Biotechnology Research Project - PCR

PART ONE:

Polymerase Chain Reaction (PCR) is a technique widely used to make many copies of a specific target DNA sample. The method allows scientists to take a very small quantity of DNA and quickly amplify it to a much larger amount so that it can be studied in detail. The basic PCR procedure requires the template DNA sample, a DNA polymerase, primers, and nucleotides. To begin, these reagents are all placed in a test tube containing a solution (often water with ionic salts, commonly $MgCl_2$). Once the test tube is prepared, it undergoes a thermal cycle that consists of three main steps:

1. Denaturation

   The first step in the process is to heat the test tube to approximately 95℃.The high heat is essential in order to break the hydrogen bonds between the complementary base pairs in the DNA. In doing so, the original double stranded DNA template is melted into two single stranded DNA molecules. This separation of the DNA is necessary to allow the primers to bind in the next step.

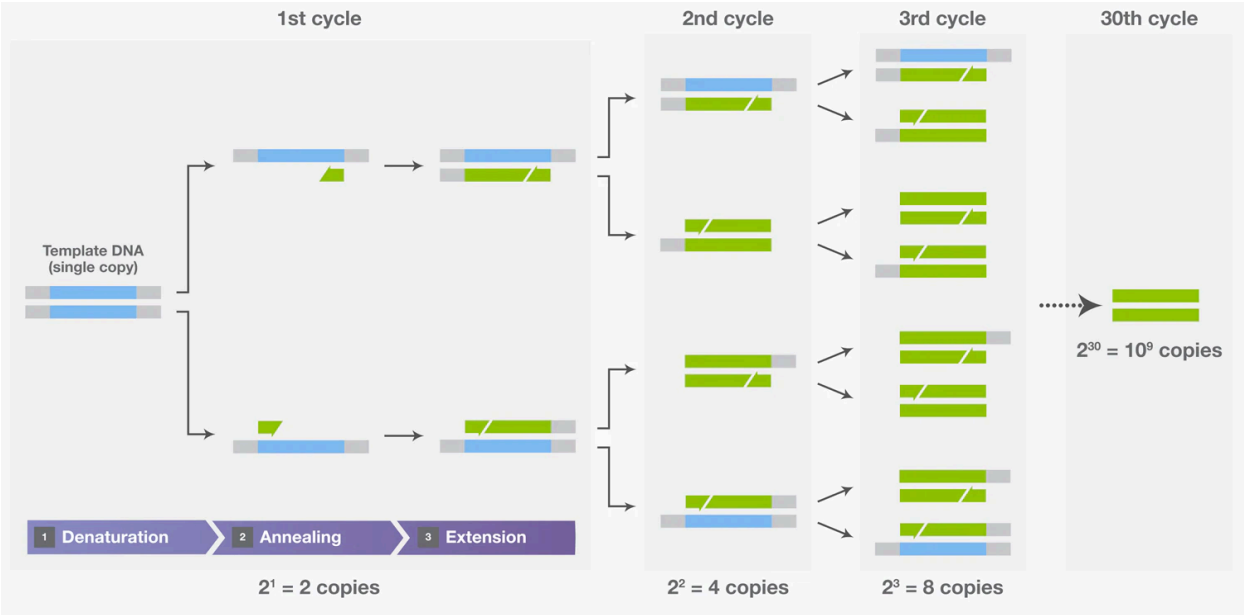
2. Annealing

   This step takes place at a lower temperature, usually between 50°C and 65℃. The exact value is mainly determined by the optimal temperature for the specific primer being used (typically about 5℃ below the primer's melting point). The temperature change facilitates the annealing, or binding, of the primers to each of the newly separated single strand molecules. A primer is a short single stranded nucleic acid sequence (usually around 20 nucleotides) that is synthesized in a lab and is complementary to a specific sequence at one end of the target fragment. Since denaturation produces two DNA template strands that run in opposite directions, two primers must be used in each PCR reaction. Each of the primers must be complementary to the 3' end of the particular target fragment on one of the strands. Because the primers are so specific, the experimenter uses them to select the exact region of the DNA that will be copied and amplified. The primers are added to the solution in very large amounts to increase the chances of them binding to the template strands, and to decrease the chances of the template strands rejoining.

3. Extension

In the final step, the temperature is increased again, this time to 72℃. This change facilitates the synthesis of new DNA strands by the DNA polymerase. However, the very high temperatures needed for PCR means that using polymerase found in humans would not be possible. Instead, the DNA polymerase most commonly used in PCR is harvested from a thermophilic bacterium that lives in hot springs and hydrothermal vents, called *Thermus Aquaticus.* The specific enzyme this bacteria produces, known as taq polymerase, can withstand extremely high temperatures. In this step of the cycle, the taq polymerase binds to the primer-template combination, and synthesizes a new complementary DNA strand by adding the free nucleotides from the solution. Like other DNA polymerases, taq polymerase adds in the 5' to 3' direction.

After starting with one double stranded template, the result of this initial cycle is two double stranded sequences of the target DNA, each containing one newly synthesized strand and one original strand. Then, the process can be repeated (usually 25-40 times) with the newly synthesized DNA. To make it easier, PCR typically takes place inside a machine called a thermal cycler, that automatically raises and lowers the temperature based on pre-programmed instructions. Each thermal cycle essentially doubles the target DNA, so it is amplified exponentially and in a very short period of time. In fact, PCR can produce billions of copies of the target DNA in just a couple of hours.



PART TWO:

As one of the most widely used molecular biology techniques, PCR has a number of different modern-day applications. One of PCR's most interesting uses is for a forensic process called DNA profiling. DNA profiling relies on analyzing short tandem repeats (also known as microsatellites), which are segments of DNA in which specific sequences of approximately 2-7 base pairs are repeated many times. Short tandem repeats (STRs) have a greater mutation rate than other areas of DNA, which results in very high genetic diversity. It is estimated that only one in every 10 trillion people will have a particular STR profile. Thus, in DNA profiling, scientists can use STRs to identify a specific individual based on their genome. The technology of PCR is critical to the profiling process, as it allows scientists to take a tiny amount of DNA, and amplify the STRs so that they can be analyzed. Using PCR, scientists can take trace amounts of DNA left at crime scenes and match it to the DNA of possible suspects.

Works Cited

"DNA Fingerprinting." *Genome.gov*, www.genome.gov/genetics-glossary/DNA-Fingerprinting#:~:text=DNA fingerprinting is a laboratory,evidence came from that suspect.

"PCR Basics." *Thermo Fisher Scientific - US*, www.thermofisher.com/ca/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/pcr-education/pcr-reagents-enzymes/pcr-basics.html.

"Polymerase Chain Reaction: PCR Process & Guide." *Sigma*, www.sigmaaldrich.com/technical-documents/articles/biology/polymerase-chain-reaction.html.

"What Is PCR?" *Science Learning Hub*, www.sciencelearn.org.nz/resources/2347-what-is-pcr.

"What Is a DNA Fingerprint?" *Facts*, The Public Engagement Team at the Wellcome Genome Campus, 2 June 2016, www.yourgenome.org/facts/what-is-a-dna-fingerprint#:~:text=PCR is a technique used,at the University of Leicester.