

Appendix A

Introduction to PCR

In 1983, Kary Mullis at Cetus Corporation developed the molecular biology technique that has since revolutionized genetic research, earning him the Nobel Prize in 1993. This technique, termed the **polymerase chain reaction (PCR)**, transformed molecular biology into a multidisciplinary research tool. Many molecular biology techniques used before PCR were labor intensive, time consuming, and required a high level of technical expertise. Additionally, working with only trace amounts of DNA made it difficult for researchers in other biological fields (pathology, botany, zoology, pharmacy, etc.) to incorporate molecular biology into their research schemes.

PCR had an impact on four main areas of biotechnology: gene mapping, cloning, DNA sequencing, and gene detection. PCR is now used as a medical diagnostic tool to detect specific mutations that may cause genetic disease, in criminal investigations and courts of law to identify suspects on a molecular level, and in the sequencing of the human genome. Prior to PCR, the use of molecular biology techniques for therapeutic, forensic, pharmaceutical, or medical diagnostic purposes was not practical or cost-effective. The development of PCR technology changed these aspects of molecular biology from a difficult science to one of the most accessible and widely used tools in genetic and medical research.

PCR and Biotechnology - What Is It and Why Did It Revolutionize an Entire Research Community?

PCR produces exponentially large amounts of a specific piece of DNA from trace amounts of starting material (template). The template can be any form of double-stranded DNA, such as genomic DNA. A researcher can take trace amounts of DNA from a drop of blood, a single hair follicle, or a corn chip and use PCR to generate millions of copies of a desired DNA fragment. In theory, only a single molecule of double-stranded template DNA is needed to generate millions of copies. Prior to the development of the PCR technique, it would have been impossible to do forensic or genetic studies with a minute sample containing only a few molecules of source DNA. The ability to amplify a precise sequence of DNA to a sufficient quantity that a researcher can analyze and manipulate is the true power of PCR.

PCR amplification requires the presence of at least one DNA template strand. In this kit, plant DNA isolated from grocery store foods provides the template strands. One of the main reasons PCR is such a powerful tool is its simplicity and specificity. All that is required are inexpensive buffers, four DNA subunits (deoxynucleotide triphosphates of adenine, guanine, thymine, and cytosine), a DNA polymerase, two DNA primers, and minute quantities of the template sequence that one wants to amplify. Specificity comes from the ability to target one specific segment of DNA (or gene) out of a complete genome through the use of sequence-specific primers.

PCR Makes Use of Two Basic Processes in Molecular Genetics

1. Complementary DNA strand hybridization
2. DNA strand synthesis via DNA polymerase

In the case of PCR, complementary strand hybridization takes place when two different oligonucleotide primers anneal to each of their respective complementary base pair sequences on the template. The two primers are designed and synthesized in the laboratory with a specific sequence of nucleotides such that they can anneal at the opposite ends and on the opposite strands of the stretch of double-stranded DNA (template) to be amplified.

Before a region of DNA can be amplified, one must identify and determine the sequence of an area of DNA upstream and downstream of the region of interest. These areas are then used to determine the sequences of oligonucleotide primers that will be synthesized and used as starting points for DNA replication. Primers are needed because DNA polymerases can only add nucleotides to the end of a preexisting chain. The DNA polymerase used in PCR must be a thermally stable polymerase because the polymerase chain reaction cycles between 59°C–94°C. The thermostable DNA polymerase (*Taq*) used in PCR was isolated from a thermophilic bacterium, *Thermus aquaticus*, which lives in high-temperature steam vents such as those found in Yellowstone National Park.

Two new template strands are created from the original double-stranded template during each complete cycle of the strand synthesis reaction. This causes exponential growth of the number of template molecules, i.e., the number of DNA strands doubles at each cycle. Therefore, after 30 cycles there will be 2^{30} , or over 10^9 , times more copies than at the beginning. Once the DNA of interest has been sufficiently amplified, it can be visualized using gel electrophoresis. This allows researchers to determine the presence or absence of the desired PCR products.

PCR Step by Step

PCR involves a repetitive series of cycles, each of which consists of template denaturation, primer annealing, and extension of the annealed primer by *Taq* DNA polymerase. Before beginning DNA amplification, genomic DNA is prepared from samples-in this lab, from plant-derived food items.

Following sample preparation, the template DNA, oligonucleotide primers, thermostable DNA polymerase (*Taq*), the four deoxynucleotides (A, T, G, C), and reaction buffer are mixed in a single micro test tube. The tube is placed into the MyCycler™ thermal cycler. These thermal cyclers contain an aluminum block that holds the samples and can be rapidly heated and cooled across wide temperature differences. The rapid heating and cooling of this thermal block is called **temperature cycling** or **thermal cycling**.

The first step of the PCR temperature cycling procedure involves heating the sample to 94°C. At this high temperature, the template strands separate (denature). This is called the **denaturation step**.

The thermal cycler then rapidly cools to 59°C to allow the primers to anneal to the separated template strands. This is called the **annealing step**. The two original template strands may reanneal to each other, or compete with the primers for the primers' complementary binding sites. However, the primers are added in excess such that the primers actually outcompete the original DNA strands for the primers' complementary binding sites.

Lastly, the thermal cycler heats the sample to 72°C for *Taq* DNA polymerase to extend the primers and make complete copies of each DNA strand. This is called the **extension step**. *Taq* polymerase works most efficiently at this temperature. Two new copies of each complementary strand are created. There are now two sets of double-stranded DNA (dsDNA). These two sets of dsDNA can now be used for another cycle and subsequent strand synthesis.

At this stage, a complete temperature cycle (thermal cycle) has been completed.

Temperature cycle = denaturation step + annealing step + extension step

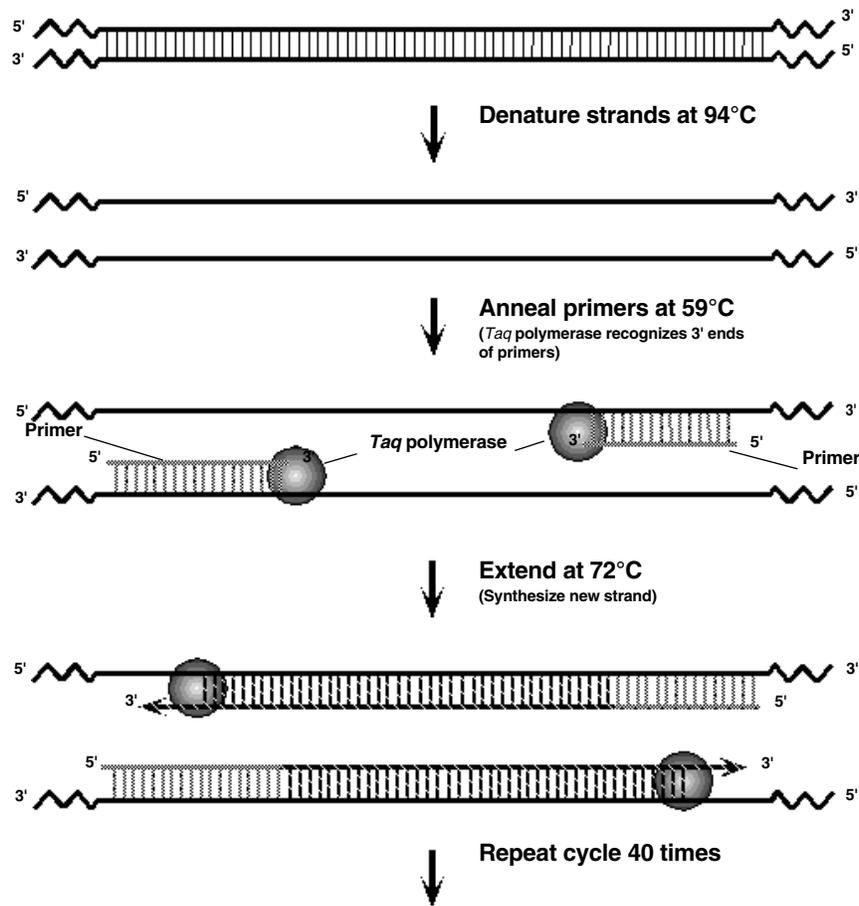


Figure A1. A complete cycle of PCR.

Usually, thermal cycling continues for about 40 cycles. After each thermal cycle, the number of template strands doubles, resulting in an exponential increase in the number of template DNA strands. After 40 cycles there will be 1.1×10^{12} more copies of the original number of template DNA molecules.

PCR generates DNA of a precise length and sequence. On the first cycle, the two primers anneal to the original genomic template DNA strands at opposite ends and on opposite strands. After the first complete temperature cycle, two new strands are generated that are shorter than the original template strands but still longer than the length of the DNA that the researcher wants to amplify. It isn't until the third thermal cycle that fragments of the precise length are generated.

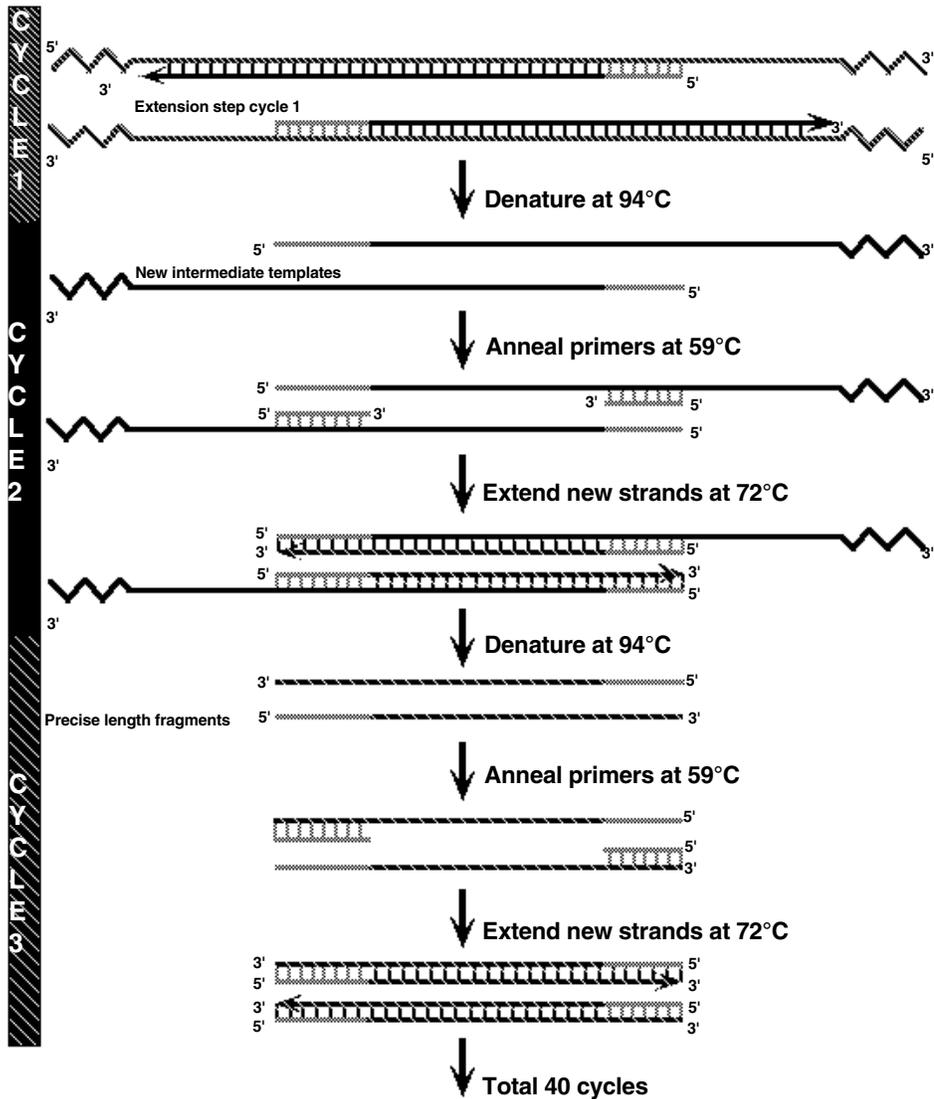


Figure A2. Generation of precise-length fragments.

It is the template strands of the precise length that are amplified exponentially (X^n , where X = the number of original template strands and n = the number of cycles). There is always one set of original long-template DNA molecules that are never fully duplicated. After each thermal cycle, two intermediate-length strands are produced, but because they can only be generated from the original template strands, the intermediate strands are not exponentially amplified. It is the precise-length strands generated from the intermediate strands that are amplified exponentially at each cycle. Therefore, if 20 thermal cycles were conducted from one double-stranded DNA molecule, there would be 1 set of original genomic template DNA strands, 20 sets of intermediate template strands, and 1,048,576 sets of precise-length template strands. After 40 cycles, there would be 1 set of original genomic template DNA strands, 40 sets of intermediate template strands, and 1.1×10^{12} sets of precise-length template strands.

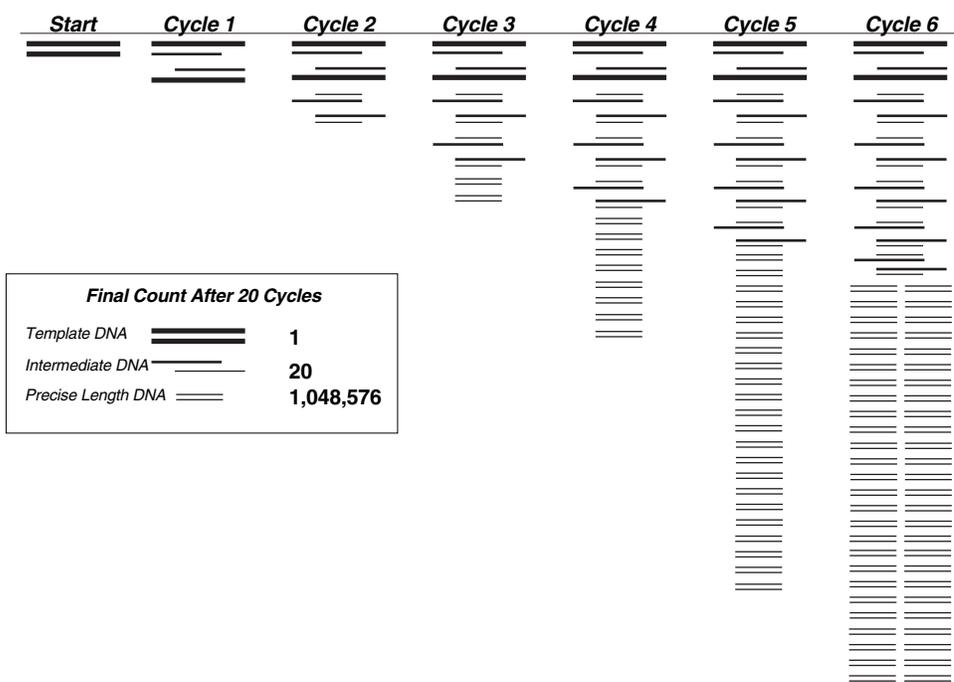


Figure A3. Schematic of PCR amplification of DNA fragments.