Polymerase Chain Reaction

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Introduction

- PCR, polymerase chain reaction, is an in-vitro technique for amplification of a region of DNA whose sequence is known or which lies between two regions of known sequence
- Before PCR, DNA of interest could only be amplified by over-expression in cells and this with limited yield

History

- 1966, Thomas Brock discovers Thermus Aquaticus, a thermostable bacteria in the hot springs of Yellowstone National Park
- 1983, Kary Mullis postulated the concept of PCR (Nobel Prize in 1993)
- 1985, Saiki publishes the first application of PCR (beta-Globin)
- 1985, Cetus Corp. Scientists isolate Thermostable Taq Polymerase (from T.Aquaticus), which revolutionized PCR

Reaction Components

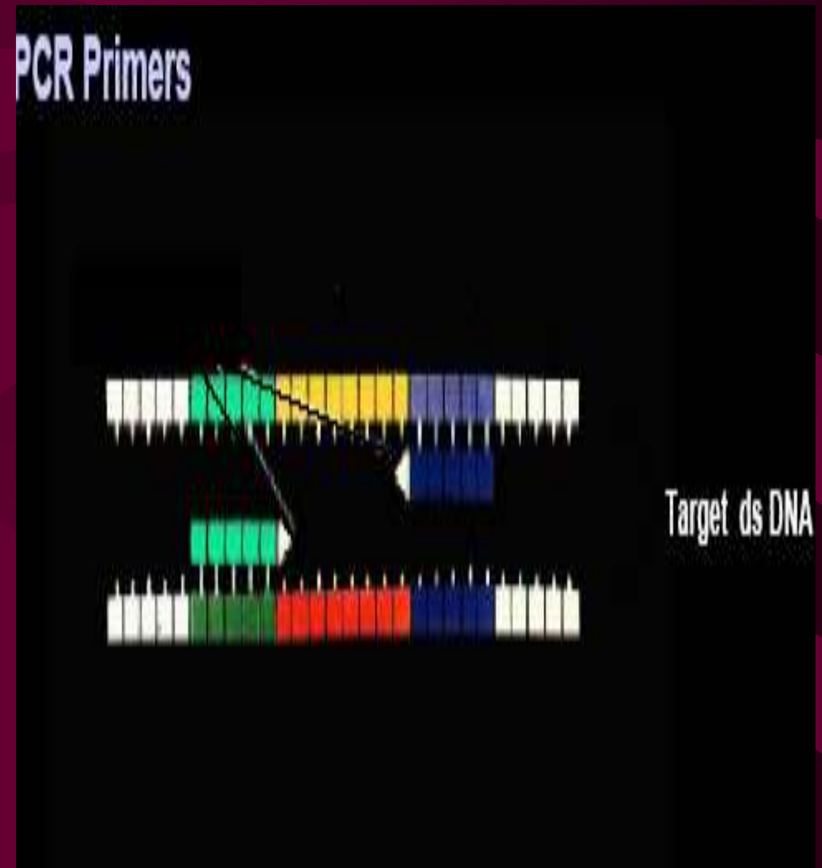
- DNA template
- Primers
- Enzyme
- dNTPs
- Mg^{2+}
- buffers

1- DNA template

- DNA containing region to be sequenced
- Size of target DNA to be amplified : up to 3 Kb

2- Primers

- 2 sets of primers
- Generally 20-30 nucleotides long
- Synthetically produced
- complimentary to the 3’ ends of target DNA
- not complimentary to each other



Primers (ctnd)

- Not containing inverted repeat sequences to avoid formation of internal structures
- 40-60% GC content preferred for better annealing
- Tm of primers can be calculated to determine annealing T^0
- $Tm = .41(\%G+C) + 16.6\log(J^+) + 81.5$ where J^+ is the concentration of monovalent ions

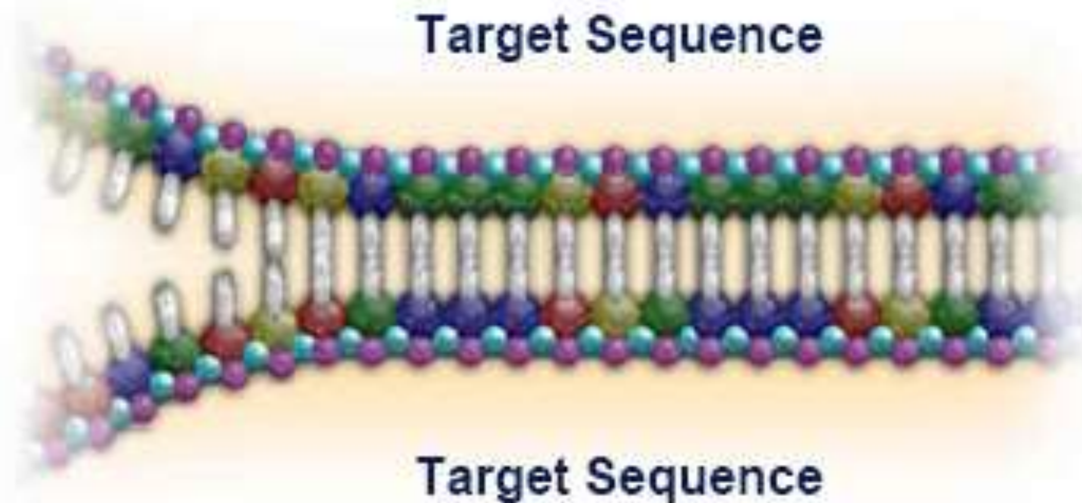
3-Enzyme

- Usually Taq Polymerase or anyone of the natural or Recombinant thermostable polymerases
- Stable at T^0 up to 95^0 C
- High processivity
- Taq Pol has 5’-3’ exo only, no proofreading

The PCR Cycle

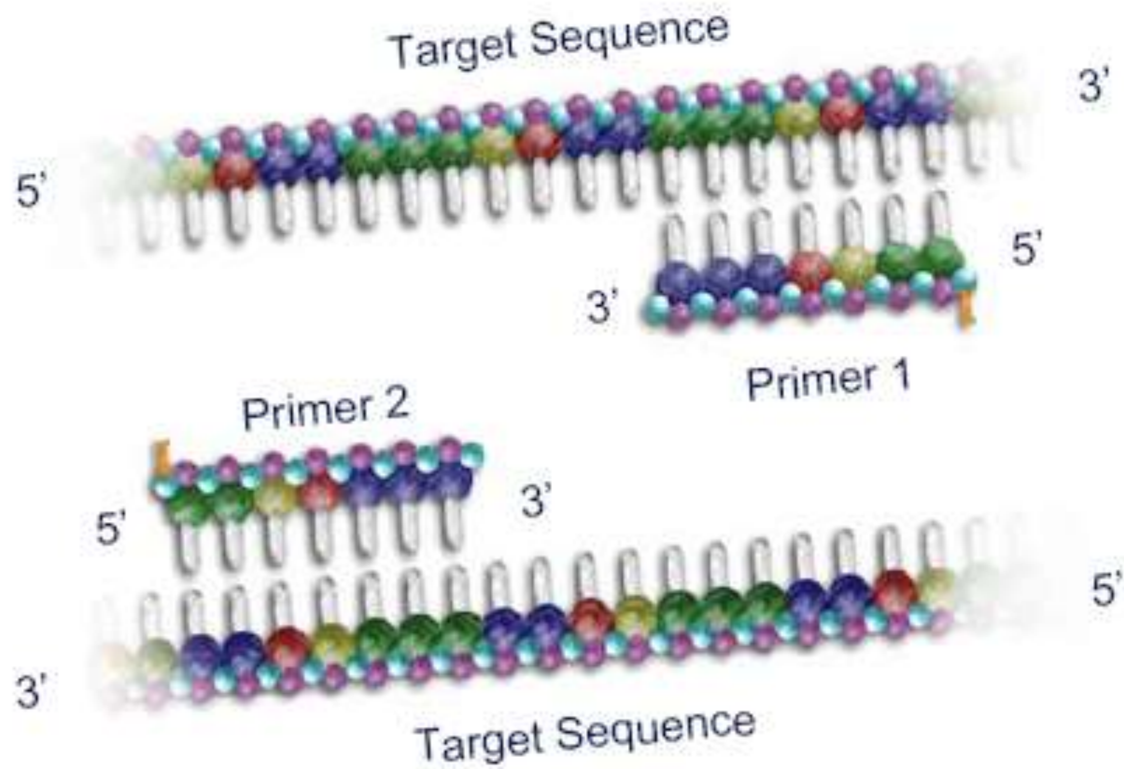
- Comprised of 3 steps:
  - Denaturation of DNA at 95^0C
  - Primer hybridization (annealing) at 40-50^0C
  - DNA synthesis (Primer extension) at 72^0C

PCR Cycle - Step 1 - Denaturation Template DNA by Heat (95°C)



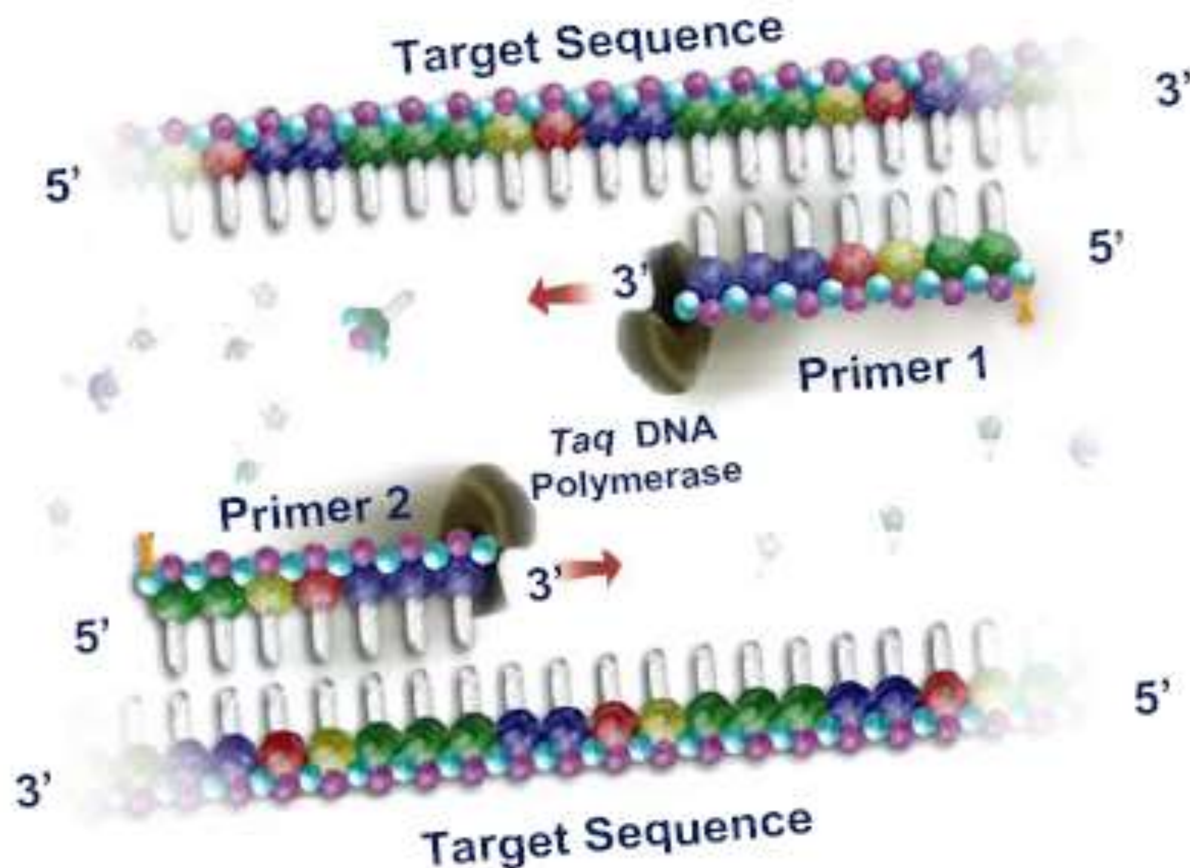
PCR Cycle - Step 2 –

Temperature is lowered (T_m) and primers anneal to target sequences



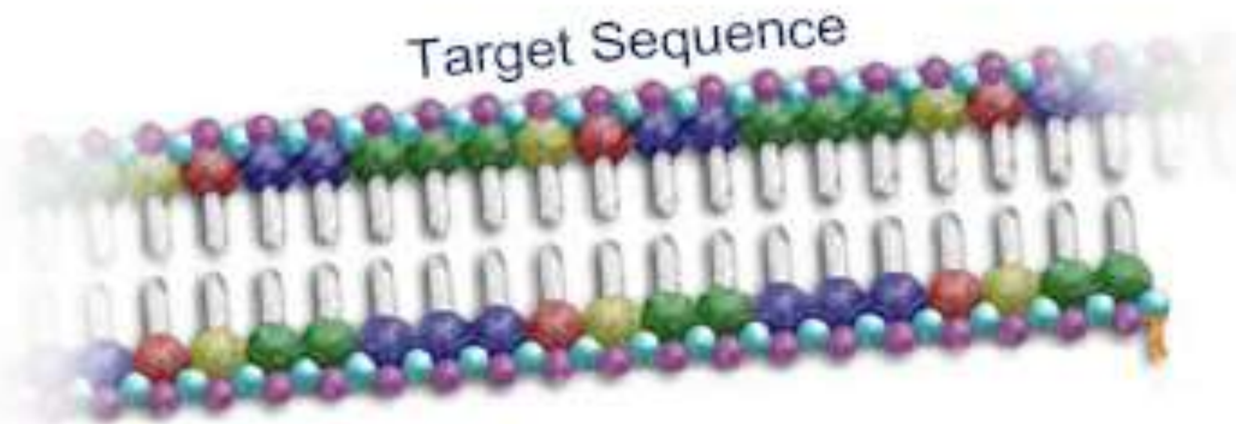
PCR Cycle - Step 3 -

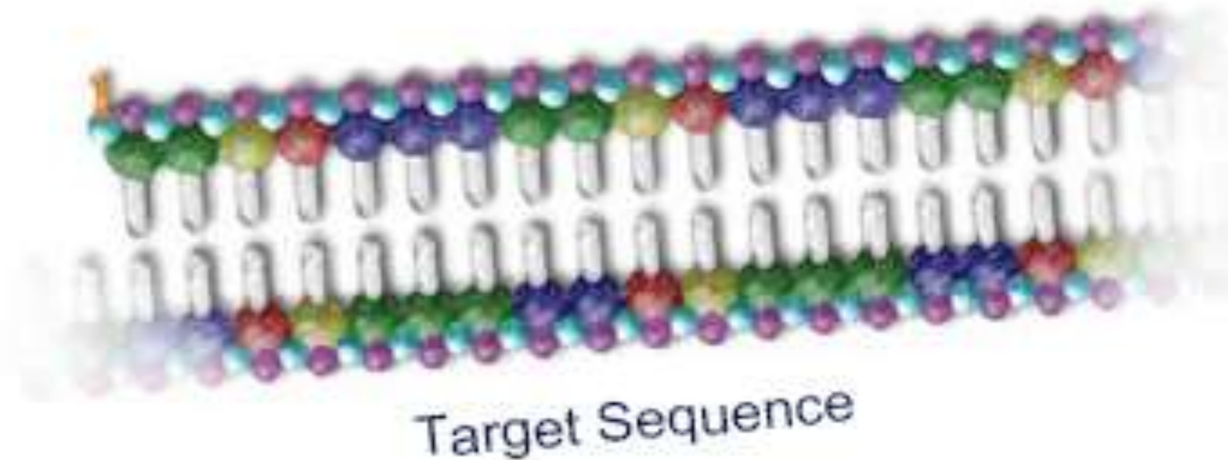
At 72 °C *Taq* DNA polymerase catalyses primer extensio[n] as complementary nucleotides are incorporated



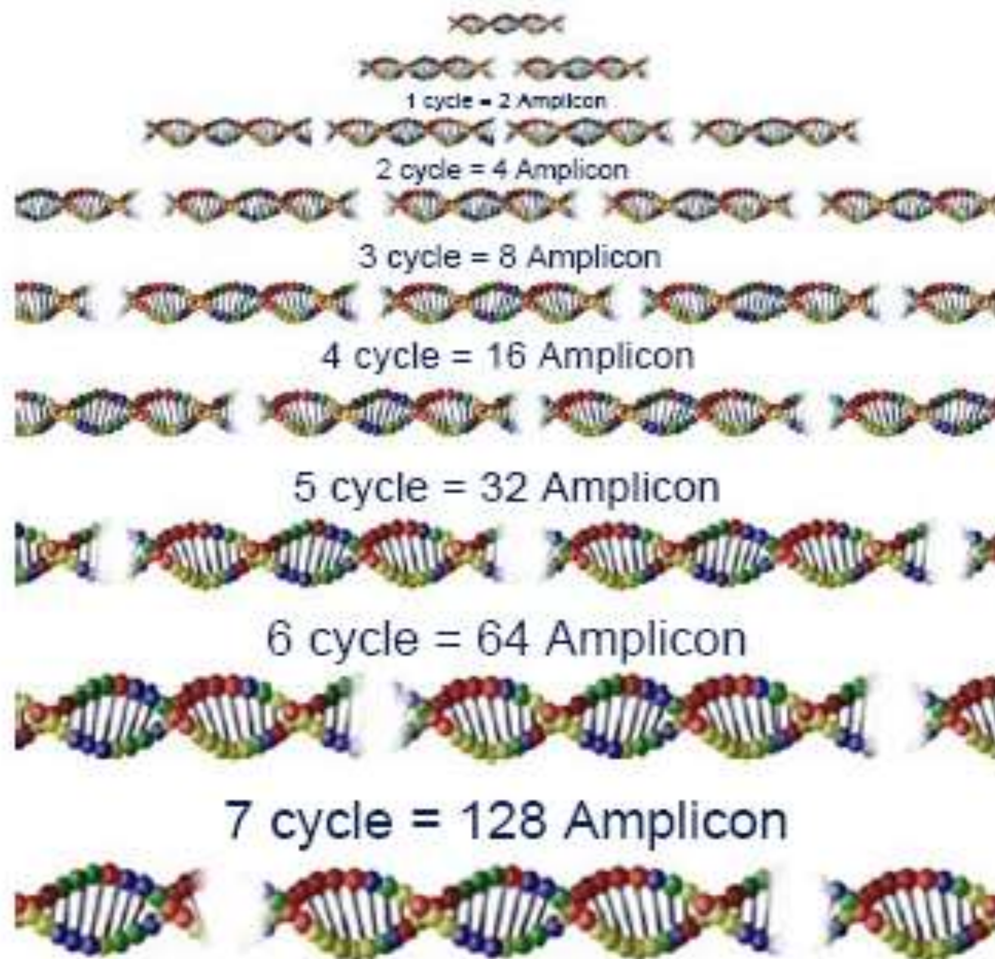
End of the 1st PCR Cycle –

Results in two copies of target sequence



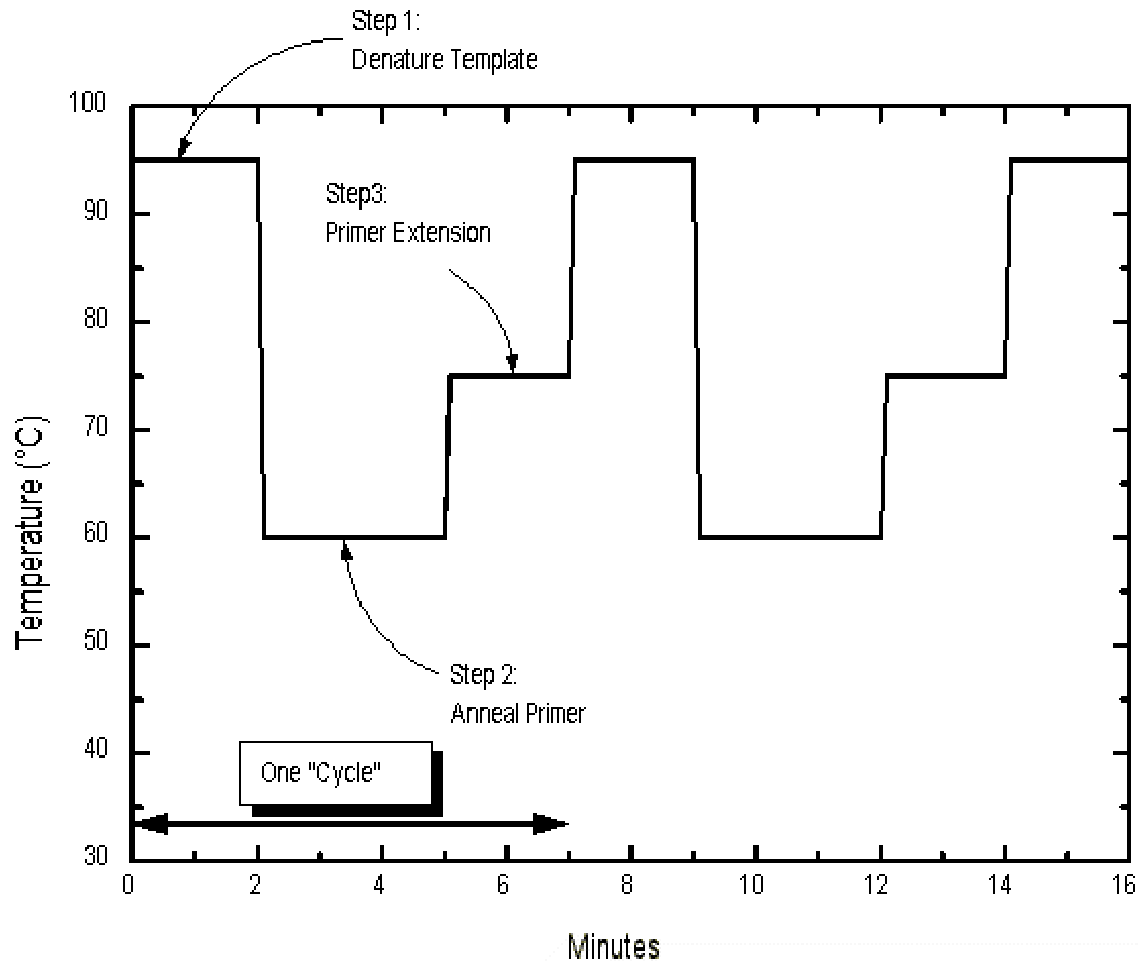


Target Amplification



No. of Cycles	No. Amplicon Copies of Target
1	2
2	4
3	8
4	16
5	32
6	64
20	1,048,576
30	1,073,741,824

Standard thermocycle

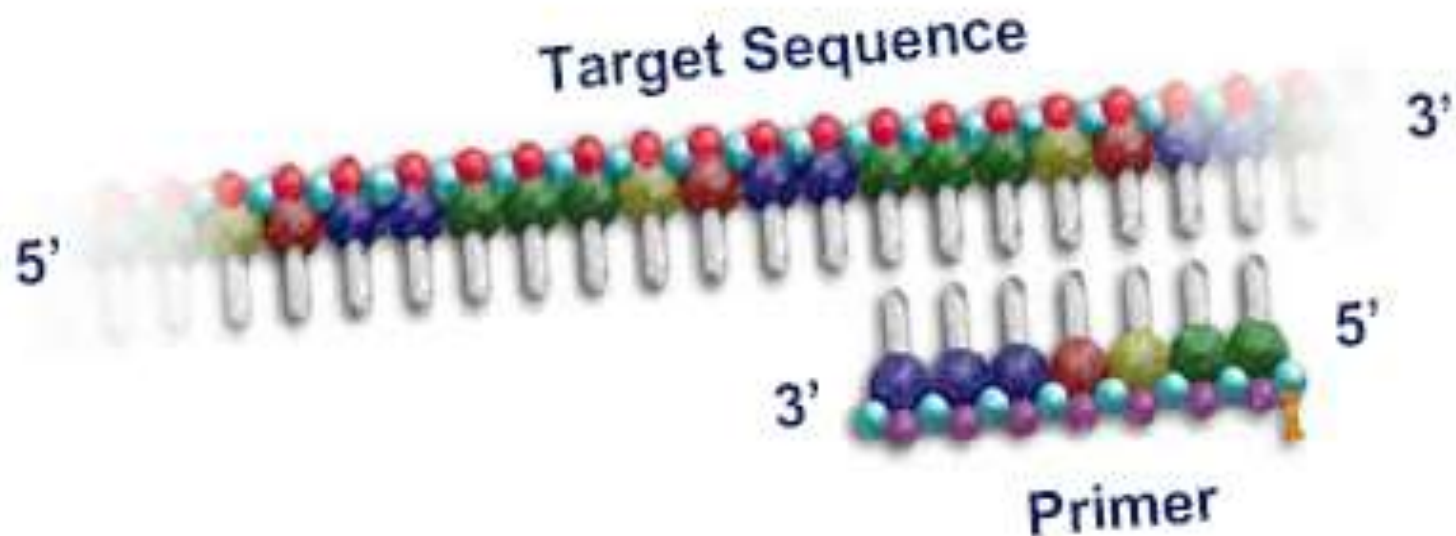


RT-PCR

- Reverse Transcriptase PCR
- Uses RNA as the initial template
- RNA-directed DNA polymerase (rTh)
- Yields ds cDNA

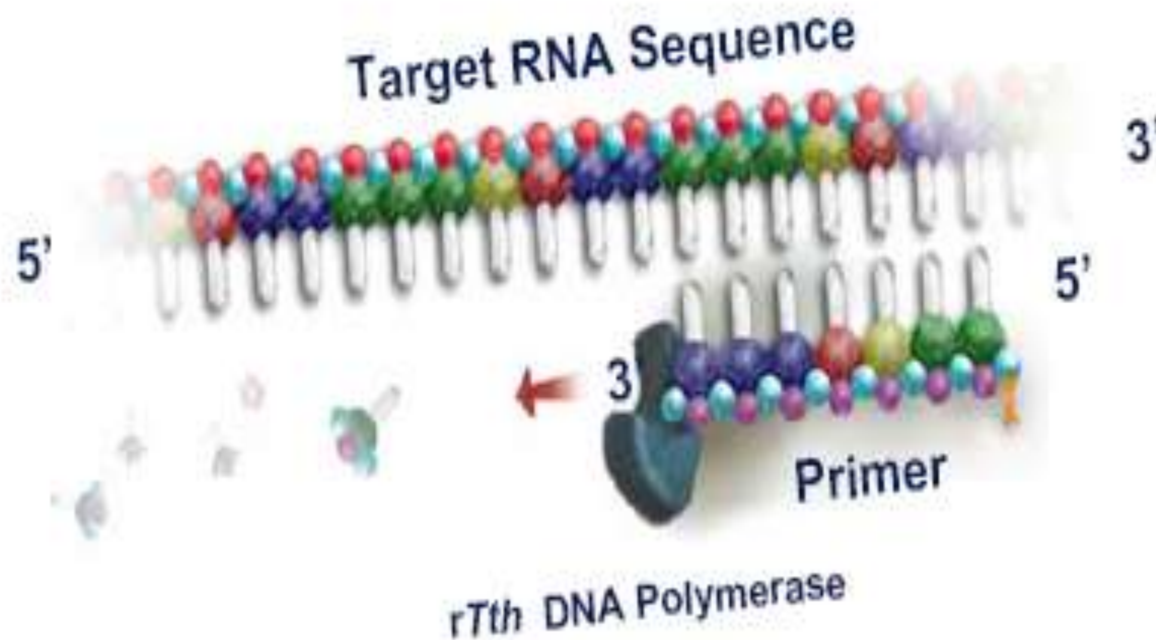
Reverse Transcription - Step 1 –

Primer Anneals to Target RNA Sequence

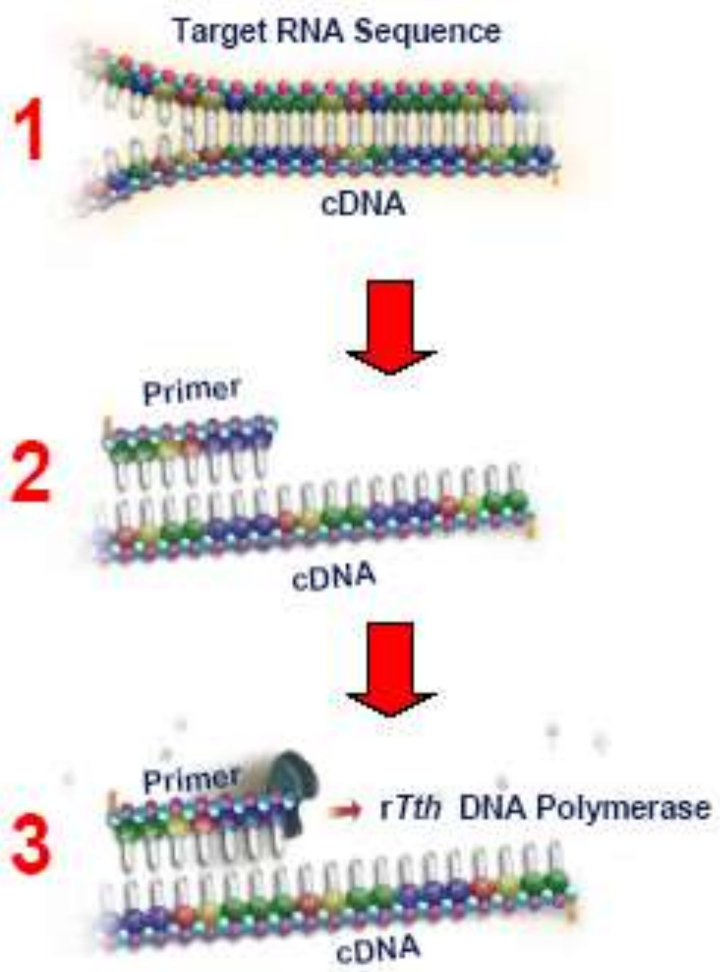


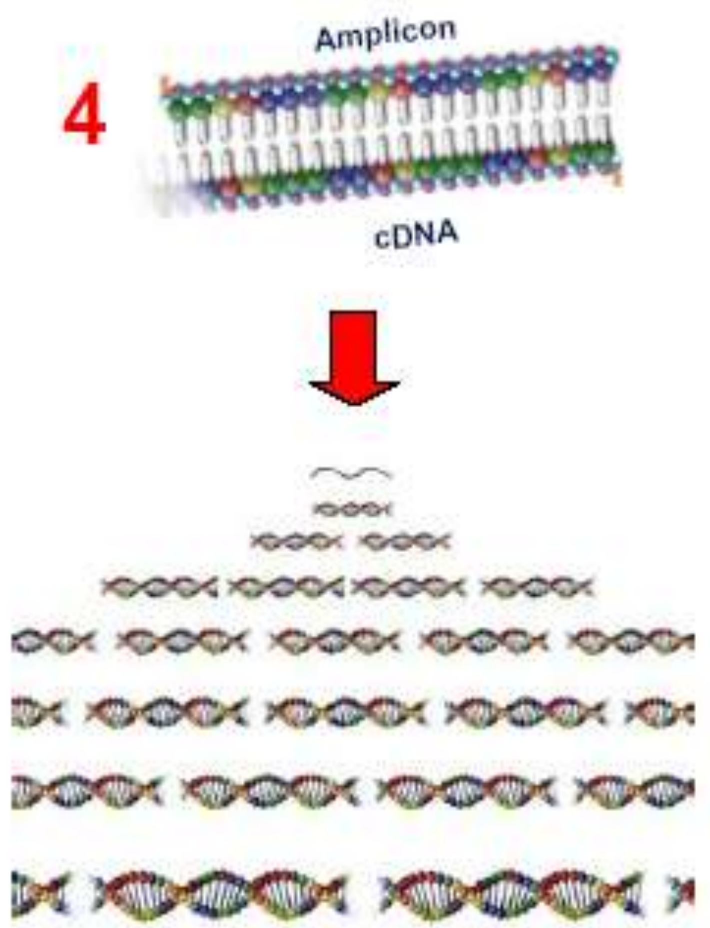
Reverse Transcription - Step 2 –

r*Tth* DNA Polymerase also has RT activity Catalyses Primer Extension by Incorporating Complementary Nucleotides



PCR Step 1 -	Denaturation by Heat
PCR Step 2 -	Annealing of Primer to cDNA
PCR Step 3 -	r*Tth* DNA Polymerase Catalyses Primer Extension
End of 1st PCR Cycle -	Yields a Double-Stranded DNA Copy (Amplicon) of the Target Sequence





Detection of amplification products

- Gel electrophoresis
- Sequencing of amplified fragment
- Southern blot
- etc...

Applications

- Genome mapping and gene function determination
- Biodiversity studies (e.g. evolution studies)
- Diagnostics (prenatal testing of genetic diseases, early detection of cancer, viral infections...)
- Detection of drug resistance genes
- Forensic (DNA fingerprinting)

Advantages

- Automated, fast, reliable (reproducible) results
- Contained :(less chances of contamination)
- High output
- Sensitive
- Broad uses
- Defined, easy to follow protocols

References

- Fundamentals of Biochem (Voet, Voet, Pratt)
- Molecular Cell Biology (Lodish, Darnell..)

Next Steps

- Summarize any actions required of your audience
- Summarize any follow up action items required of you

THANKS