Third stag **Medical Genetics**

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Lab 5 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is an in-vitro technique for amplification of specific region of DNA whose sequence is known, or which lies between two regions of known sequence, The PCR amplifies only a chosen segment (the target sequence) within the original DNA template, not the whole template DNA molecule.

History of PCR

- Before PCR, DNA of interest could only be amplified by over-expression in cells and this with limited yield
- 1966, Thomas Brock discovers Thermus Aquaticus, a thermostable bacterium in the hot springs of Yellowstone National Park
- 1983, Kary Mullis postulated the concept of PCR (Nobel Prize in 1993)
- 1985, Saiki publishes the first application of PCR (beta-Globin)
- 1985, Cetus Corp. Scientists isolate Thermostable Taq Polymerase (from T. aquaticus), which revolutionized PCR.

Reaction Components

PCR is used in clinical diagnosis, genetic analysis, genetic engineering, and forensic analysis. In particular, PCR has revolutionized and speeded up the whole area of recombinant DNA technology.

The components involved in the polymerase chain reaction are as follows:

1. The original DNA molecule that is to be copied is called the **template** and the segment of it that will actually be amplified is known as the **target sequence**. A trace amount of the DNA template is sufficient.

2. Two **PCR primers** are needed to initiate DNA synthesis. These are short pieces of single-stranded DNA that match the sequences at either end of the target DNA segment.

- Not containing inverted repeat sequences to avoid formation of internal structures
- not complimentary to each other
- 40-60% GC content preferred for better annealing
- Tm of primers can be calculated to determine annealing T0
- Tm= .41(%G+C) + 16.6log(J+) + 81.5 where J+ is the concentration of monovalent ions

3. The enzyme **DNA polymerase** is needed to manufacture the DNA copies. The PCR procedure involves several high temperature steps so a heat resistant DNA polymerase is required. This came originally from heat resistant bacteria living in hot springs at temperatures up to 90°C. **Taq polymerase** from **Thermus aquaticus** is most widely used.

4. A supply of nucleotides **(dNTPs)** is needed by the polymerase to make the new DNA. these are supplied as the nucleoside triphosphates.

5. Finally we need a **PCR machine** to keep changing the temperature The PCR process requires cycling through several different temperatures. Because of this, PCR machines are sometimes called thermocyclers.

6- buffer and Mg2+

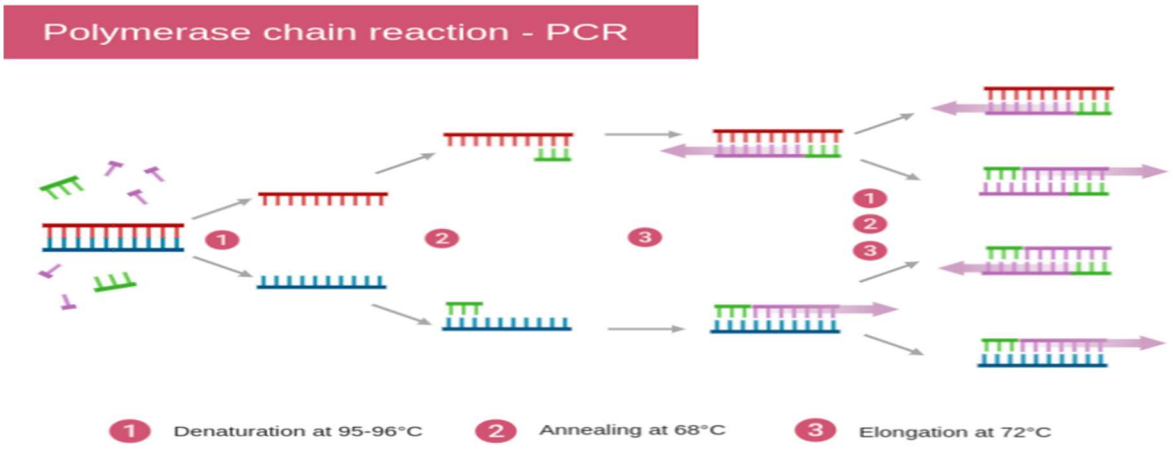
The PCR Cycle

Comprised of 3 steps:

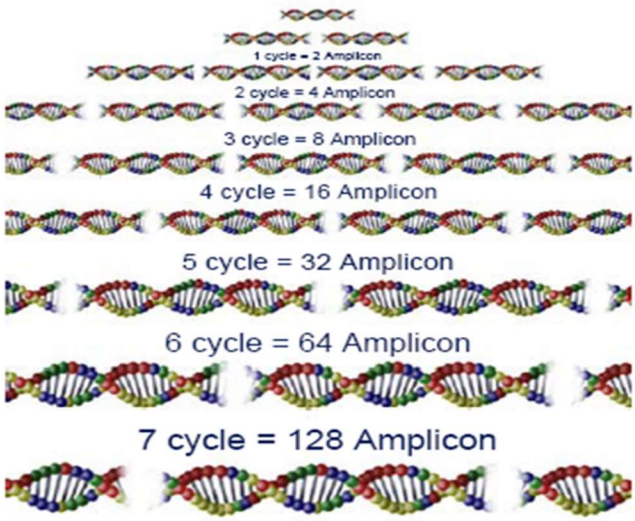
1 - **Denaturation** of DNA at 950C

2-**Primer hybridization**(**annealing**)at40-500C

3- **DNA synthesis** (Primer extension) at 720C.



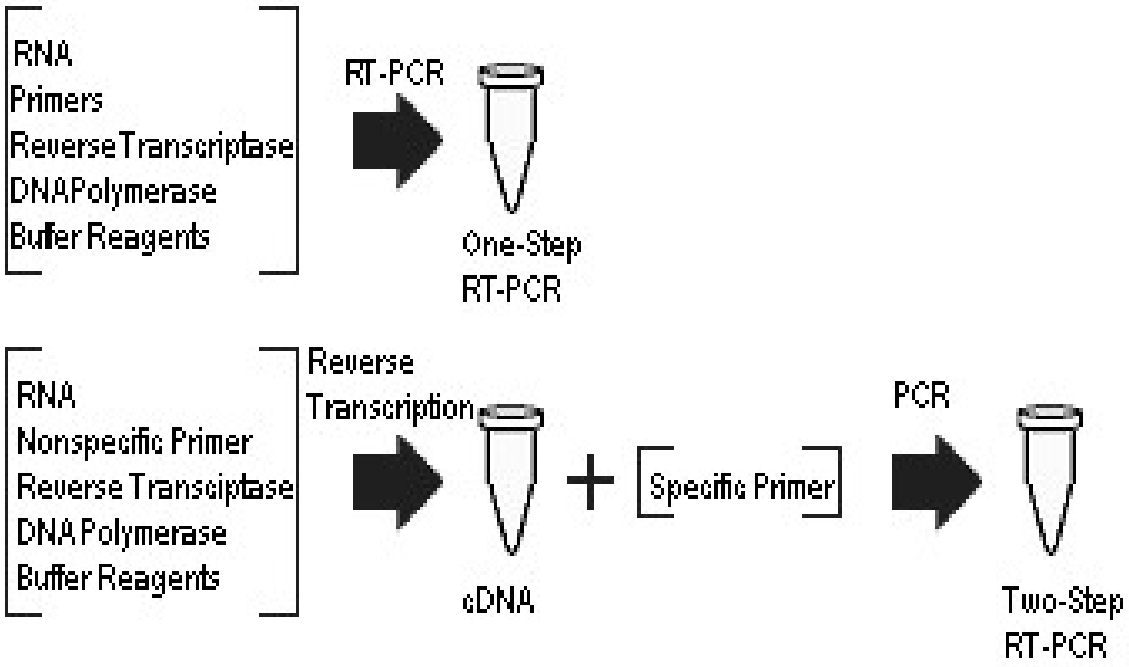
Target Amplification



No. of Cycles	No. Amplicon Copies of Target
1	2
2	4
3	8
4	16
5	32
6	64
20	1,048,576
30	1,073,741,824

Reverse Transcriptase PCR (RT-PCR)

Reverse Transcriptase PCR Uses RNA as the initial template, yields ds cDNA, Real time RT–PCR is a nuclear-derived method for detecting the presence of specific genetic material in any pathogen, including a virus. Originally, the method used radioactive isotope markers to detect targeted genetic materials, this technique allows scientists to see the results almost immediately while the process is still ongoing, whereas conventional RT–PCR only provides results at the end of the process. Real time RT–PCR is one of the most widely used laboratory methods for detecting the COVID-19 virus, Ebola virus and Zika virus.



Detection of amplification products

1- Gel electrophoresis
2- Sequencing of amplified fragment
3- Southern blot …. ect

Advantage of PCR

- Automated, fast, reliable (reproducible) results
- Contained :(less chances of contamination)
- High output
- Sensitive
- Broad uses
- Defined, easy to follow protocols.

Application of PCR

- Genome mapping and gene function determination
- Biodiversity studies (e.g. evolution studies)
- Diagnostics (prenatal testing of genetic diseases, early detection of cancer, viral infections...)
- Detection of drug resistance genes
- Forensic (DNA fingerprinting)