

Polymerase Chain Reaction (PCR) – modified from Open Stax Biology (<https://openstax.org/details/books/biology>)

PCR is a technique used to amplify specific regions of DNA for further analysis. PCR is used for many purposes in laboratories, such as the cloning of gene fragments to analyze genetic diseases, identification of contaminant foreign DNA in a sample, and the amplification of DNA for sequencing.

Polymerase chain reaction, or PCR, is used to amplify or make copies of a specific sequence of DNA. It relies on the temperatures ranges at which the DNA double-helix becomes thermally unstable. Above certain temperatures (say 90°C) the two strands of DNA separate from each other when their hydrogen bonds break. The process of the two strands separating is called “melting” and is temporary; one the temperature cools down hydrogen bonds can reform to build a new complement to each strand.

For PCR to make DNA copies, short pieces of DNA called primers, complementary to each end of the target sequence, are combined with a DNA template, a thermostable DNA polymerase enzyme, and deoxynucleotides (dNTPs). The primers in PCR are made of DNA, but during DNA replication in a living cell RNA primers are actually used! The standard PCR enzyme is Taq polymerase, a DNA polymerase isolated from the thermostable bacterium *Thermus aquaticus*, which is able to withstand the high temperatures used in PCR. *Thermus aquaticus* grows in the Lower Geyser Basin of Yellowstone National Park. Taq polymerase works the same as a DNA polymerase in other living organisms, just at a higher temperature of 72°C. Standard PCR reactions involve DNA ‘template,’ forward and reverse primers, dNTPs, DNA polymerase, buffering salts, and water. Reverse transcriptase PCR (RT-PCR) is similar to PCR except that it uses a template of “copy DNA” (cDNA) made by reverse transcribing an RNA template before PCR begins.

In a standard PCR, the temperatures for each step are:

Step 1. Denaturation at 94-95°C

Step 2. Annealing at the optimal temperature for the specific primer, usually between 50–60°C

Step 3. Elongation at 72°C.

The number of cycles can vary from 15–35.

