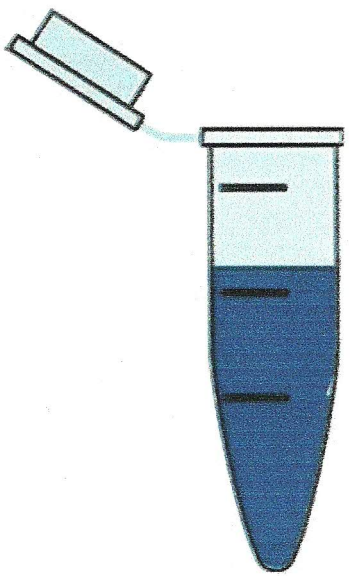
POLYMERASE CHAIN REACTION (PCR)

- the most common DNA amplification method in molecular biology
- developed in 1983 by Kary Mullis
- relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA
- enables to produce millions of copies of a specific DNA sequence in approximately two hours
- used in medical and biological research labs for a variety of applications:
  - DNA cloning for sequencing,
  - DNA-based phylogeny,
  - functional analysis of genes,
  - diagnosis of hereditary diseases,
  - identification of genetic fingerprints (used in forensic sciences and paternity testing)
  - detection and diagnosis of infectious diseases

PCR REACTION COMPONENTS



- DNA template (isolated genomic DNA)
- DNA polymerase
- primers (forward and reverse)
- deoxynucleotide triphosphates (dNTPs) mixture
- PCR buffer
- $MgCl_2$
- nuclease-free water

DNA template

the sample DNA that contains the target sequence. At the beginning of the reaction, high temperature is applied to the original double-stranded DNA molecule to separate the strands from each other.

DNA polymerase

a type of enzyme that synthesizes new strands of DNA complementary to the target sequence. The first and most commonly used of these enzymes is *Taq* DNA polymerase (from *Thermis aquaticus*), whereas *Pfu* DNA polymerase (from *Pyrococcus furiosus*) is used widely because of its higher fidelity when copying DNA. Although these enzymes are subtly different, they both have two capabilities that make them suitable for PCR: 1) they can generate new strands of DNA using a DNA template and primers, and 2) they are heat resistant.

Primers

short pieces of single-stranded DNA that are complementary to the target sequence. The polymerase begins synthesizing new DNA from the end of the primer.

Nucleotides (dNTPs or deoxynucleotide triphosphates)

single units of the bases A, T, G, and C, which are essentially "building blocks" for new DNA strands.

(http://www.ncbi.nlm.nih.gov/probe/docs/techpcr/)

PCR PROCEDURE

- initial denaturation (94–96°C, 1-9 min.)

- a series of 20–40 cycles consisting of:

  - **DENATURATION**

  Double-stranded DNA is heated to 94°C-98°C for 20–30 seconds. During this period, the double-stranded DNA template helix melts open into two single-stranded templates (by disrupting the hydrogen bonds between complementary bases).

  - **ANNEALING**

  The reaction is cooled to 50–65°C for 20–40 seconds. Sequence-specific primers anneal to the single-stranded DNA template. During this step, DNA polymerase attaches to the primer-template hybrid and begins to incorporate complementary nucleotides.

  - **EXTENTION/ELONGATION**

  The temperature is raised slightly to 65°C-75°C. The optimal temperature for Taq DNA polymerase is 72 °C. During this phase, DNA polymerase extends the sequence-specific primer with the incorporation of nucleotides that are complementary to the DNA template.

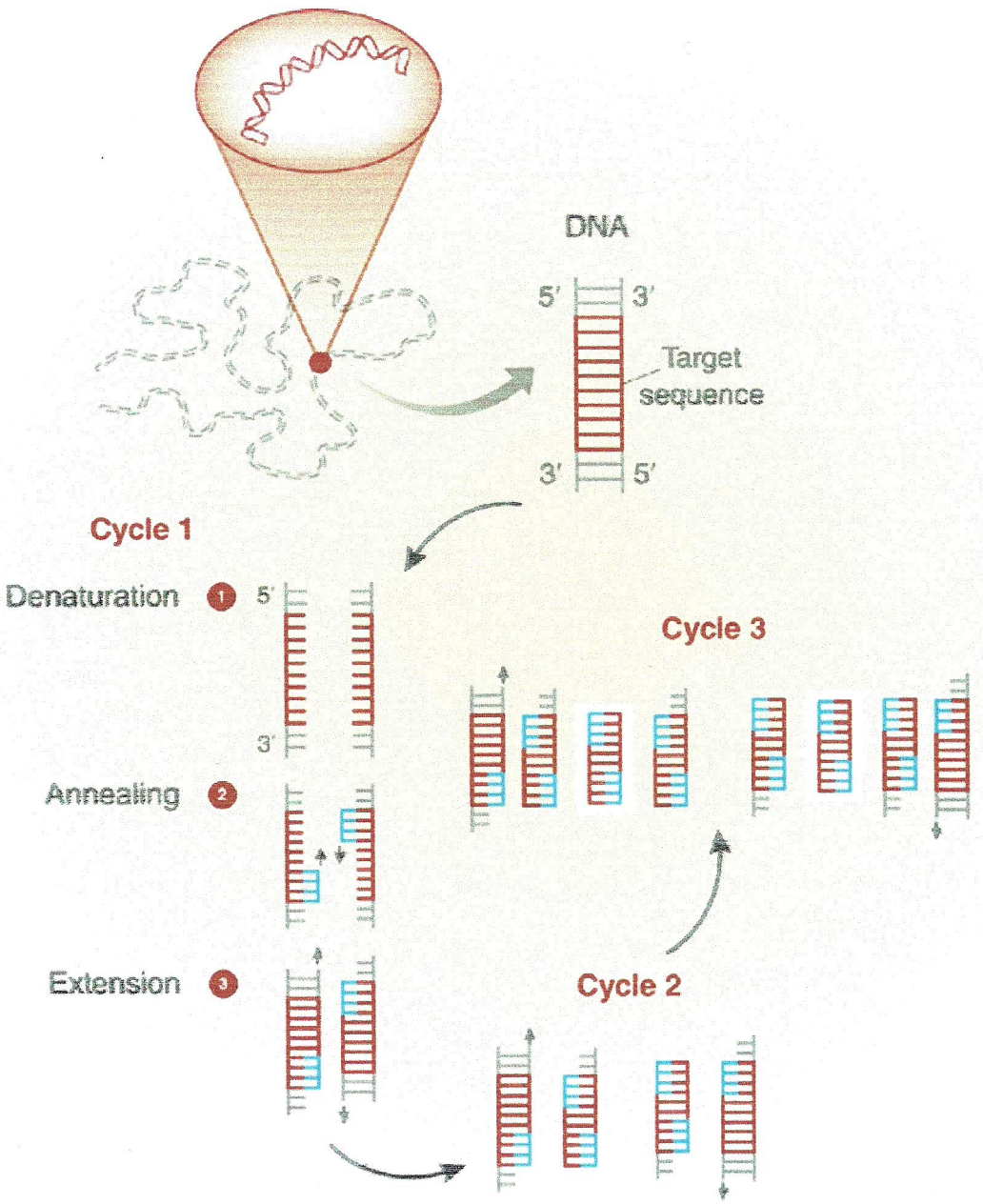
- final elongation (70–74°C)

  This step ensures that any remaining single-stranded DNA is fully extended.

- final hold (4–15°C)

  This step at may be employed for short-term storage of the reaction.

PCR PROCEDURE

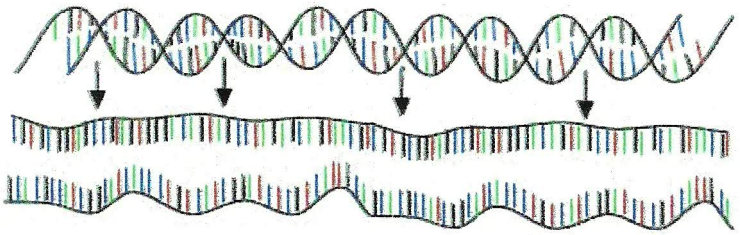


Schematic presentation of the polymerase chain reaction principle

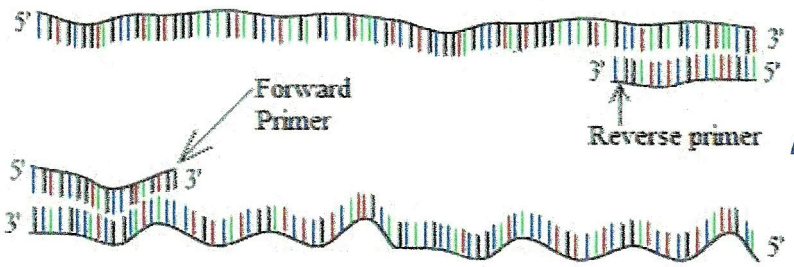
(http://www.nature.com/jid/journal/v133/n3/fig_tab/jid20131f1.html#figure-title)

PCR PROCEDURE

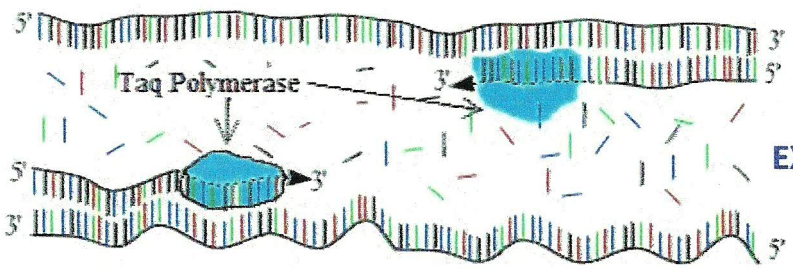
A SINGLE CYCLE OF PCR



DENATURATION



ANNEALING



EXTENTION/ELONGATION

(http://en.wikibooks.org/wiki/Structural_Biochemistry/Polyermase_Chain_Reaction/How_PCR_is_Performed; modified)