King Saud University
College of Science
Biochemistry Department

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PCR

INTRODCTION:

- Polymerase chain reaction was invented by **Kary Mullis**. He was awarded the **Noble Prize in Chemistry in 1993-1994** for his invention.

- Polymerase chain reaction (PCR) is a molecular biology technique for enzymatically replicating DNA without using a living organism, such as E.coli or Yeast.

INTRODCTION:

- The name, polymerase chain reaction, comes from the **DNA polymerase** used to amplify (replicate many times) a piece of DNA by *in vitro* enzymatic replication.

- PCR is used to amplify specific regions of a DNA strand. This can be a single gene, just a part of a gene, or non-coding sequence.

Principle of the PCR:

PCR, as currently practiced, requires several basic components. These components are:

- **DNA template**, which contains the region of the DNA fragment to be amplified .

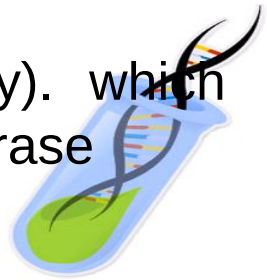
- **Oligonucleotide primers**, Two primers (one for each strand) are generally used. which determine the beginning and end of the region to be amplified

Principle of the PCR:

- **Thermostable DNA polymerase** , These polymerases can tolerate the high temperatures (94 ° or higher) needed to melt (denature) double-stranded DNA. such as Taq polymerase , an enzyme originally isolated from the bacterium Thermus aquaticus. This DNA polymerase enzymatically assembles a new DNA strand from DNA building blocks,

- **Deoxnucleotide tri-phosphate** , (dNTPs). A mixture of all four nucleotides (dATP, dCTP, dGTP, and dTTP) is provided. from which the DNA Polymerase builds the new DNA

- **Buffer**, contain Mg2+ (required for polymerase activity). which provides a suitable chemical environment for the DNA Polymerase



DNA template

Buffer Mg2+

Taq polymerase

primers

5”GGCTGATGCATG3”

5”CGTATTATCGTAT3”

dNTPs

D H2O

The PCR is commonly carried out in a reaction volume of 15-100 µl in small reaction tubes (0.2-0.5 ml volumes) in a thermal cycler.

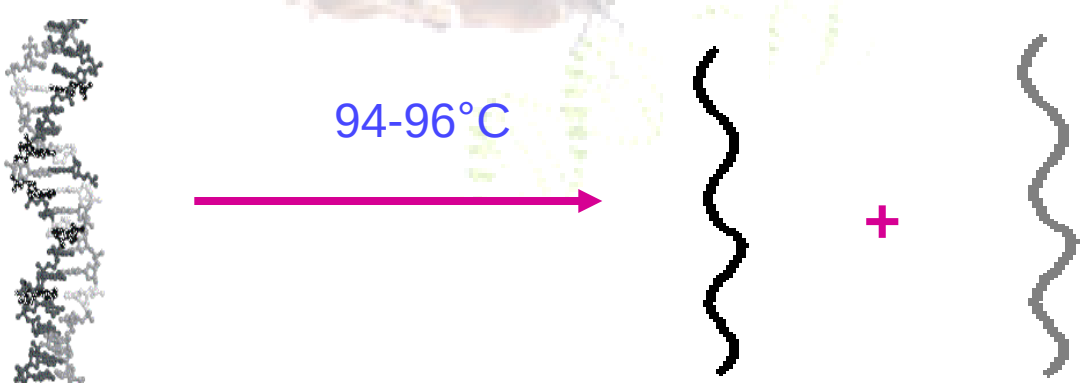
The thermal cycler allows heating and cooling of the reaction tubes to control the temperature required at each reaction step

Denaturation step :

The double-stranded DNA has to be heated to 94-96°C (or 98°C if extremely thermostable polymerases are used) in order to separate the strands.

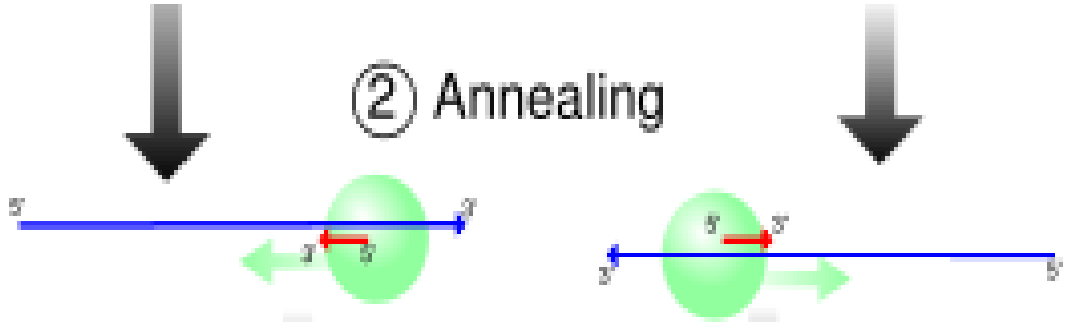
it breaks apart the hydrogen bonds that connect the two DNA strands.

Time: usually 1-2 minutes, but up to 5 minutes. Also certain polymerases are activated at this step.



Annealing step:

The reaction temperature is lowered to 50-65°C for 20-40 seconds allowing annealing of the primers to the single-stranded DNA template.
Typically the annealing temperature is about 3-5 degrees Celsius below the Tm of the primers used.
Stable DNA-DNA hydrogen bonds are only formed when the primer sequence very closely matches the template sequence.
The polymerase binds to the primer-template hybrid and begins DNA synthesis .

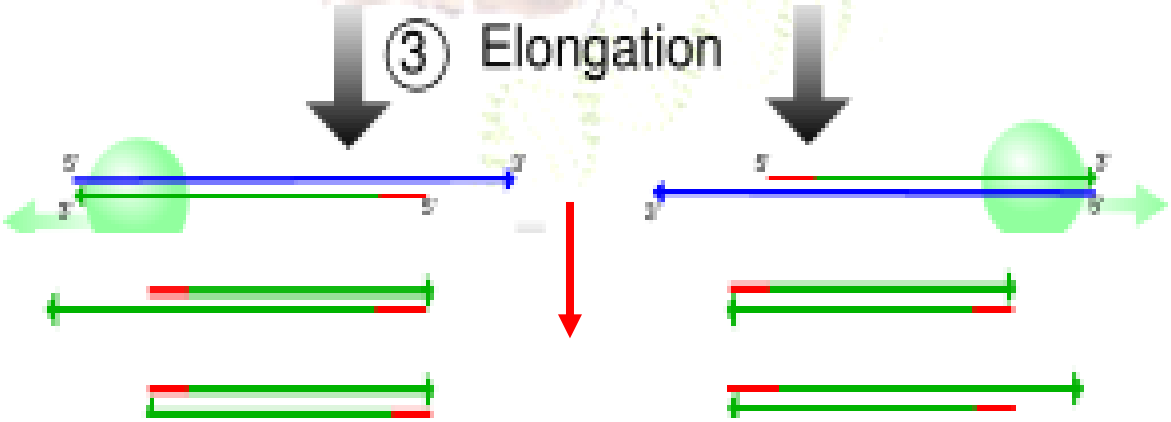


Extension/elongation step :

The temperature at this step depends on the DNA polymerase used ; Taq polymerase has its optimum activity temperature at 75-80°C.

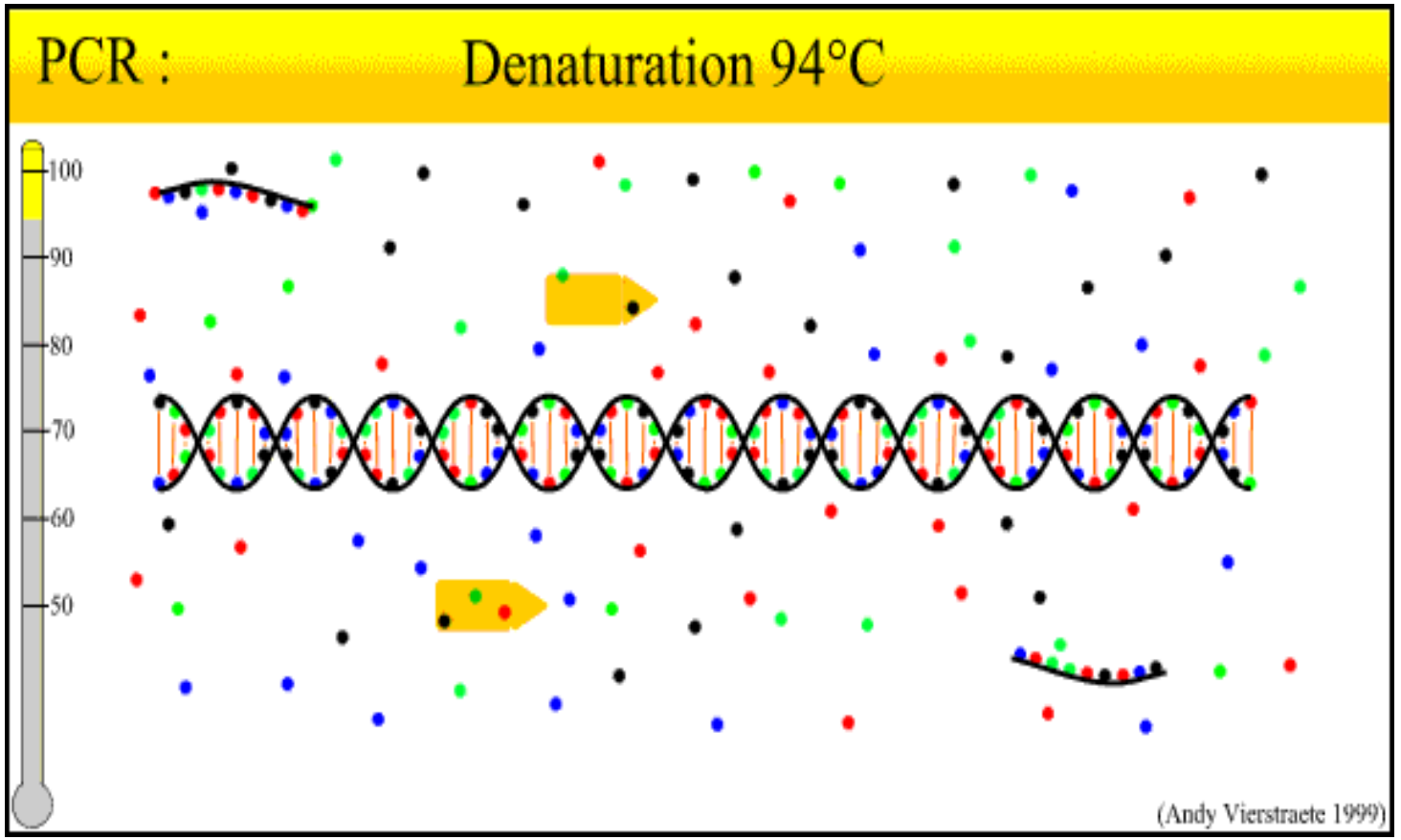
commonly a temperature of 72°C is used with this enzyme. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTP's that are complementary to the template in 5' to 3' direction.

The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified .



Final elongation :

This single step is occasionally performed at a temperature of 70-74°C for 5-15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended .


PCR :
Denaturation 94°C
100
90
80
70
60
50
(Andy Vierstraete 1999)