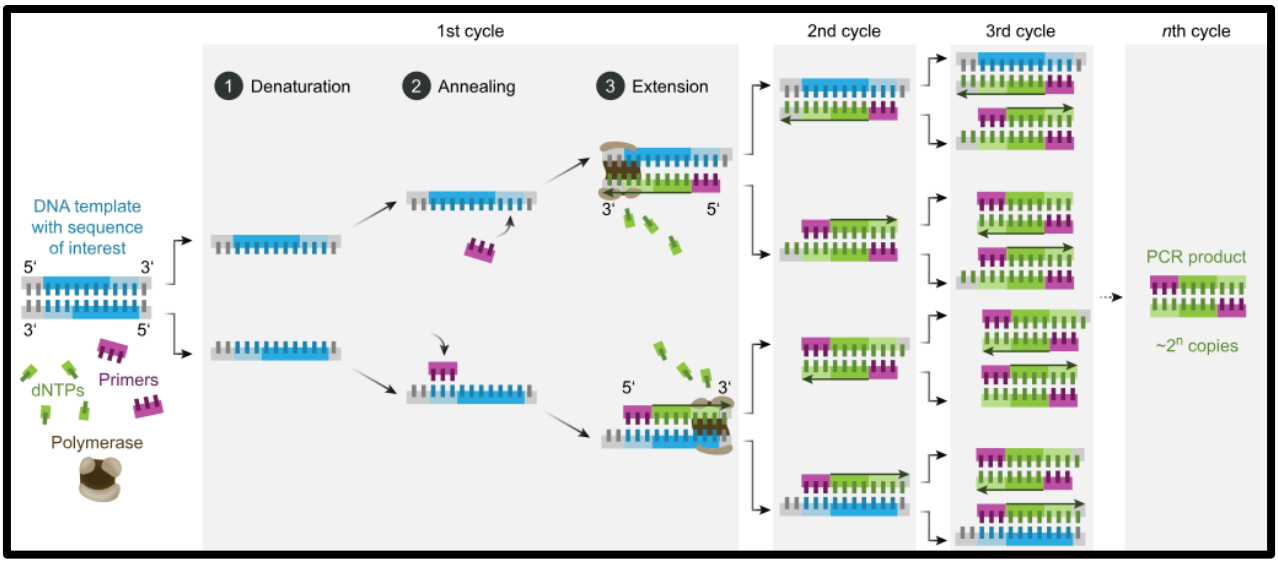
Polymerase Chain Reaction (PCR)

- Polymerase Chain Reaction (PCR) is a powerful method for amplifying particular segments of DNA, distinct from cloning and propagation within the host cell. This procedure is carried out entirely biochemically, that is, in vitro.
- PCR was invented by Kary Mullis in 1983. He shared the Nobel Prize in chemistry with Michael Smith in 1993.

Principle of PCR



PCR uses the enzyme DNA polymerase that directs the synthesis of DNA from deoxynucleotide substrates on a single-stranded DNA template. DNA polymerase adds nucleotides to the 3` end of a custom-designed oligonucleotide when it is annealed to a longer template DNA. Thus, if a synthetic oligonucleotide is annealed to a single-stranded template that contains a region

complementary to the oligonucleotide, DNA polymerase can use the oligonucleotide as a primer and elongate its 3` end to generate an extended region of double stranded DNA.

1. Denaturation

The DNA template is heated to 94° C. This breaks the weak hydrogen bonds that hold DNA strands together in a helix, allowing the strands to separate creating single stranded DNA.

2. Annealing

The mixture is cooled to anywhere from 50-70° C. This allows the primers to bind (anneal) to their complementary sequence in the template DNA.

3. Extension

The reaction is then heated to 72° C, the optimal temperature for DNA polymerase to act. DNA polymerase extends the primers, adding nucleotides onto the primer in a sequential manner, using the target DNA as a template.

With one cycle, a single segment of double-stranded DNA template is amplified into two separate pieces of double-stranded DNA. These two pieces are then available for amplification in the next cycle. As the cycles are repeated, more and more copies are generated and the number of copies of the template is increased exponentially.

A basic PCR set-up requires several components and reagents, including:

- **DNA template** that contains the DNA target region to amplify.

- **DNA polymerase**; an enzyme that polymerizes new DNA strands; heat-resistant Taq polymerase is especially common, as it is more likely to remain intact during the high-temperature DNA denaturation process two DNA primers that are complementary to the 3′ (three prime) ends of each of the sense and anti-sense strands of the DNA target (DNA polymerase can only bind to and elongate from a double-stranded region of DNA; without primers, there is no double-stranded initiation site at which the polymerase can bind)
- **Specific primers** that are complementary to the DNA target region are selected beforehand and are often custom-made in a laboratory or purchased from commercial biochemical suppliers.
- **Deoxynucleoside triphosphates**, or dNTPs (sometimes called "deoxynucleotide triphosphates"; nucleotides containing triphosphate groups), the building blocks from which the DNA polymerase synthesizes a new DNA strand.
- **A buffer solution** providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.
- **Bivalent cations**, typically magnesium (Mg) or manganese (Mn) ions; $Mg^{2}+$ is the most common, but $Mn^{2}+$ can be used for PCR-mediated DNA mutagenesis, as a higher $Mn^{2}+$ concentration increases the error rate during DNA synthesis; and monovalent cations, typically potassium (K) ions.

Applications of PCR

The following are the applications of PCR:

Medicine

- Testing of genetic disease mutations.

- Monitoring the gene in gene therapy.
- Detecting disease-causing genes in the parents.

Forensic Science

- Used as a tool in genetic fingerprinting.
- Identifying the criminal from millions of people.
- Paternity tests

Research and Genetics

- Compare the genome of two organisms in genomic studies.
- In the phylogenetic analysis of DNA from any source such as fossils.
- Analysis of gene expression.
- Gene Mapping

References

- Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). *Molecular biology of the cell*. New York: Garland Science.
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[The information, including the figures, will be used solely for academic purpose.]