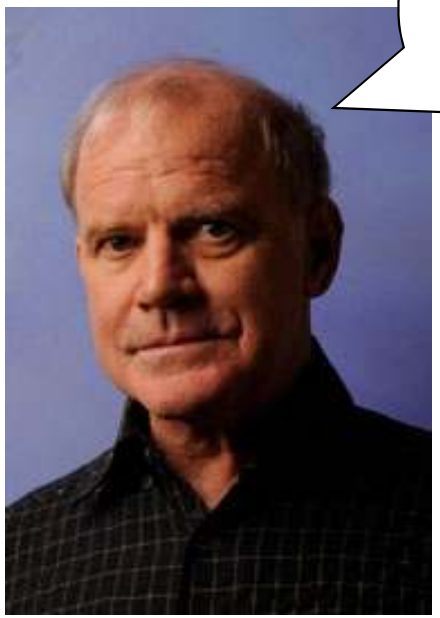
Subhadipa 2021

PCR

Polymerase Chain Reaction

In short……

- **PCR (Polymerase Chain Reaction)** is a revolutionary method developed by **Kary Mullis in the 1980s.**
- PCR is based on using the ability of **DNA polymerase to synthesize new strand of DNA complementary** to the offered template strand.
- Because DNA polymerase can add a nucleotide only onto a preexisting 3'-OH group, it **needs a primer to which it can add the first nucleotide.**
- This requirement makes it possible to delineate a **specific region of template sequence that the researcher wants to amplify.**
- At the end of the PCR reaction, the specific sequence will be **accumulated in billions of copies (amplicons).**



"lets you pick the piece of DNA you're interested in and have as much of it as you want" (Mullis, 1990).

(December 28, 1944 – August 7, 2019)

In recognition of his invention of the polymerase chain reaction technique, he shared the **1993 Nobel Prize in Chemistry with Michael Smith and was awarded the Japan Prize in the same year.**

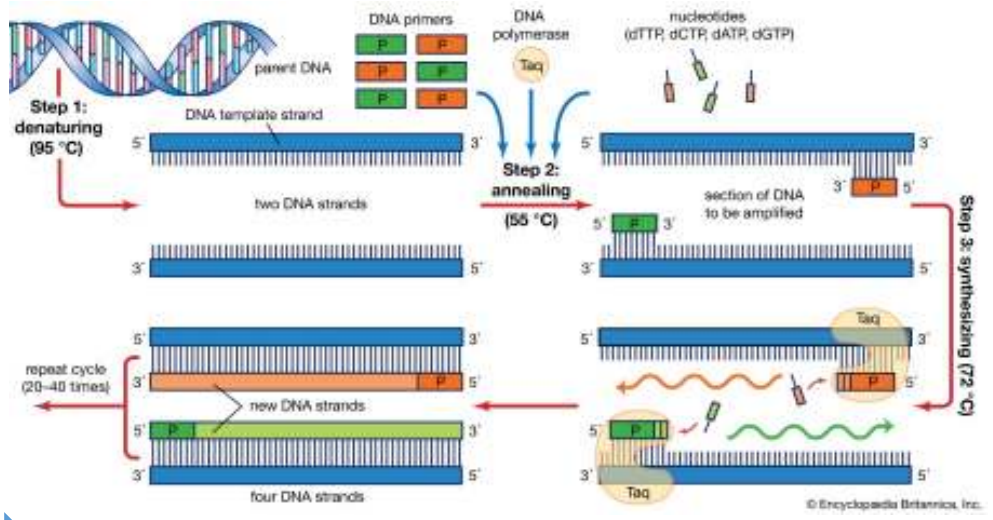
Significance of PCR

1. PCR can be performed using **source DNA** from a **variety of tissues and organisms, including peripheral blood, skin, hair, saliva, and microbes.**
2. The technique allows **trace amount of the DNA molecule to be amplified many times**, in an **exponential manner** from complex mixture.
3. It can **enzymatically replicate DNA (i.e clone) without using a living organism,** such as *E. coli* or yeast.
4. It **allows replication of a specific region** (say a sequence present in the genome that contains the gene of interest) of template sequence that the researcher wants to amplify.

Principle of the PCR

Tissue collection

DNA extraction
(whole genome)



PCR makes it possible to obtain, by in vitro replication, **multiple copies of a DNA fragment** from an extract.

Matrix DNA can be:

1. Genomic DNA
2. Complementary DNA obtained by RT-PCR from a messenger RNA extract (poly-A RNA)
3. or mitochondrial DNA.
4. Viral/Bacterial DNA

Three phases of PCR:

I. a denaturation phase
II. a hybridization/annealing phase with primers, and
III. an elongation phase.

Note: The products of each synthesis step serve as a template for the following steps, thus exponential amplification is achieved.

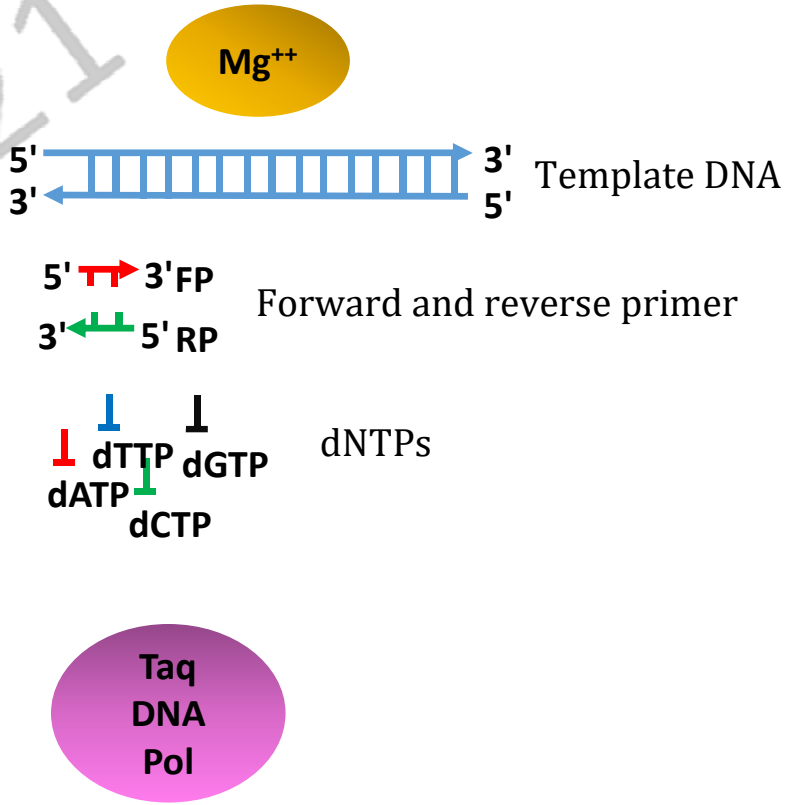
Principle of the PCR

- The whole process of PCR involves **three main events, Denaturation, Annealing and Elongation.**
- A **DNA fragment of interest is used as a template** and a **pair of primers** which are short oligonucleotides complimentary to the both strands of the template DNA.
- The purpose of primer is to initiate the DNA synthesis in the direction of 5' to 3'.
- The number **of amplified DNA or the amplicons increases exponentially per cycle thus one molecule of DNA gives rise to 2,4,8,16 and so forth.**
- This continuous doubling is carried out by a specific enzyme called **DNA polymerase** which sits at the unfinished double stranded DNA created by template DNA and primer.
- For further extension of the DNA, the polymerase enzyme require supply of other DNA-building blocks such as the **nucleotides consisting of four bases Adenine (A), Thymine (T), Cytosine (C) and Guanine (G).**

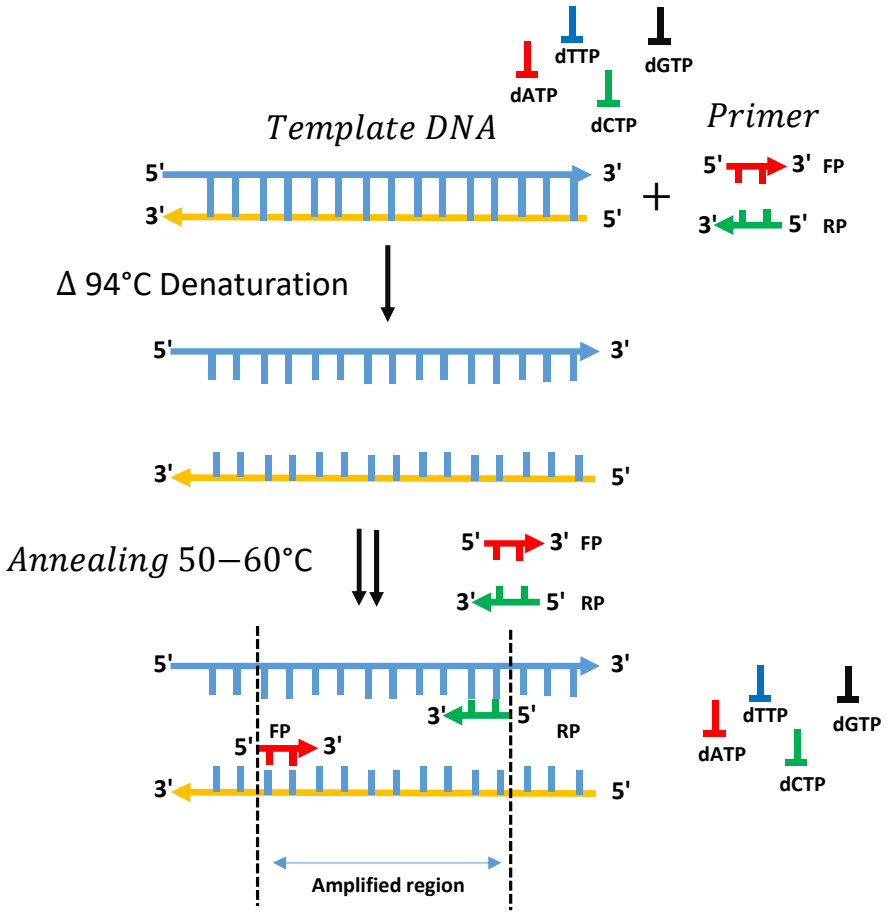
Reaction Mixture

PCR requires several basic components in a mixture called **REACTION MIXTURE**. These components are:

1. **DNA template,** or cDNA which contains the region of the DNA fragment to be amplified.
2. **Two primers**, which determine the beginning and end of the region to be amplified.
3. **Taq DNA polymerase,** which copies the region to be amplified.
4. **Nucleotides (the four deoxyribonucleoside triphosphates (dNTPs)** from which the DNA-Polymerase for new DNA
5. **Buffer (containing Mg^{++}),** which provides a suitable chemical environment for the DNA-Polymerase.

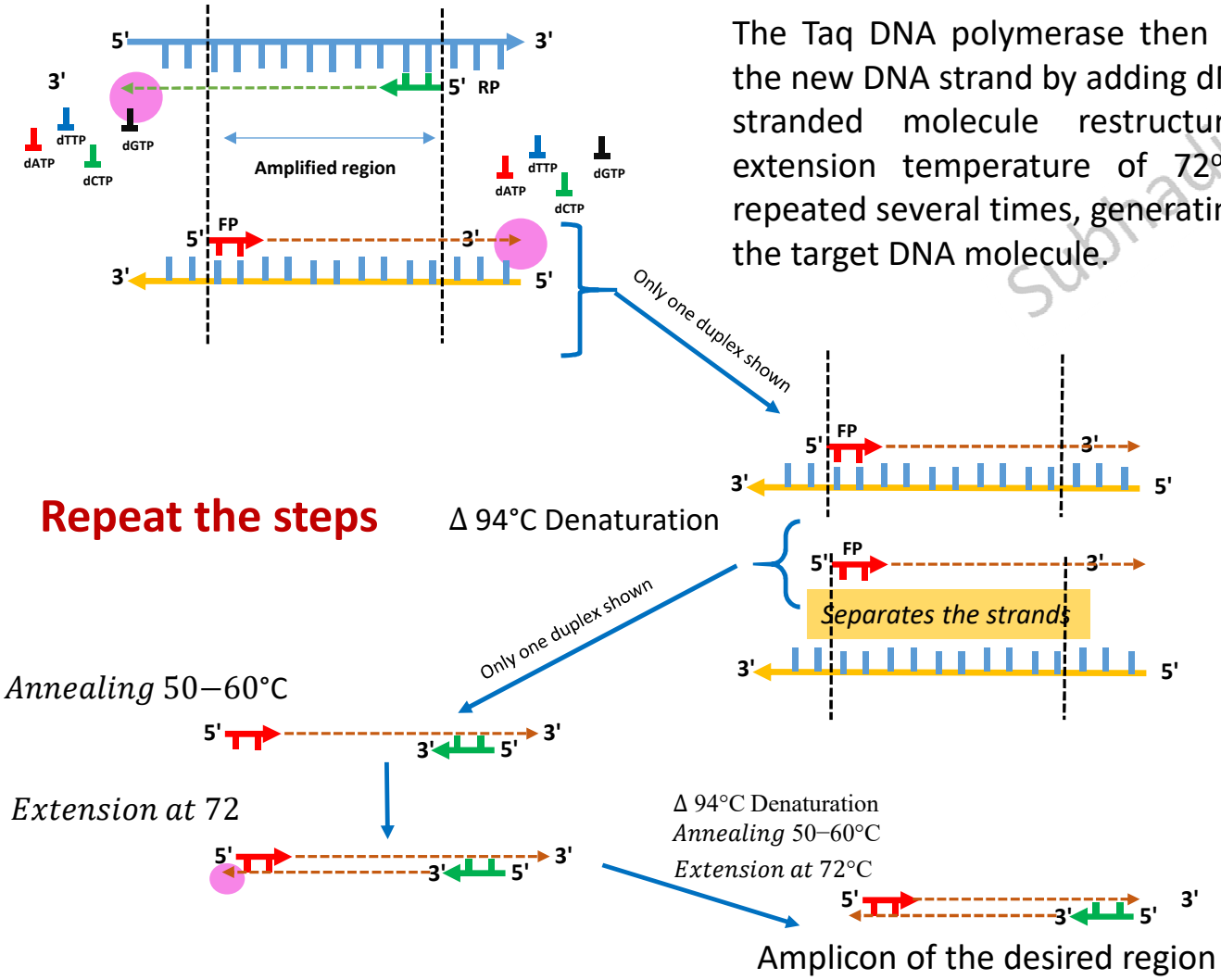


Steps of PCR



Initial denaturation occurs at 90-95°C for 3-5 minutes, where the two strands of the double stranded target DNA molecule separate by braking inter strand H-bonds

At the **annealing** temperatures in the range of 50-65°C, the complementary forward and reverse primers bind at the 3' end of the flanking regions of the separated single stranded target DNA molecule.



The Taq DNA polymerase then extends **(extension)** the new DNA strand by adding dNTPs and the double stranded molecule restructures itself at the extension temperature of 72°C. This process is repeated several times, generating multiple copies of the target DNA molecule.

As a whole