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Independent Study Mentorship 3A

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**Research Assessment #7**

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Aldridge, Susan. "Polymerase Chain Reaction (PCR)." Biotechnology: In Context, edited by Brenda Wilmoth Lerner and K. Lee Lerner, Gale, 2012. In Context Series. Science in Context, link.galegroup.com/apps/doc/IECUZQ506679329/SCIC?u=j043905010&xid=84f77a44. Accessed 1 Dec. 2017.

**Assessment:**

 This past week, I have finally come to a decision on what to do with my Original Work. For my Original Work, I want to conduct different DNA fingerprinting experiments to create a guide on which process will be faster and more efficient to use depending on the circumstance. However, I am not very knowledgeable in this area, so my very first step is to conduct research. My main goal with this research assessment is to learn about the Polymerase Chain Reaction and how to conduct the experiments.

 Initially, I had trouble finding an article that talks about the procedure of the Polymerase Chain Reaction. It is a very specific part of DNA Fingerprinting, and after many searches, I found one called “Polymerase Chain Reaction (PCR)” by Biotechnology. I came upon many key points while conducting this research. It allowed me to grasp more information and gave me a couple of tips and exactly what kind of equipment I will need. Previously, I did not know exactly how I was gonna conduct this experiment for my Original Works, but now, I know I will need to get an Automated Thermal Cycler. It made me realize that I need to budget my time. I do not have a lot of time left before my Original Work is completely due. The article also gave me insight on how to conduct my experiment without the advanced technology but it will be more tedious and have more plausible ways of error. The article enlightened me with the types of microscopic material I will need. It also informed me about a special type of bacteria called Thermus aquaticus which is a needed so I can get a heat-stable polymerase. This heat-stable polymerase so the enzyme, DNA Polymerase, will not be denatured. It will be critical to conduct this part with caution because enzymes are sensitive to temperature and there are multiple steps in my experiment that involve changing temperature. This research also emphasized that I will need a professional in this career field to help me with my experiment. There are so many other aspects of Polymerase Chain Reaction that I will not be to analyze due to my inexperience in that area.

The research I have conducted gave me a vivid insight on the procedure of Polymerase Chain Reaction. I noticed how I was able to easily understand how to conduct my experiment due to my previous knowledge I attained from my AP Biology class two years ago. I have gained valuable information from this article and am excited about this experiment in the near future and for the results, it has to bring to help me finish my Original Work. Aforetime, I had much confusion on the differences between the different types of DNA Fingerprinting procedures I will be conducting. Now, I believe I understand a bit on how I will conduct my experiment for this specific reaction and it allowed me to understand on a deeper level the main points of the procedure and their respective purpose. The research overall was very beneficial to my Original Work as well as my ISM journey through Chemical Engineering. Now I know what to aim for and how to proceed with my Original Work.

## **Polymerase Chain Reaction (PCR)**

*Biotechnology: In Context*, 2012 Updated: August 25, 2017

Introduction

The [polymerase chain reaction](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs) (PCR) is a way of amplifying [DNA](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs) fragments by using [enzymes](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs). It allows a billion-fold increase of the amount of DNA available for analysis in just a few hours. A single gene is just a tiny fraction of the whole DNA in a genome but, with PCR, it can be picked out from a sample and studied in detail.

Because DNA consists of two complementary strands, these can be separated and used as templates for the synthesis of two new strands. Primers are used to mark off the sequence along the genome that is to be amplified. Each cycle of PCR doubles the number of DNA molecules, and the reaction is applicable to the tiniest amounts of DNA, even a single molecule. The use of a heat stable enzyme known as Taq polymerase allows PCR to be carried out much more rapidly than previously. The polymerase chain reaction has become one of [molecular biology](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs)'s most widely used tools. Its applications include preimplantation diagnosis, forensics, molecular archaeology, and diagnosis of viral diseases. All of these rely upon the ability to utilize tiny samples of target DNA. Advances such as real-time PCR allow a quantitative dimension to be introduced to PCR analysis, which is proving useful in diagnostics and [gene expression](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs) studies.

## **Words to Know**

**Denaturation**

The unfolding of the three-dimensional structure of a biological molecule, such as a protein, on exposure to increased temperature or other unfavorable change in condition. Denaturation leads to loss of biological function.

#### **Molecular archaeology**

The study of DNA extracted from archaeological specimens, such as bones and mummies.

#### **Preimplantation genetic diagnosis (PGD)**

A procedure carried out in conjunction with in vitro fertilization in which DNA from an embryo is subjected to PCR and analyzed for the presence of mutations that could lead to single-gene disorders

#### **Primer**

A strand of DNA, made by chemical synthesis, that binds to the start or end of a single-stranded DNA target sequence in PCR and therefore starts off the action of DNA polymerase in copying the strand.

#### **Real-time PCR**

A method of PCR that allows quantitation of the DNA in a sample, by labeling the target with a measurable fluorescent tag.

#### **Reverse transcriptase**

An enzyme that copies [RNA](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs) into its complementary DNA molecule.

#### **Reverse transcriptase PCR**

Often abbreviated to RT-PCR, not to be confused with real-time PCR, reverse transcriptase PCR allows amplification of RNA. Reverse transcriptase converts RNA to DNA, which is then subject to the usual PCR method.

#### **Taq polymerase**

A heat stable DNA polymerase that is used to copy DNA in PCR.

## **Historical Background and Scientific Foundations**

The polymerase chain reaction (PCR) was discovered by Kary Mullis (1944–) of the Cetus Corporation in the United States in 1983, and he was awarded a Nobel Prize for this work in 1993. The method exploits the complementary nature of the two strands in the DNA molecule, which is a polymer consisting of a sequence of four nucleotide monomers. Each nucleotide contains one of four bases: adenine (A), thymine (T), cytosine (C) and guanine (G). In 1952, molecular biologists Francis Crick (1916–2004), an Englishman, and American James Watson (1928–) put the finishing touches to their three-dimensional structural model of DNA when they realized that it was weak hydrogen bonding, known as base-pairing, between A and T and C and G, which holds the [double helix](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs) of the molecule together. This base-pairing principle lies at the heart of PCR.

A typical PCR reaction begins with heating the original DNA sample to 193°F to 212°F (90°C to 100°C), which separates the two strands into single strands as the weak hydrogen bonds between the bases break. A cooling step to 86–149°F (30–65°C) follows, and two primer DNA molecules are added to the sample. The primers find the two ends of the target DNA sequence, which is only a small segment of the whole DNA molecule, and bind to them. Now the target sequence is marked at both ends by a short segment of double-stranded DNA. The enzyme DNA polymerase uses these two segments as starting points for synthesis of two complementary strands, one for each single strand, with single [nucleotides](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs) that are also added to the mixture. The result is that two single strands of DNA have become two double strands.

Thus one cycle of PCR makes two molecules of DNA from the original one. If the cycle is repeated, the amount of target DNA doubles again. As the cycles are repeated, the amount of DNA increases in a geometric fashion, doubling each time. Within a few hours, one nanogram of DNA becomes a milligram, which is sufficient for hundreds of DNA analytic tests. Previously, DNA could only be amplified by [cloning](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs) the sequence in bacteria, which took many days to produce the same amounts as are available in hours using PCR.

When Mullis first tried out PCR in the laboratory he uncovered a major limitation of the technique: Enzymes tend to undergo denaturation on heating. Each time the PCR mixture was heated in the first step of the cycle, the DNA polymerase molecule broke down, and a fresh amount of enzyme had to be added. This slowed down the reaction and made the whole process too expensive for widespread application. The problem was solved by the introduction of a heat-stable polymerase from the bacterium *Thermus aquaticus*. Thomas Brock (1926–) of Indiana University had discovered this microbe, which thrives at a temperature of 212°F (100°C), in the Great Fountain region of Yellowstone Park in 1965. It can survive life in these hot springs because its enzymes, including its DNA polymerase, are stable to heat. Taq polymerase is added to a PCR reaction at the start and continues acting throughout all the cycles required, whatever the temperature.

The second important advance in PCR was the introduction of automated thermal cyclers. Manual PCR requires a laboratory worker to move reaction mixtures between water baths at different temperatures, which is time-consuming, tedious, and prone to human error. The cycler is basically a metal block into which reaction tubes are placed and taken through a computer-controlled series of temperature changes. In a thermal cycler, a PCR cycle takes about five minutes. Many more samples thus can be processed than would be possible with manual PCR.

## **Impacts and Issues**

PCR has made many applications involving tiny amounts of DNA possible. One key advantage is that the original DNA need not be pure: A mixture will give good results because the primers, if well chosen, allow specific amplification of only the target sequence. A major PCR application is preimplantation genetic diagnosis (PGD), in which embryos are screened for genetic disease as part of an in vitro fertilization (IVF) process. The process involves taking a cell from an early embryo and using PCR to amplify the DNA, which is then tested for mutations linked to single-gene disorders, such as cystic fibrosis, thalassaemia, and sickle cell anemia, if there is known to be a family history and therefore a risk to the unborn child. All the embryos created by the IVF process are screened in this way, and only those shown to be free of [mutation](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs) are placed in the womb. The mother then can embark upon her pregnancy confident that she is carrying a child free of the genetic disease. The application of PGD replaces diagnosis of genetic disease by biopsy of the developing fetus later in the pregnancy, where difficult decisions about termination may have to be made.

Another technology made possible by PCR is molecular archaeology, which is the analysis of tiny fragments of ancient DNA. In an early example, researchers in Florida recovered DNA from a 7,000-year-old human preserved in a peat bog. Analysis revealed a gene sequence that was not similar to those of other Native Americans. This is just one example of how molecular archaeology is being used to reconstruct the population history of America. Other work has involved ancient DNA from insects trapped in amber and leaves and even dinosaurs. Dinosaur expert Jack Horner (1946–), of Montana State University, uncovered a 65-million-year-old skeleton from a *Tyrannosaurus rex* and was able to extract DNA from blood cells in an unfossilized section of the animal's femur. This showed an intriguing similarity to bird, rather than reptile, DNA, suggesting that perhaps modern birds evolved from dinosaurs.

PCR also has made a huge contribution to forensic science. The ability to amplify minute DNA samples from a crime scene for DNA profiling has meant that much more information can be extracted from forensic [evidence](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs). Therefore, a single hair root, or a tiny amount of blood, semen, or saliva, can be amplified for analysis. The DNA profile then is matched against DNA profiles of convicted criminals held in the Federal Bureau of Investigation's Combined DNA Index. Thousands have been convicted with the help of such DNA profiling evidence. Equally, forensic DNA profiling can also help prove a person's innocence.

The primers used in PCR are specific for known DNA sequences so they can be used to detect their presence in a sample, which has led to applications in medical diagnostics. For instance, viral DNA can be detected directly in a blood sample, instead of waiting for antibodies to the virus to develop. As well as amplifying DNA, PCR can now also be used to make copies of RNA molecules using a technique called reverse-[transcription](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs) PCR. The RNA molecules first are converted into their complementary DNA sequence using the enzyme reverse transcriptase. This DNA sample then can be amplified in the usual way. Therefore, reverse transcriptase PCR can be used to detect RNA viruses such as human immunodeficiency virus (HIV), hepatitis C, mumps, and measles virus. Reverse transcriptase PCR gives results for HIV just seven days after exposure, which allows time for prophylactic treatment. It is also used to monitor treatment with anti-viral drugs in both HIV and hepatitis C. If viral load decreases, then this indicates treatment is effective. If it does not decrease, then treatment can be altered accordingly.



Originally, PCR was used just to detect the presence of a particular DNA sequence. However, with a development known as real-time PCR the method has been made quantitative. The principle of the method is to add a fluorescent dye to the PCR reaction mixture. The dye is either specific to the target sequence or to double-stranded rather than single-stranded DNA. Either way, the signal from the fluorescent tag grows as the PCR reaction proceeds, allowing the reaction to be followed in real time and also the quantification of the original amount of target DNA. Application of real-time PCR has helped improve the diagnosis of infectious disease by quantifying the amount of virus in a sample.

Real-time PCR is often combined with reverse transcriptase PCR so that the amount of RNA in a sample can be determined. When [genes](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs) are expressed, they produce RNA that is translated into protein. Therefore determination of RNA levels gives useful information about gene expression in a tissue. There is increasing interest in how the pattern of gene expression differs in health and disease. This avenue of research sheds new light on cancer, helping identify subtypes of a tumor with different molecular signatures or gene expression profiles. Ultimately, this kind of information will help target treatments more accurately to a patient's tumor, which is the primary aim of the emerging science of pharmacogenomics.

PCR is a powerful tool in many molecular biology applications, but it does still have some limitations. For instance, PCR requires knowledge of at least a part of the target sequence so that the complementary primers can be constructed. Therefore it cannot be applied to the discovery and investigation of unknown DNA sequences. Another potential problem is that the sensitivity of PCR, with its ability to amplify such tiny amounts, makes it highly susceptible to contamination. Thus, DNA from the skin of laboratory workers or from the surrounding air might enter the reaction mixture and be amplified along with the target DNA. This is why the design of the primers for the target DNA in a PCR reaction is so important; they must be as specific as possible to the target so that only that sequence, and not any other sequence, is amplified. The issue of contamination is particularly important when it comes to the forensic applications of PCR. If proven, contamination could render a vital piece of evidence inadmissible.

PCR with standard Taq polymerase can amplify a DNA fragment only around 2,000 base pairs long. However, adding in a second DNA polymerase has helped to push this limit up to around 50,000 base pairs, which extends the range of applications somewhat. A further limitation on PCR is that Taq polymerase is not able to proofread. When DNA is copied, it is important that the sequence is copied accurately with the correct nucleotide being brought into the sequence each time. Other [DNA polymerases](https://docs.google.com/document/d/163Cv9Ovq1s0mZq-oBEPcK181Elw6rLHpyuUr4o5mUAA/edit#bookmark=id.gjdgxs) do have this ability, so that few mistakes are made. Therefore, although Taq polymerase is valuable for its ability to withstand higher temperatures, it is not ideal. Under standard PCR conditions, it will incorporate an incorrect nucleotide about once in every 20,000 base pairs in a sequence. However, advances in enzyme technology have led to the introduction of heat-stable DNA polymerases, which do have proofreading abilities and are able to produce more accurate PCR products.

## **Further Readings**

### **Books**

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### **Web Sites**

“Polymerase Chain Reaction (PCR).” *National Human Genome Research Institute*. <http://www.genome.gov/10000207> (accessed August 9, 2017).

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