Polymerase Chain Reaction (PCR)

- Polymerase chain reaction (PCR) is a technique in molecular biology used to amplify (multiply) a single copy or a few copies of a piece of DNA, generating thousands to millions of copies of that particular DNA sequence.
- PCR 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 of DNA.

Steps involved in PCR:

Step 1: Denaturation

As in DNA replication, the two strands in the DNA double helix need to be separated. The separation happens by raising the temperature of the mixture, causing the hydrogen bonds between the complementary DNA strands to break. This process is called denaturation. Denaturation takes place at about **93-95^{0}C.**

Step 2: Annealing

Primers bind to the target DNA sequences and initiate polymerisation. This can only occur once the temperature of the solution has been lowered. One primer binds to each strand. Annealing takes place at about 50 -70^{0}C. (Meting temperature – 5^{0}C).

Step 3: Extension

New strands of DNA are made using the original strands as templates. A DNA polymerase enzyme joins free DNA nucleotides together. This enzyme is often Taq polymerase, an enzyme originally isolated from a thermophilic bacteria called *Thermus aquaticus*. The order in which the free nucleotides are added is determined by the sequence of nucleotides in the original (template) DNA strand. Extension takes place about **70-75^{0}C**

Conclusion:

- The result of one cycle of PCR is two double-stranded sequences of target DNA, each containing one newly made strand and one original strand.
- The cycle is repeated many times (usually 20–30) as most processes using PCR need large quantities of DNA. It only takes 2–3 hours to get a billion or so copies.