

Principle of PCR

The polymerase chain reaction (PCR) is a widely used molecular biology technique that amplifies a specific DNA sequence. The principle of PCR involves a cyclic process that repeatedly synthesizes new DNA strands using a DNA template, DNA primers, and a heat-stable DNA polymerase enzyme. The basic steps of PCR are as follows:

Denaturation: The PCR reaction begins with a denaturation step, where the DNA template containing the target sequence is heated to a high temperature (typically around 94-98°C). This causes the double-stranded DNA to separate into two single strands, effectively denaturing the DNA.

Annealing: After denaturation, the reaction temperature is lowered to allow the DNA primers to bind (anneal) to the complementary sequences on each of the single-stranded DNA templates. The primers are short, synthetic DNA sequences that flank the target region and provide a starting point for DNA synthesis. The annealing temperature depends on the melting temperature (T_m) of the primers and is typically a few degrees Celsius below the T_m .

Extension: Once the primers are annealed, the temperature is raised to an optimal range for the DNA polymerase enzyme (usually around 72°C). The DNA polymerase enzyme synthesizes new DNA strands by adding complementary nucleotides to the 3' end of the primers, extending the DNA in a 5' to 3' direction. The DNA polymerase used in PCR is typically a heat-stable enzyme, such as Taq polymerase, which is derived from the thermophilic bacterium *Thermus aquaticus*.

Cycling: The three steps of denaturation, annealing, and extension are repeated for a specific number of cycles, typically 20 to 40 cycles. Each cycle doubles the amount of the target DNA sequence. As a result, the DNA amplification is exponential, leading to the rapid accumulation of the target DNA.

By the end of the cycling process, a large number of DNA copies of the target sequence have been produced. These copies can be further analyzed or utilized for various applications, such as DNA sequencing, cloning, genotyping, and gene expression analysis.

It is important to note that PCR requires a specific primer design to ensure specific amplification of the target sequence and to minimize non-specific amplification. Several marker based PCRs are

adopted to perform plant genotyping, including RAPD, ISSR, and SSR primers. Additionally, appropriate reaction conditions, such as buffer composition, magnesium concentration, and cycle parameters, must be optimized to achieve reliable and efficient PCR amplification.