PCR and Real-Time PCR

Basic Molecular Biology Modules - Principle of PCR

Polymerase chain reaction or PCR is a technique for amplifying specific DNA fragments from a DNA template.
PCR happens in three basic steps: denaturation, annealing and extension.

Denaturation is the first step of PCR. In this step, heat is applied to the template to separate double-stranded DNA into two single strands.

Following the denaturation step is the annealing step. The temperature is decreased so that the primers can anneal to the complementary sequences on the DNA templates.

The temperature of the annealing step depends on the melting temperatures of the primer.

Selection of the annealing temperature is critical. Typically, the optimum annealing temperature is 3-5 degrees Celsius below the melting temperature.

Too high of an annealing temperature prevents optimal binding of the primers to the templates while too low of an annealing temperature can lead to non-specific binding and, subsequently, non-specific PCR products.

Extension occurs after the primers hybridize to the templates. The temperature of this step is adjusted to the optimum temperature where the DNA polymerase functions best.

In extension, DNA polymerase adds nucleotides to the 3’ ends of the primers that sit on the templates following the base-pair complementarity rule.

The length of the extension steps depends on the extension rate of the polymerase used and the length of the desired PCR product.

These three steps comprise 1 PCR cycle. The cycle is repeated multiple times allowing generation of a large number of copies of a specific DNA fragment.