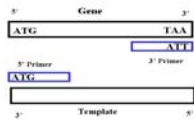


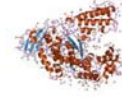
PCR: (Polymerase Chain Reaction)

Amplifies DNA,
generating 1,000's to
millions of copies of a
particular DNA sequence
(gene)

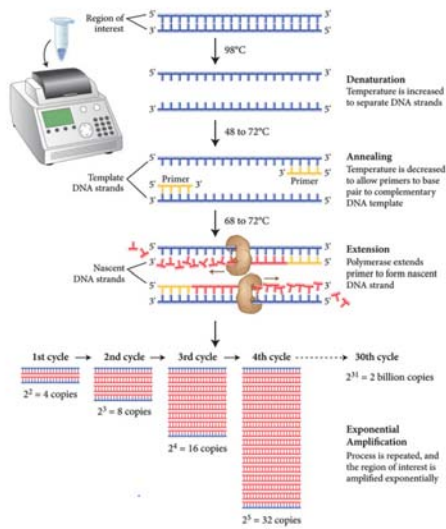
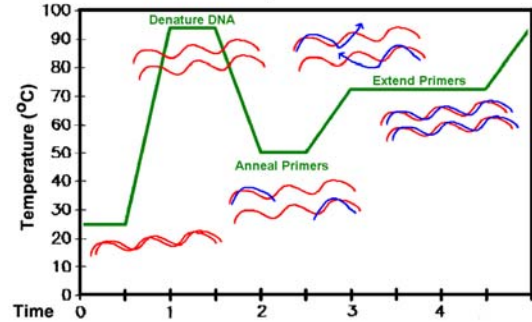
Oligonucleotides = Primers



Taq Polymerase



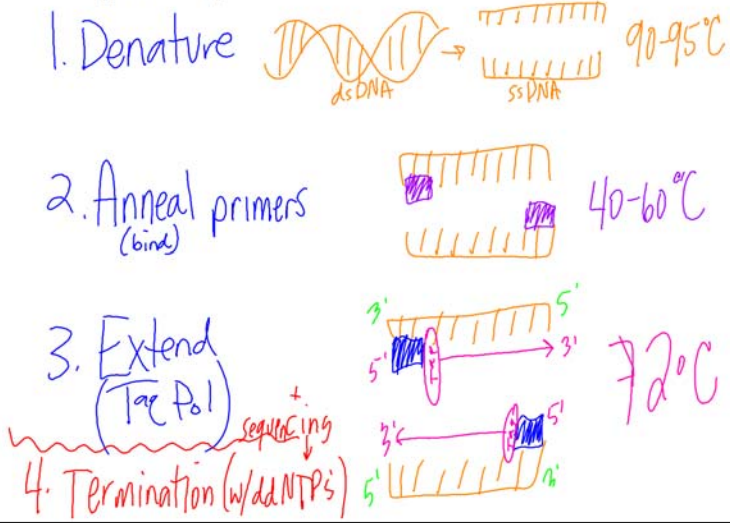
DNA polymerase named after the thermophilic bacterium *Thermus aquaticus* (lives in hot springs & hydrothermal vents)



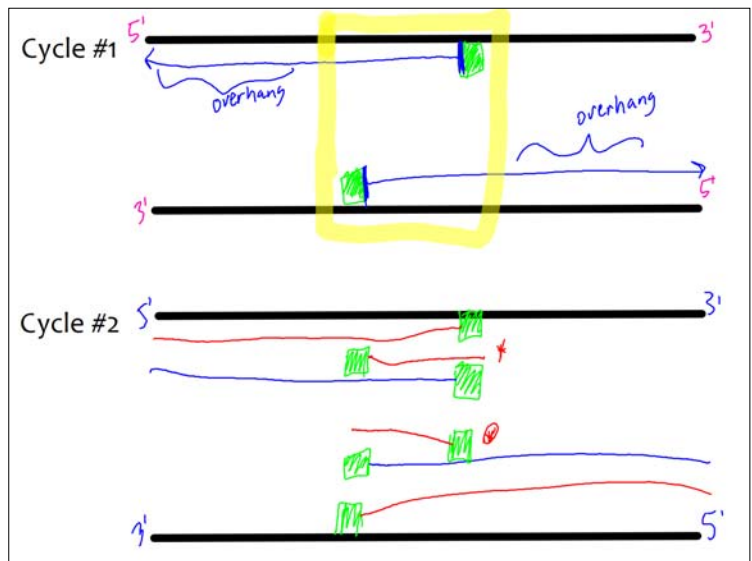
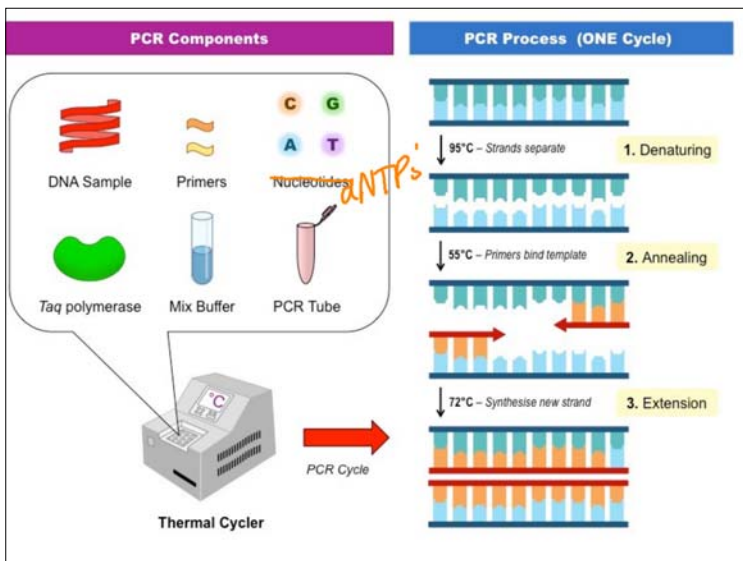
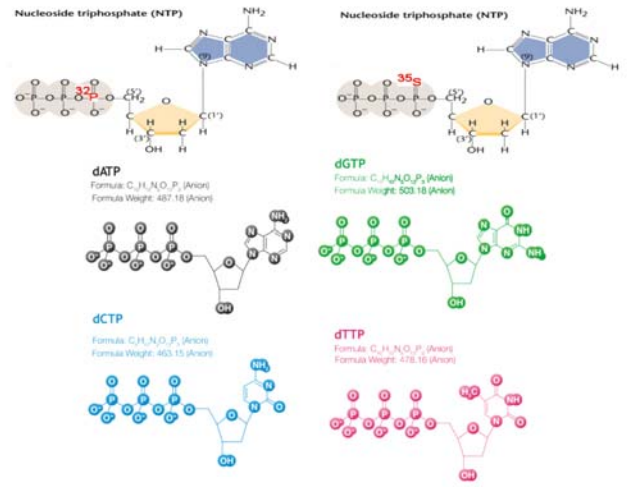
PCR Tools & Ingredients:

- Thermocycler
- DNA sample
- microcentrifuge tubes
- Taq Pol
- primers (oligonucleotides)
 - ← forward
 - ← backward
- dNTP's

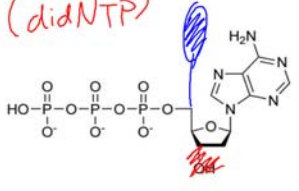
PCR Steps & Temps:



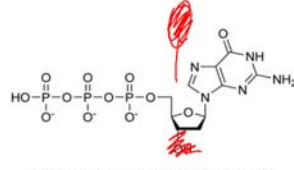
dNTP's = Nucleoside Triphosphate



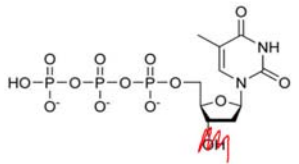
ddNTP's = terminator (lack 3'OH, fluorescent tag)



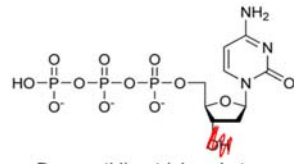
Deoxyadenosine triphosphate (dATP)



Deoxyguanosine triphosphate (dGTP)



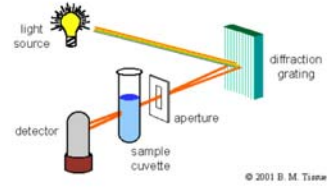
Deoxythymidine triphosphate (dTTP)



Deoxycytidine triphosphate (dCTP)

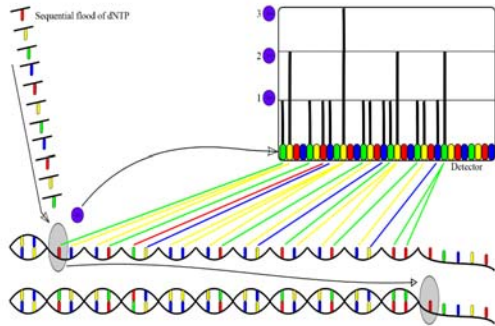
Spectrophotometer:

instrument which measures the amount of light of a specified wavelength which passes through a medium

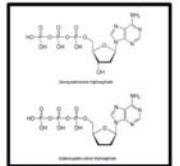
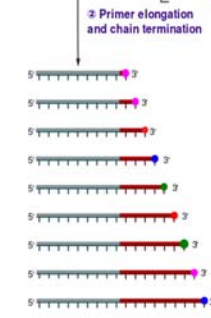
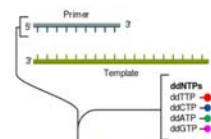


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Cycle Sequencing



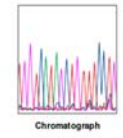
- 1 Reaction mixture
 - Primer and DNA template
 - DNA polymerase
 - ddNTPs with fluorochromes
 - dNTPs (dATP, dCTP, dGTP, and dTTP)



3 Capillary gel electrophoresis separation of DNA fragments



4 Laser detection of fluorochromes and computational sequence analysis



DNA Replication

vs.

PCR

- natural (nucleus) → mitosis
- DNA Pol III (5' → 3')
- Topoisomerase, Helicase, ssbp's
- RNA primers (primase) & DNA Pol I
- Temp: ~99°F (37°C)
- 1 full copy of DNA → 2
- dNTP's only
- Ligase (Okazaki fragments)

- artificial (thermocycler)
- Taq Pol (5' → 3')
- heat
- DNA primer (oligonucleotide)
↳ lab
- 95°C, 40-60°C, 72°C
- 1 full copy of DNA →
1,000,000's of gene
- ddNTP's to seq.
(diagnosis)