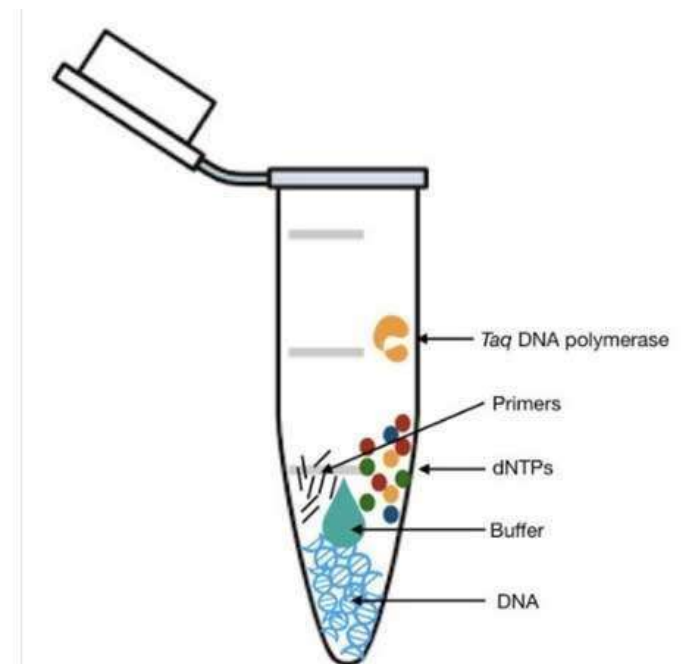
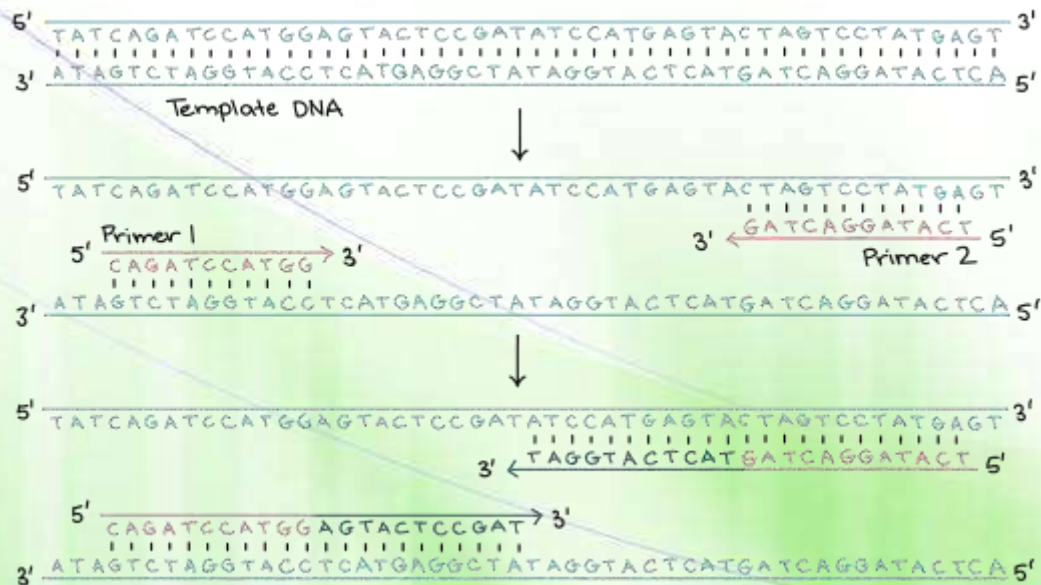
- Polymerase chain reaction (PCR) allows the DNA from a selected region of a genome to be amplified a billionfold, effectively "purifying" this DNA away from the remainder of the genome.
- It is extremely sensitive; it can detect a single DNA molecule in a sample.



Components of PCR reaction



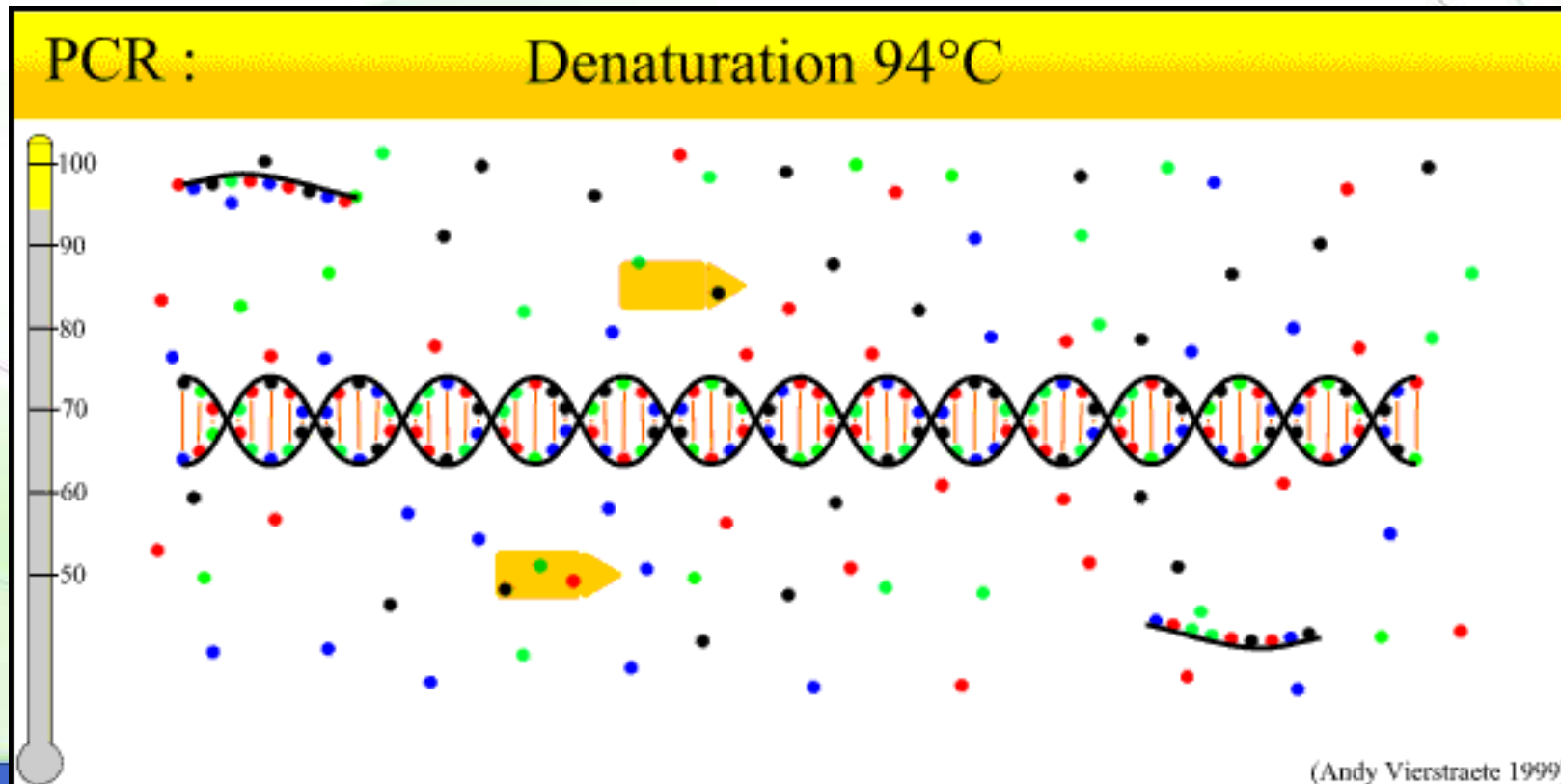
- The DNA template
- A pair of DNA primers
 - The 15-25 nucleotides-long primers should surround the target sequence.
- All four deoxyribonucleoside triphosphates
- A heat-stable DNA polymerase



The PCR cycles



- Denaturation (at 95°C): DNA is denatured into single-stranded molecules.
- Annealing (50°C to 70°C): The primers anneal (bind, hybridize) to the DNA.
- Polymerization or DNA synthesis (at 72°C): optimal for the polymerase.



The DNA polymerase

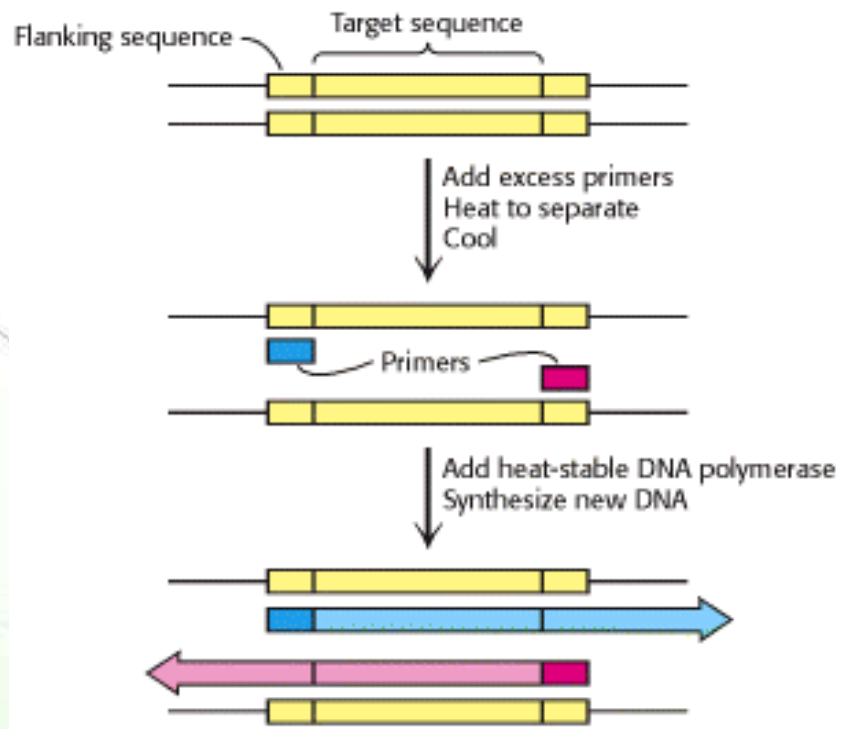


- Suitably heat-stable DNA polymerases that have been obtained from microorganisms whose natural habitat is hot springs.
- For example, the widely used Taq DNA polymerase is obtained from a thermophilic bacterium, *Thermus aquaticus*, and is thermostable up to 95°C.

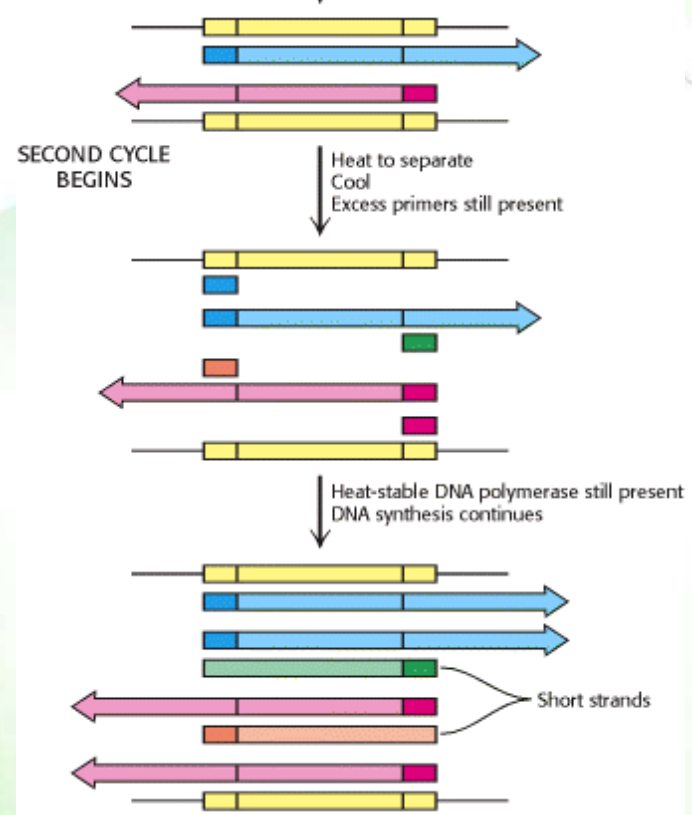




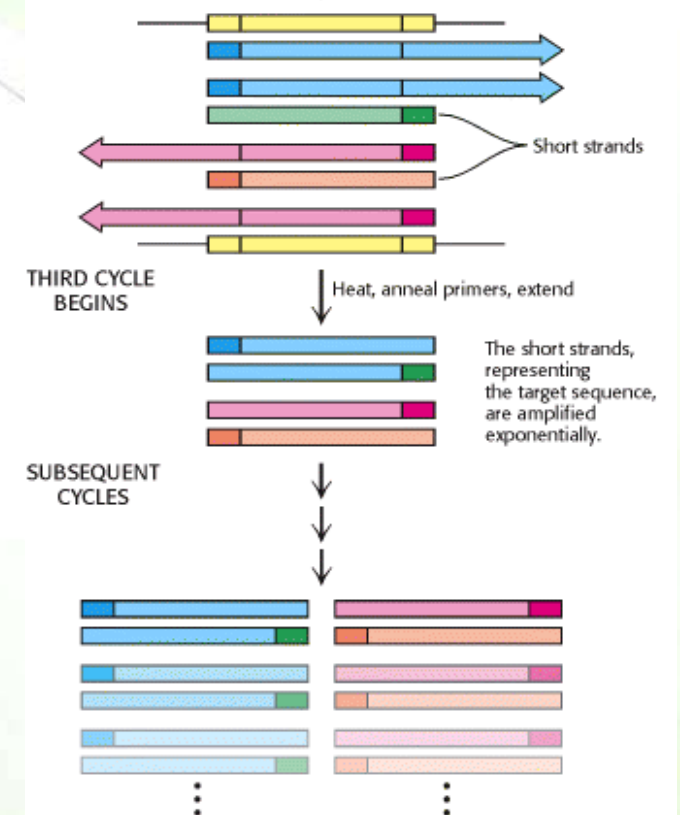
FIRST CYCLE BEGINS



SECOND CYCLE BEGINS



THIRD CYCLE BEGINS



PCR cycles



- 20-30 cycles of reaction are required for DNA amplification.
 - The products of each cycle serve as the DNA templates for the next products, 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.

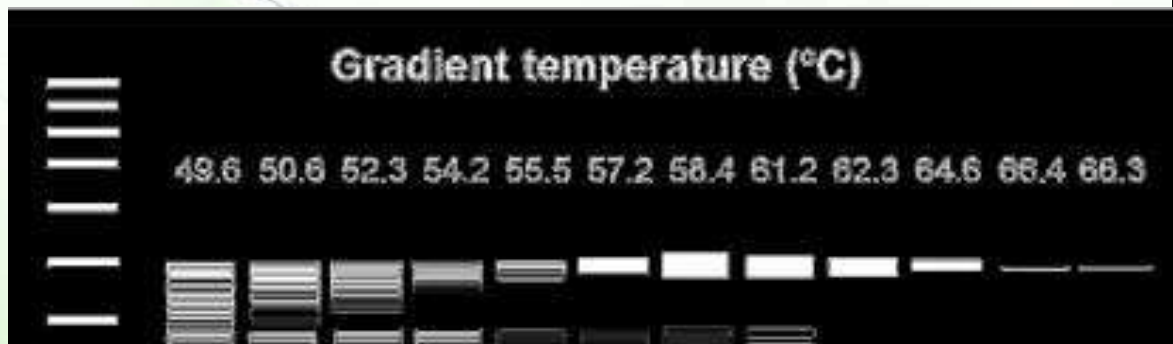
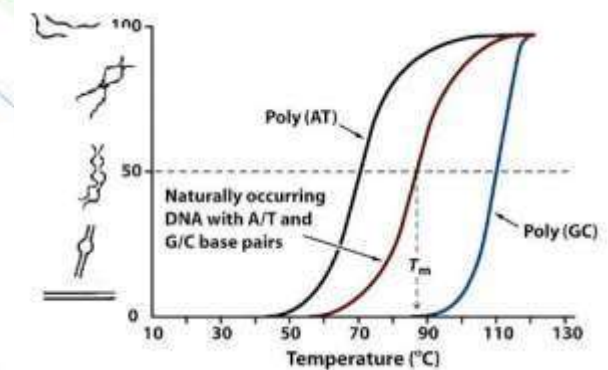
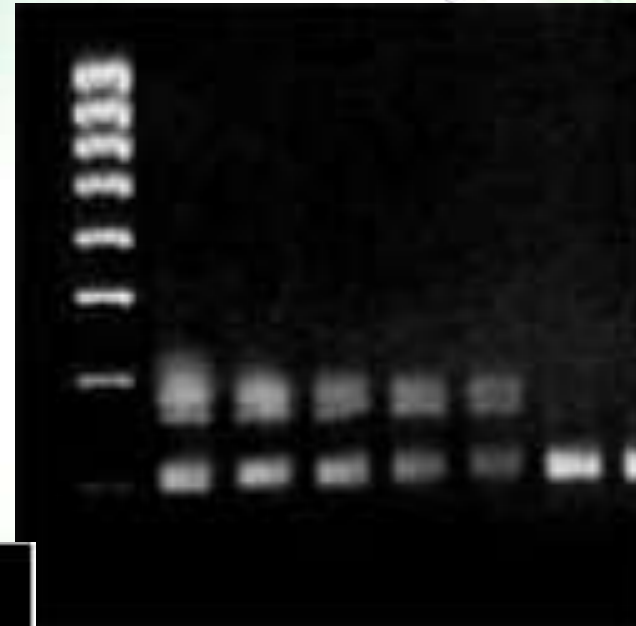


Importance of primers



- 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?

Annealing temperature





Polymerase Chain Reaction

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Uses of PCR



- Molecular fingerprinting
- Genotyping
- Genetic matching
- Detection of Mutations
- Prenatal diagnosis
- Cloning
- Detection of organisms
- Classification of organisms
- Mutagenesis
- Molecular archeology



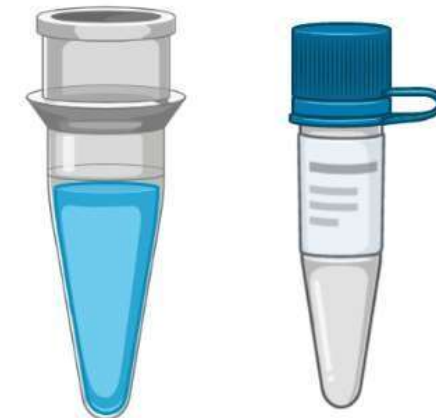
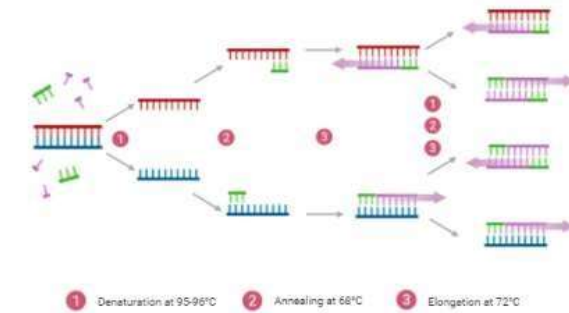
Types of PCR with definition and uses

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

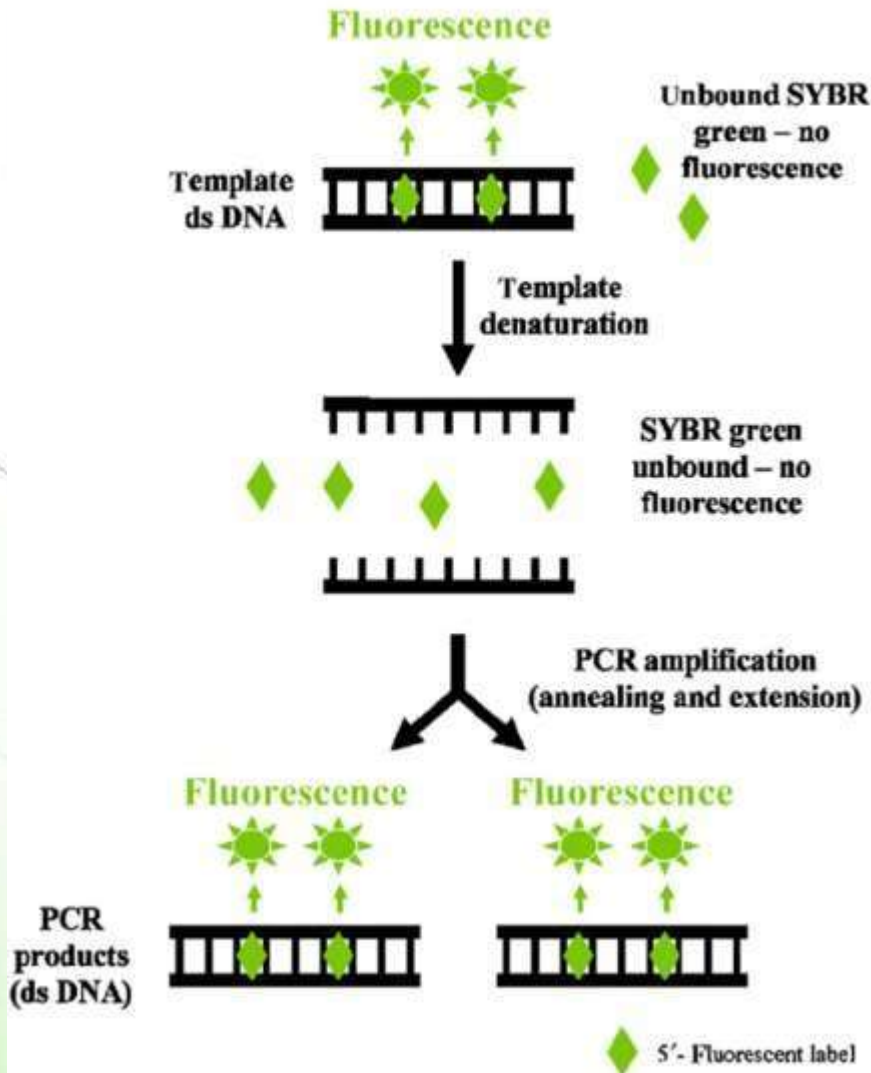
Polymerase chain reaction - PCR



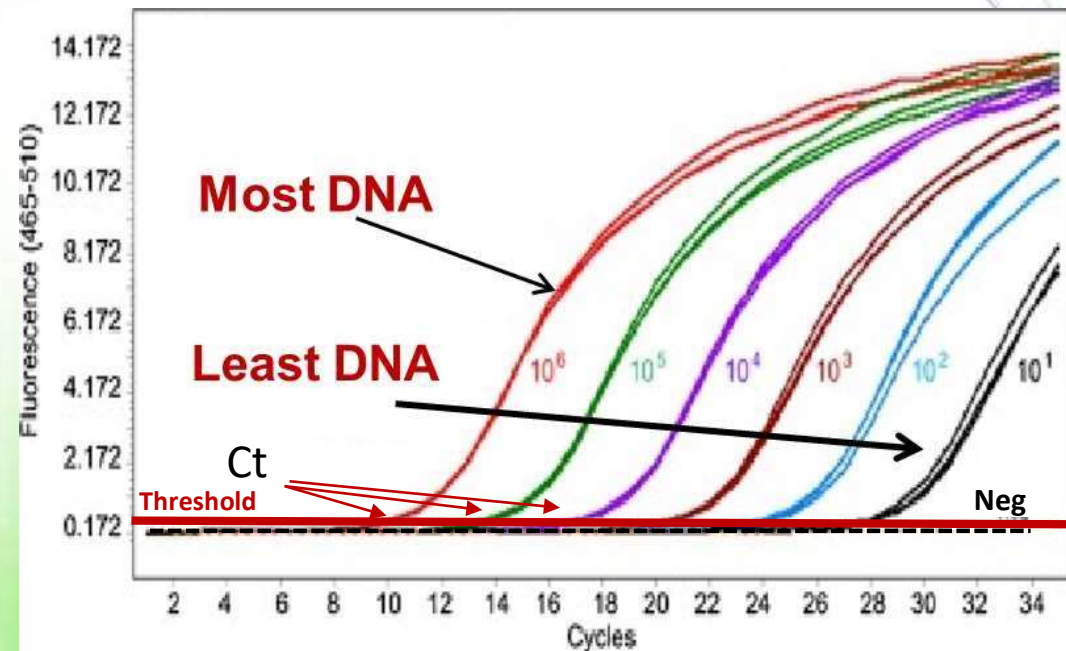
Real-time quantitative PCR (qPCR)



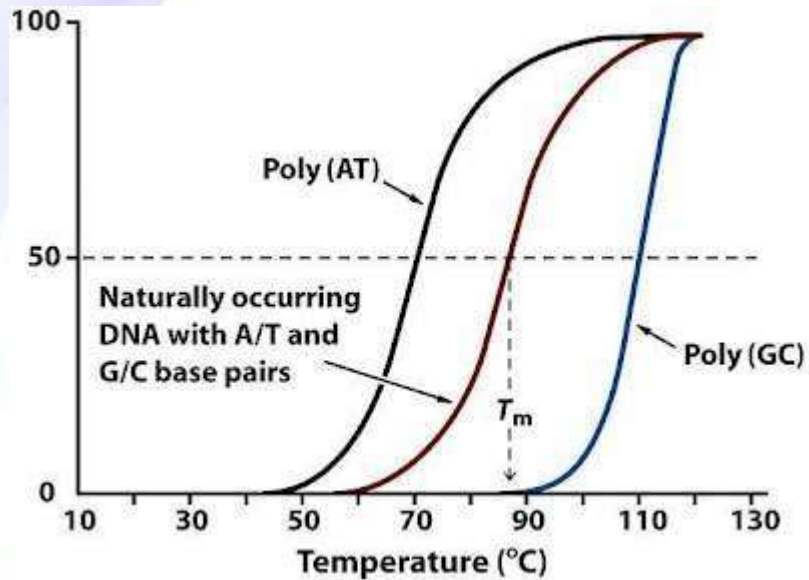
(a) SYBR green assays



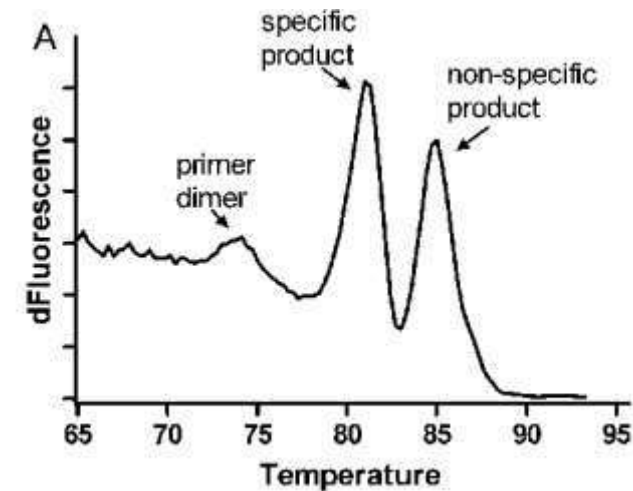
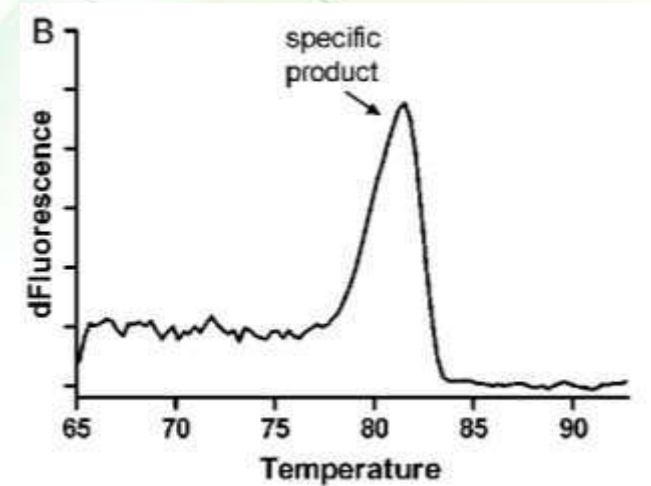
- SYBR green binds to double-stranded DNA and fluoresces only when bound.
- A way of relative quantitation of amount of DNA in a sample is by amplifying it in the presence of SYBR green.
- The higher the amount of DNA, the sooner it is detected.
- Threshold cycle (Ct) tells us at which cycle the signal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

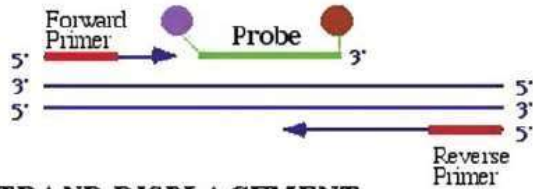


Taqman qPCR

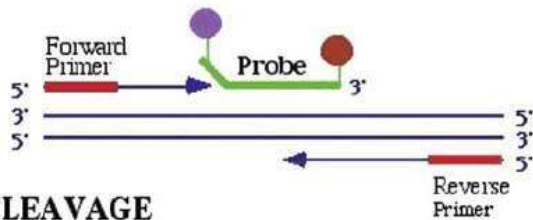


● Reporter
● Quencher

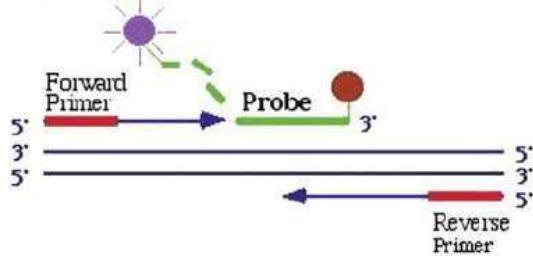
POLYMERIZATION



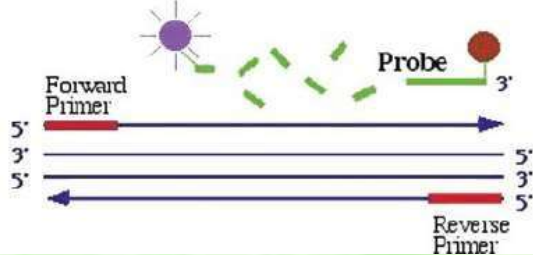
STRAND DISPLACEMENT



CLEAVAGE



POLYMERIZATION COMPLETED



Advantages (versus SYBR chemistry)

- More specific
- More sensitive
- More reproducible
- Multiplexing

