Background on Polymerase Chain Reaction (PCR)

Since its introduction in 1985, polymerase chain reaction (PCR) has become a powerful tool in research and genetic analysis. PCR is a method for making many copies of a targeted DNA sequence in a test tube. The power of PCR lies in its ability to quickly copy and selectively amplify a few strands of DNA that, because of their scarcity, are difficult to manipulate or examine. After DNA has been amplified, it can be manipulated or analyzed using standard molecular biology techniques including gel electrophoresis, cloning, sequencing, and restriction enzyme analysis. PCR revolutionized many applications where access to only small amounts of DNA had previously limited the analysis that could be performed. These applications include forensic examination of DNA, molecular genetic analysis, and analysis of ancient DNA specimens.

The development of PCR is based on the understanding of DNA replication in cells. Although the processes of cellular DNA replication and PCR are not identical, they share many similarities.

During the first step in cellular DNA replication, enzymes unwind and separate, or denature, the DNA double helix into single strands. The first step in PCR, the denaturation step, also involves separating the DNA double helix into single strands. However, in the case of PCR, this is achieved not by enzymes, but by heating the DNA to high temperatures.

In the next step of cellular DNA replication, small complementary stretches of RNA are synthesized against the DNA template. DNA polymerase then uses these small stretches of DNA-bound RNA and DNA molecules as "primers" to initiate DNA synthesis.

Taq polymerase, the DNA polymerase that replicates DNA during PCR, also requires primers to begin synthesis. In place of the small RNA and DNA molecules synthesized as primers in cellular DNA replication, PCR uses short (usually 20 to 30 base pairs), synthetic, single-stranded fragments of DNA called oligonucleotides. In order to amplify a specific region of DNA, two different primers (forward and reverse) are added to the PCR reaction. These primers bracket the region to be amplified and are complementary to opposite strands. These two synthetic primers hydrogen bond and form base pairs with their complementary sequences in the target DNA during the annealing step of the PCR reaction, when the temperature of the PCR reaction is lowered to enable hydrogen bonding to occur.

In cellular replication, the DNA polymerase adds nucleotides complementary to the original DNA strand to the 3' end of the RNA and DNA primers. The original DNA strand acts as a template for the new strand of DNA. Through this mechanism, DNA polymerase creates a new DNA molecule, identical, but complementary, to the original DNA strand.

The analogous process in the PCR reaction occurs during the extension step. During the extension step, Taq polymerase uses the original strands of DNA in the test tube as the template and adds nucleotides complementary to those in the template strand to the 3' end of the synthetic primer. The specific location of the synthetic primers ensures that the Taq polymerase replicates only a specific target region of DNA.

In summary, the PCR replication cycle consists of three steps:

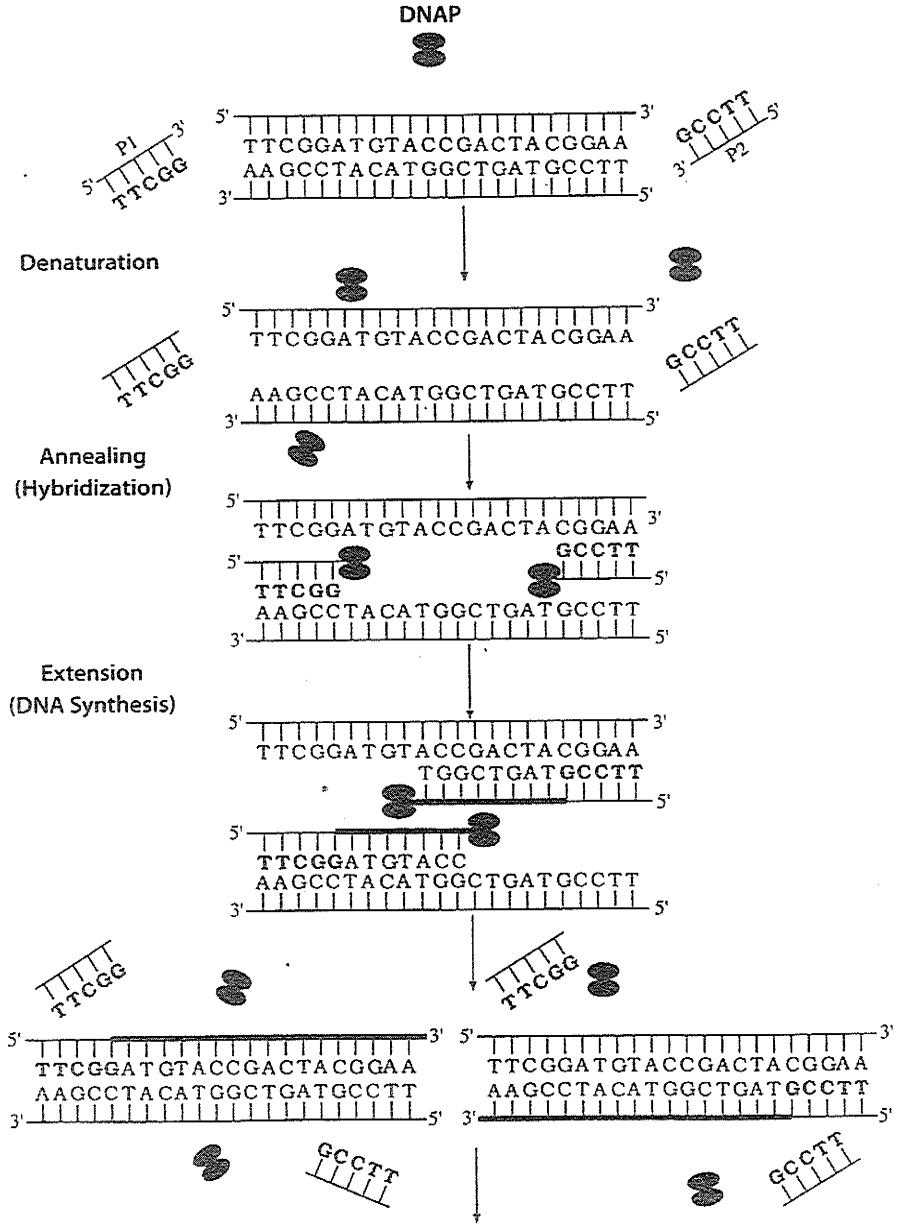
1. the denaturation step, during which the two strands of the double-stranded template are separated.
2. the annealing step, during which the primers anneal in preparation for DNA replication.
3. the extension step, during which DNA replication occurs.

This replication cycle is repeated many times (20–40 times is typical). If the reaction works at 100% efficiency, the number of copies of the target DNA sequence doubles with every cycle. Therefore, if you start a reaction with one double-stranded copy of DNA, the predicted number of target DNA molecules is

$2n$, where n is the number of cycles performed. This means that in as few as 20 cycles, under ideal conditions, you can create over one million copies from one initial, double-stranded DNA segment.

Originally, PCR was performed manually, using a timer and three water baths set to the needed denaturing, annealing, and extension temperatures. Because the DNA polymerase first used for PCR was heat sensitive, after every denaturing step, new polymerase had to be added to the reaction. Two innovations made automated sequencing possible. First, the use of a heat-stable polymerase (Taq polymerase) from the hot spring-inhabiting bacterium *Thermus aquaticus* eliminated the need for new polymerase after each step. Then, the development of thermal cyclers with automatic temperature control eliminated the need to move the reactions from one temperature to another.

A good animation demonstrating PCR can be found on the Dolan DNA Learning Center Web site at **http://www.dnalc.org/ddnalc/resources/pcr.html.**



The polymerase chain reaction amplifies a segment of DNA lying between two primer sequences. P1, P2: primers. DNAP: DNA polymerase (Taq polymerases)

Note: For simplicity's sake, in this depiction of PCR the length of the primer is shorter than what would actually be used.