Polymerase chain reaction

Introduction

Nearly every experiment or test in a modern biology lab relies on a technique called *polymerase chain reaction* or PCR. PCR is a powerful tool that allows scientists to make copies of specific sections of DNA.

The DNA sequence that a scientist is interested in is typically relatively short and infrequent in an organism's vast amount of DNA. The human genome is more than 3.2 billion base pairs long, and identifying a small genetic sequence that might be just a few hundred bases is like searching for a needle in a haystack. PCR allows scientists to make billions of copies of only the region of DNA they are interested in, effectively creating a giant pile of needles that dwarfs the original haystack.

DNA replication in a test tube

Copying DNA in a test tube using PCR follows the principles of DNA replication inside living cells. DNA replication relies on the fact that the bases on both strands of DNA are complementary to each other. If there is an adenine (A) base on one strand, it will always pair with a thymine (T) on the other strand. Likewise, guanine (G) always pairs with cytosine (C). If you know the sequence of bases on one strand, you can infer the other complementary strand. In DNA replication the strands are pulled apart, and each serves as a template for constructing a new complementary strand using the base-pairing rules. The result of DNA replication is two double-stranded DNA molecules that are identical to the starting DNA. In each resulting DNA molecule, one of the strands is the original template, while the other one is a newly synthesized complementary strand.

Remember that the goal of PCR is to copy a specific region of DNA, which is referred to as the *target sequence*. In order to copy the target sequence using PCR, scientists add the following reagents to a test tube:

Template DNA: The starting DNA is called the template. The template DNA will be different in every experiment, and could come from any organism—alive or dead. Even trace amounts of DNA that have been left behind can be used as the starting point for PCR. From this complex mix of DNA, only the target sequence will be copied billions of times.

DNA polymerase: DNA polymerases are enzymes that can read a strand of DNA and make a complementary copy following the base-pairing rules. In PCR, we typically use a particular DNA polymerase called *Taq* polymerase. *Taq* polymerase is used because it originally comes from thermophilic (heat-loving) bacteria, and it is therefore stable and active at the high temperatures used in PCR.

Primers: Primers are short (about 20 to 30 bases), single-stranded pieces of synthetic DNA that can bind to complementary regions in the template DNA. In PCR, scientists design two primers that flank the DNA they want to copy. One primer (usually called the *forward primer*) will bind at the start of the target sequence. The second primer (usually called the *reverse primer*) will bind to the other strand of DNA at the end of the target sequence. To go back to the needle in the haystack analogy, it is the primers that find the specific target sequence within the vast amount of template DNA. Primers are necessary for the DNA polymerase to begin its copying job, as we will explain below.

dNTPs: *Nucleotides* are the building blocks of DNA and are composed of a phosphate group, a sugar, and one base (A, T, C, or G). The term dNTPs refers to a mix of all four nucleotide building blocks. The polymerase uses these DNA building blocks to build a new strand of DNA.

Buffer: A buffer is a solution that maintains a stable pH. PCR buffer maintains a pH that enables polymerase function. PCR buffer also contains ions required for polymerase function.

PCR steps

During DNA replication in the cell, a single copy of all the DNA in the nucleus is made. In contrast, in PCR, only a specific region of DNA is copied, and the copying process repeats many times. Scientists control the copying process in PCR by changing the reaction temperature using a machine called a *thermal cycler*. The thermal cycler runs through three steps, each requiring a precise temperature.

Step 1 - Denaturation: To separate the DNA strands, the samples are heated to around 95°C. The links that hold the complementary strands of DNA together, called hydrogen bonds, break at this high temperature. Scientists use the term *denaturation* to describe when a biological molecule loses some of its structure. When complementary DNA strands separate, they have denatured.

Step 2 - Annealing: To allow the primers to bind to each strand of the target sequence, the sample is cooled to between 50°C and 65°C. The precise temperature depends on the sequences of the primers used. When complementary strands of nucleotides bind via hydrogen bonds, they are said to *anneal*. At annealing temperature, hydrogen bonds can form between the primers and the template DNA, creating short segments of double-stranded DNA that flank the target sequence. The target sequence is now primed to be copied.

Step 3 - Extension: To allow the polymerase to copy the DNA, the reaction mixture is heated to around 72°C. This is the temperature at which the *Taq* polymerase used in PCR can function optimally. Because *Taq* polymerase adds nucleotides starting from the primers, extending the DNA chain, the third step of PCR is called the extension step.

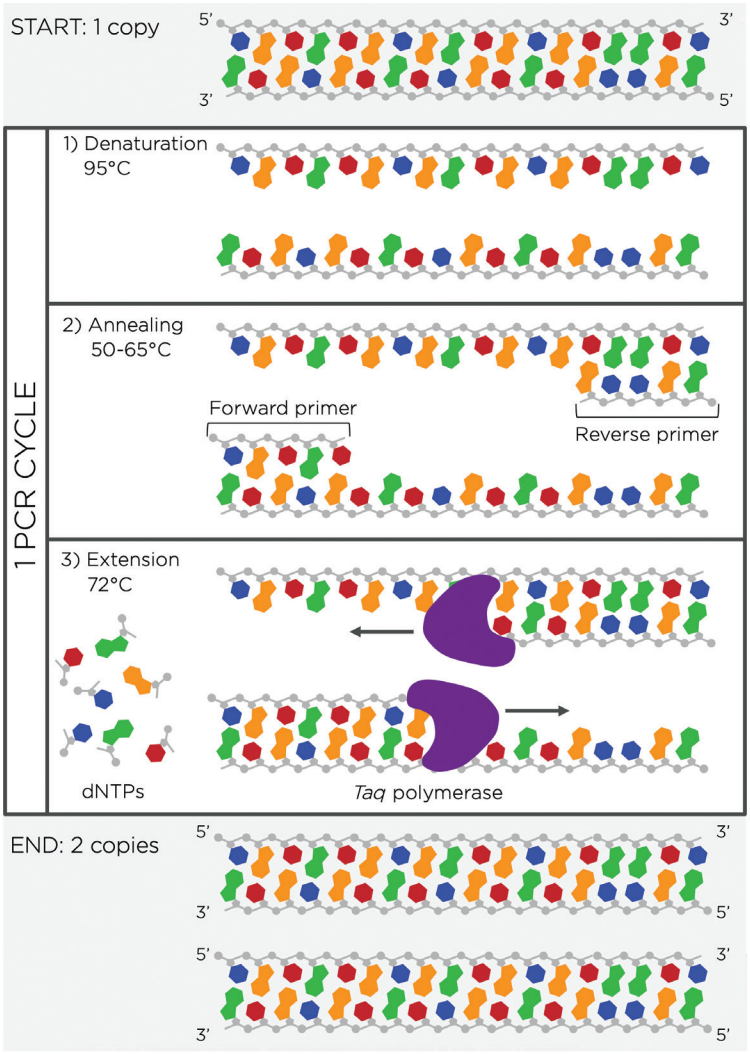

Figure 1. Depiction of one PCR cycle

After these three steps, we will have doubled the number of copies of the target sequence (Figure 1). But we don't stop there. The thermal cycler repeats the process, and the newly made DNA fragments will also be copied in the following cycle. After one cycle, for every copy of our target sequence that we started with, we will have two copies; after two cycles, we will have four. Then 8, 16, 32, etc. This type of exponential copying of the DNA sequence is like a chain reaction, the "C" and "R" in PCR. This three-step cycle, denaturation, annealing, and extension, typically repeats around 30 times, making roughly one billion new molecules of the target sequence for each initial copy.

Importance of PCR

Using PCR to make billions of copies of specific DNA sequences revolutionized molecular biology and even won its inventor the Nobel Prize. Despite being developed in the 1980s, PCR remains a versatile molecular biology technique that is used in nearly every experiment or test involving DNA, from detecting viral infections to preparing samples for DNA sequencing.