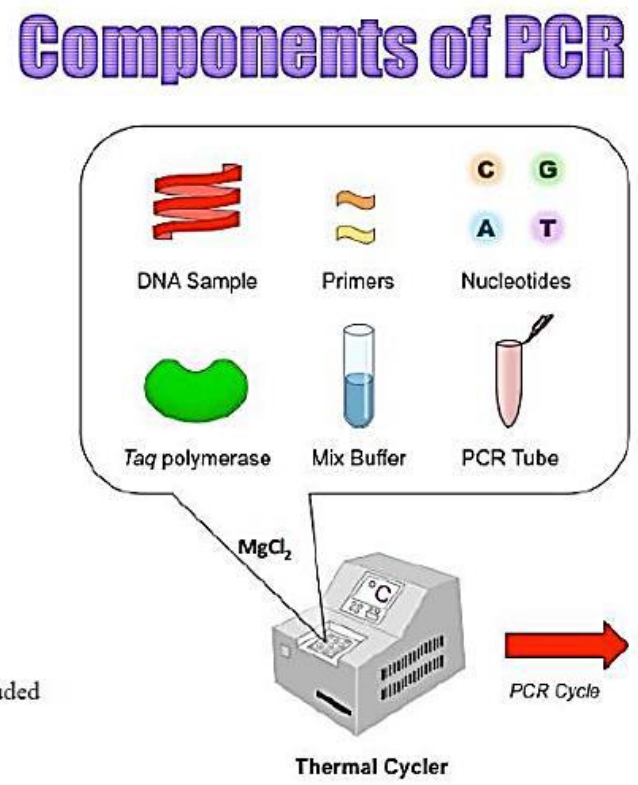
Diagnosis of Viral infections by PCR

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.

Reaction Components

- DNA template
- Primers
- Enzyme
- dNTPs
- Mg2+
- buffers



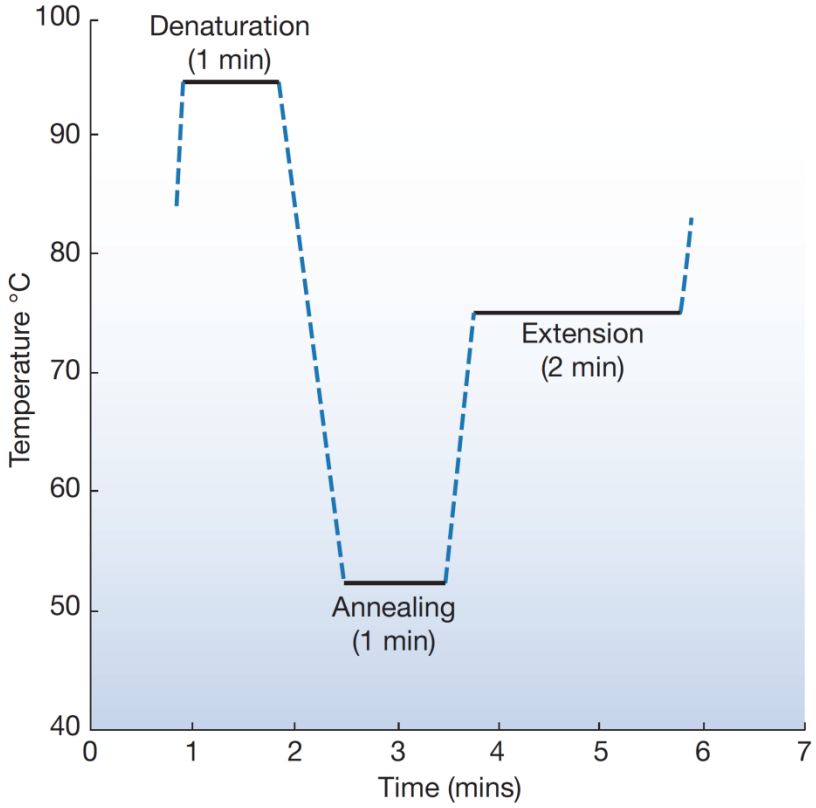
Additional reagents may included

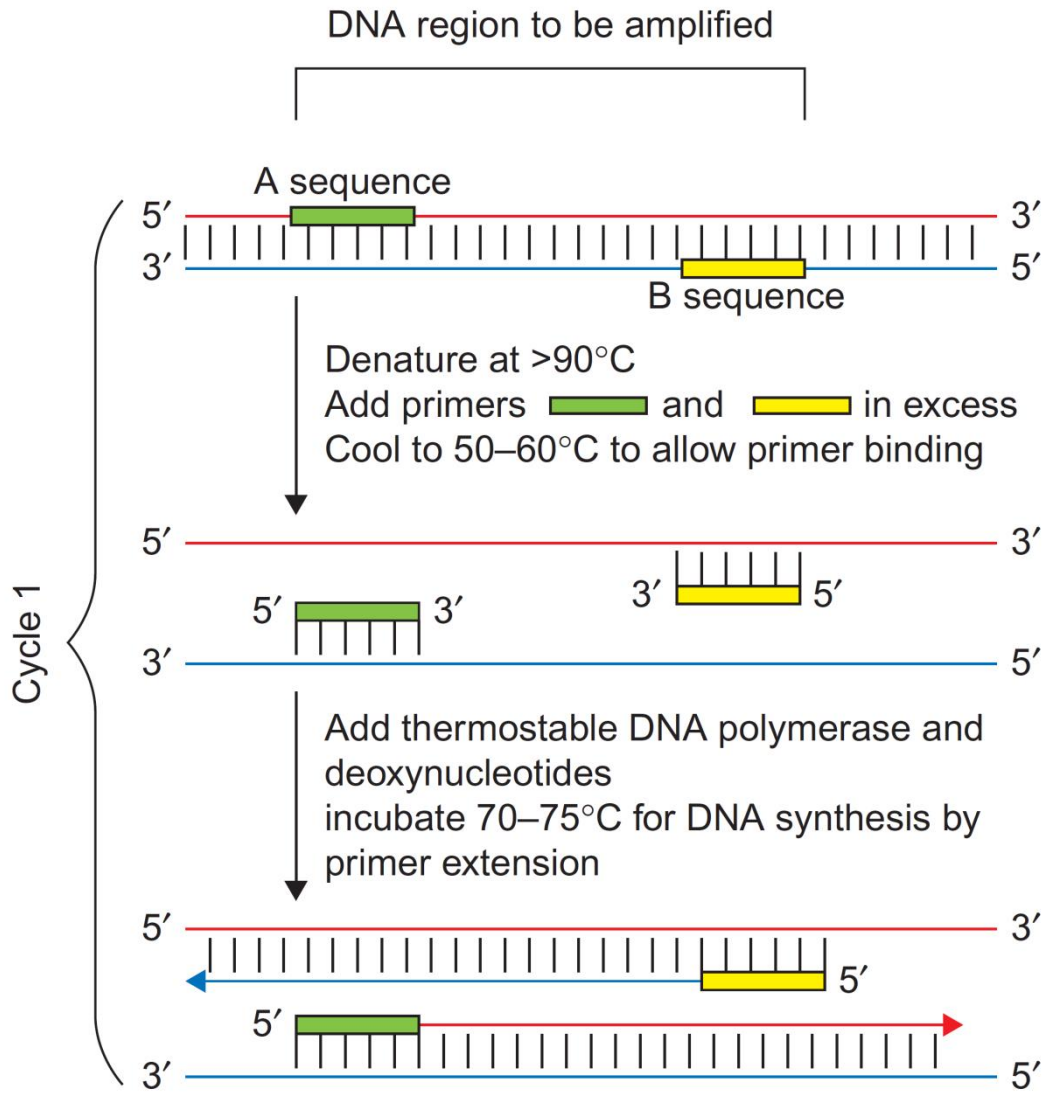
The PCR Cycle comprised of 3 steps:

-Denaturation of DNA at 95 ^{o}C

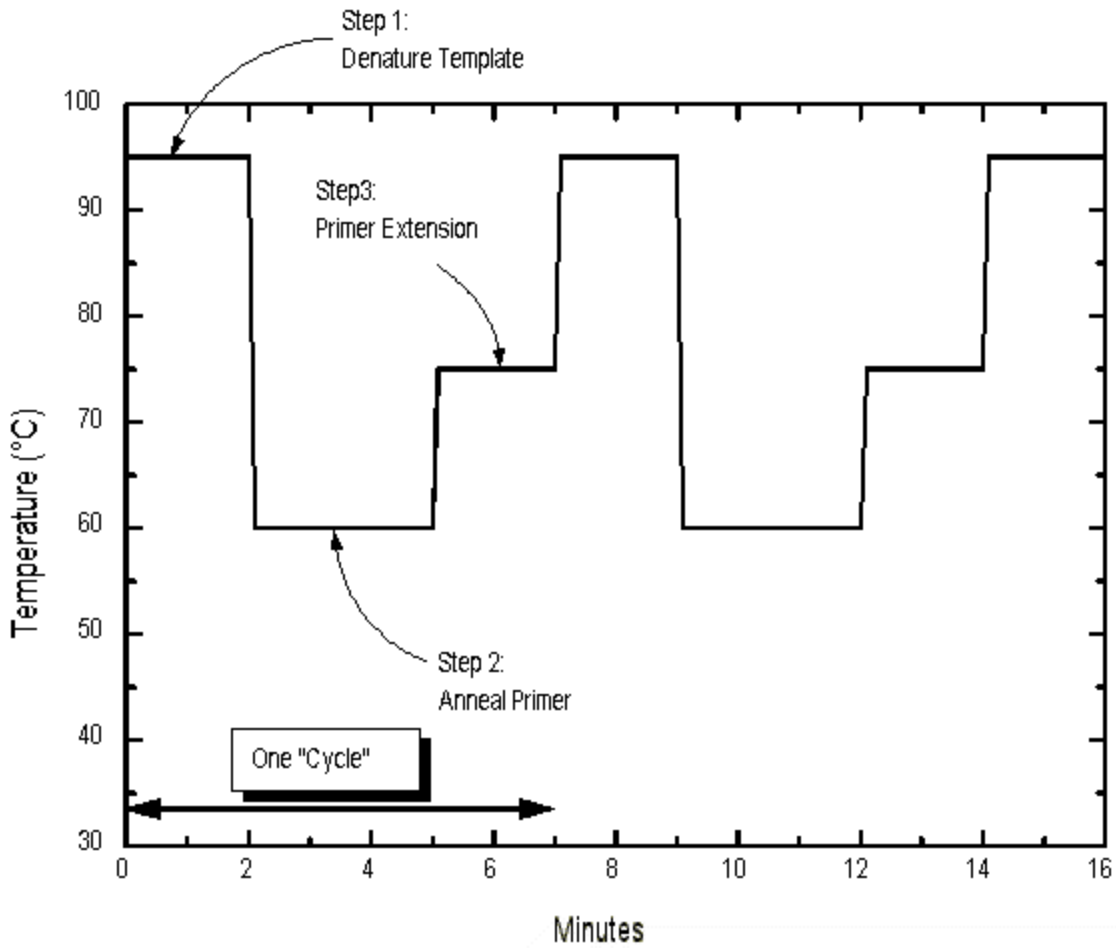
-Primer hybridization (annealing) at 40-50 ^{o}C

-DNA synthesis (Primer extension) at 72 ^{o}C



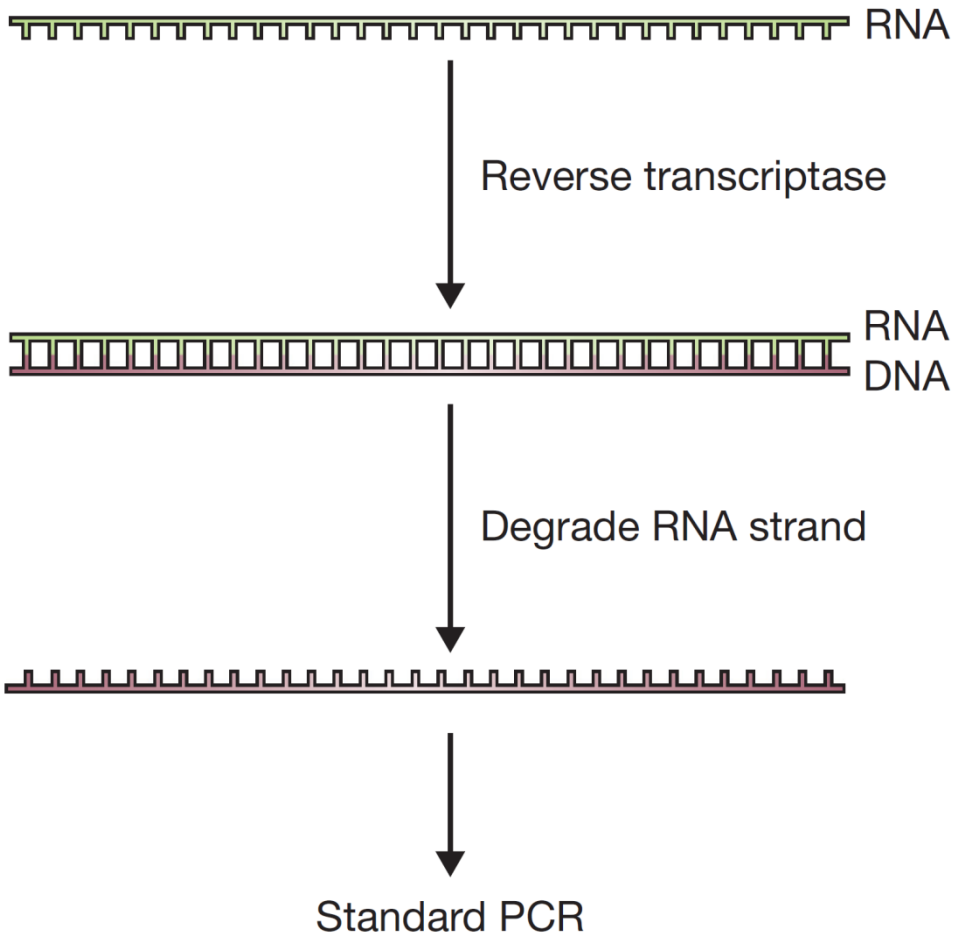

DNA region to be amplified
A sequence
5′
3′
3′
5′
B sequence
Denature at >90°C
Add primers and in excess
Cool to 50–60°C to allow primer binding
Cycle 1
Add thermostable DNA polymerase and deoxynucleotides
incubate 70–75°C for DNA synthesis by primer extension

Standard thermocycler



RT-PCR

- Reverse Transcriptase PCR
- Uses RNA as the initial template (*RNA virus, mRNA transcripts*)
- RNA-directed DNA polymerase (*Reverse transcriptase*)
- Yields ds cDNA


RNA
Reverse transcriptase
RNA
DNA
Degrade RNA strand
Standard PCR

Detection of amplification products

- Gel electrophoresis
- Sequencing of amplified fragment