## UNLEASHING THE POWER OF EXPONENTIAL GROWTH—THE POLYMERASE CHAIN REACTION

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In the early 1980s the fruits of the molecular biology revolution were beginning to be realized. Geneticists were uncovering the genetic defects that lead to many hereditary diseases, and the newly burgeoning biotechnology industry was eager to provide physicians with simple diagnostic tests for such diseases. However, the best method available for detecting abnormal genes, Southern hybridization, required sizable DNA samples and several days to perform. In this environment, one of the most powerful molecular biology techniques known was born: the polymerase chain reaction, or PCR.

## Background

Researchers in the human genetics department of a young biotechnology company were trying to develop a practical method for the prenatal diagnosis of sickle-cell anemia. The molecular defect that causes most cases of this disease is a single nucleotide change in the sixth codon of the gene encoding the protein  $\beta$ -globin, one of the subunits of hemoglobin. Kary Mullis, a molecular biologist at the company, had an idea for a molecular method that would amplify specific DNA sequences. The detection of a single nucleotide change, as occurs in sickle-cell anemia, was the perfect test for his ideas.

Mullis's idea was an extension of known techniques for synthesizing specific pieces of DNA in vitro using chemically synthesized oligonucleotides and purified DNA polymerase, the enzyme that catalyzes the synthesis of DNA. First, a short oligonucleotide whose sequence was complementary to a portion of the target DNA was synthesized. Next, a fragment of DNA

containing the target sequence was isolated using restriction endonucleases, enzymes that catalyzed the cleavage of DNA at specific sequences. The isolated DNA fragment was then heated to denature the double-stranded helix into two single-stranded DNA molecules. At this point, the oligonucleotide was added to the DNA and allowed to anneal to the complementary region, thereby creating a primer-template complex, one of the substrates for DNA polymerase. The other substrates, the four deoxynucleotide triphosphates (dNTPs), were then added, so that DNA synthesis could occur. Although this method was useful for producing radioactively labeled pieces of DNA, it could not amplify a DNA sequence, only replicate it.

## **The Experiment**

Mullis designed a method that would actually amplify the amount of target DNA, a prerequisite for detecting a small DNA sequence within a large complex sample of genomic DNA. For instance, the human genome conations  $3 \times 10^9$  nucleotides of coding sequence. Molecular diagnosis of sicklecell anemia requires the detection of one altered nucleotide in one gene among the rest of the genome. To accomplish this, the region of the genome containing the alteration must be amplified.

Basing his work on the sequence of the  $\beta$ -globulin gene, which was known, Mullis designed primers that would anneal at sequences both upstream and downstream from the disease causing mutation. One primer was complementary to the coding strand, known as the (+) strand, the second was complementary to the noncoding, or (-), strand. When the primers were added to a sample of denatured genomic DNA along with DNA polymerase and the four dNTPs, DNA synthesis occurred across the region of the mutation from both of the original strands, producing two new double-stranded DNA molecules. Thus the DNA between the primer sites was doubled, not simply replicated as in the older method. Mullis realized that each cycle of DNA-primer annealing and DNA synthesis would yield twice as much target DNA as the previous cycle (see Figure). A chain reaction would ensue and the amount of DNA in the sample would grow exponentially. He called his technique the polymerase chain reaction (PCR) to reflect the mechanism by which amplification was occurring.

The first published test of the PCR made use of upstream and downstream oligonucleotide primers that flanked a 110-bp region of the  $\beta$ globin gene; the target region included the mutation found in sickle-cell anemia. These primers were mixed with samples of amniotic fluid that had been previously typed for the presence or absence of the mutation. After the samples were put through 20 cycles of heat denaturation, cooling to allow annealing, and DNA synthesis or primer elongation, the amount of  $\beta$ -globin target DNA in the samples was found to be enriched more than one million times  $(2^{20})$  compared with the initial samples. The exponential expansion of the DNA was easily demonstrated by comparing the same sample after 15 and 20 cycles. It was clear that the additional five cycles greatly increased the amount of DNA produced in the reaction. Next, Mullis tested the ability of the PCR to detect small quantities of DNA. He found that after



Schematic of the polymerase chain reaction (PCR) to amplify the  $\beta$ -globin gene. In this case, one oligonucleotide primer is complementary to the (+) strand and hybridizes downstream of the mutation that leads to sickle-cell anemia; the other primer is complementary to the (-) strand and hybridizes upstream of the mutation. Repeated cycles of DNA denaturation, primer annealing, and DNA synthesis amplify the target sequences between the primer-binding sites.

20 cycles, the  $\beta$ -globulin gene could be detected starting with a genomic DNA sample as small as 20 ng, which was 50 times smaller than the samples in the original tests. This finding implied that the PCR could be used in a variety of situations where only a small amount of DNA was available, contributing to the widespread use of the technique today.

## Discussion

Development of the PCR relied on two key insights. First, that a DNA

sequence could be amplified, not just replicated, if synthesis were carried out from both the coding and noncoding strands. Second, that a target DNA sequence would "grow" like dividing bacteria in a culture if the amplification cycle was repeated several times in succession. By employing this relatively simply methodology, Mullis developed one of the most powerful techniques in molecular biology.

The advantages of PCR were obvious from the first report. Almost

instantly, it became a standard technique used in all fields of biology and medicine, as well as the forensic sciences. Today, the technique is known not only to biologists, but also to people in all walks of life. In 1993, just eight years after his first report on the PCR, Kary Mullis was awarded the Nobel Prize in Chemistry for developing this revolutionary technique.