Found in every living organism, enzymes are naturally produced proteins that regulate the rate of chemical reactions occurring in cells. Enzymes are responsible for many thousands of biochemical processes and are vital for cell growth and the production and use of energy within cells. Enzymes also play a vital role in industrial applications and microbiology research. By providing the foundation for many of the advances in biotechnology, enzymes are now revolutionizing health care and agriculture industries.

Unlocking Genetic Information
The current biotechnology revolution began only a few decades ago when scientists discovered they could use certain enzymes to unlock the genetic information contained in a cell’s chromosomes. Found in the genes of DNA strands, a sequence of nucleotides provide cells with instructions on how to build the proteins and amino acids that are used to carry out biological functions. Study of this sequence information has already led to major advances in cloning, forensic identification, and cancer research. It may also lead to therapeutic treatment and custom drug design for other diseases currently without a cure.

Thermally Stable Enzymes Needed for DNA Amplification
The techniques used to obtain sequencing information involve a series of processes to tear apart a cell and break open its components to reveal the underlying genes. This is done with the help of an enzyme called DNA polymerase. DNA polymerase also plays a critical role in another important genetic process that is used to create multiple copies of a known sample of DNA. This process, called amplification, works by placing an isolated sample of DNA in a solution of bases, primers, and DNA polymerase. The solution is then heated, which causes the double-stranded DNA to separate into two single strands. The mixture is then cooled so the primers can bind to

The enzymes initially used for sequencing and amplification were not thermally stable and tended to break down when subjected to the heat necessary to separate the DNA strands.
pre-established sites along each of the separate DNA strands. When the mixture is reheated, the DNA polymerase moves along the single strands of DNA and attaches the bases to form two identical, double strands of DNA. Repeating the heating and cooling cycle doubles the strands, and after about 30 cycles, the single fragment of DNA is sufficiently amplified to provide enough material for sequencing and further research.

The enzymes initially used for sequencing and amplification were not thermally stable and tended to break down when subjected to the heat necessary to separate the DNA strands. As a result, the polymerization process required constant monitoring to insure that adequate enzyme amounts were present.

Scientists discovered that microbes inhabiting the hot springs of Yellowstone Park produce a heat tolerant DNA polymerase. Although this enzyme, commercially developed as “Taq,” was thermally stable for amplification of DNA, it often proved inaccurate for DNA sequencing. As the problems with Taq became apparent, the search for superior enzymes became a major quest.

**ATP Supports the Search for a More Effective Enzyme**

In 1993, U.S. Biochemical Corporation (USB) applied to ATP to study the commercial potential of enzymes extracted from newly discovered microorganisms found living in the superheated waters of thermal vents on the deep ocean floor. Researchers hoped that microorganisms living in such extreme temperatures could be used to produce an enzyme that was both thermally stable and more accurate than Taq.

Working together with the University of Maryland’s Center of Marine Biotechnology (COMB), Amersham’s initial objective was to collect a large number of exotic hyperthermophiles from the superheated waters found in thermal vents along the ocean floor. Hyperthermophiles actually thrive in extremely high temperatures (80°–110°C), high pressures (@3300 l bps), and highly caustic solutions made up of sulfur and other minerals.

The unusual properties that make hyperthermophiles so appealing also make them extremely difficult and expensive to work with. The researchers designed special equipment and improved methods to allow the tasks of isolating and purifying the new enzymes to be carried out in an environment that matches their deep-sea habitat. Once the new enzymes were available, researchers screened them to determine how their properties compare to Taq.

As the work developed, researchers found that the DNA polymerases from deep-sea hyperthermophiles, like Pyrococcus furiosus, did not outperform Taq. Indeed, none of the enzymes they produced was found to be superior to Taq. As researchers continued their study, they began to realize that the search for the Holy Grail of enzymes was not yielding results from the ocean depths.

**Scientific Discovery Leads Amersham To Redirect Research Efforts**

Two years after the project began, a discovery published in the *Proceedings of the National Academy of Sciences* by Tabor and Richardson showed how scientists could re-engineer Taq to achieve greater fidelity and accuracy of sequence data.1 By creating a single amino acid change after originating this project, USB was purchased by U.K.-based Amersham International. Amersham pledged full commitment to this project using USB’s original plan and U.S.-based personnel and facilities. The ATP awarded $1.6 million to USB, and determined that continuation of the project after the company was purchased by Amersham International would be in the U.S. economic interest.

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in a polymerase, Tabor and Richardson showed that they could affect the enzyme’s ability to discriminate among nucleotides, and thereby produce uniform sequence signals. This important discovery meant that the structure of an enzyme such as Taq could be reengineered to provide the discrimination functions that lead to accurate, uniform sequence signal intensity. It wasn’t the Holy Grail, but it was very close.

So profound was this discovery that Amersham immediately stopped its effort to screen deep-sea hyperthermophilic enzymes and set about to reproduce the work of Tabor and Richardson. They soon discovered that these
results could not be replicated with hyperthermophilic enzymes, but they could reengineer other thermophilic enzymes to produce properties superior to Taq.

**Faster Development of ThermoSequenase**

With the knowledge that naturally occurring hyperthermophilic enzymes were not viable alternatives to reengineered thermophilic enzymes, Amersham licensed Tabor and Richardson’s technique and produced ThermoSequenase, a DNA polymerase that is both thermostable and produces amplified DNA sequences of uniform signal intensity.² With ATP’s support, the development of ThermoSequenase was advanced by at least six months. Former director of the Human Genome Project, Dr. David Smith of the Department of Energy, singles out the timely development of ThermoSequenase in 1995 as being critical to the Human Genome Project...

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ThermoSequenase is now incorporated into Amersham’s leading line of sequencing reagent kits. Currently, these kits account for sales of over $15 million per year and are expected to reach sales of $60 million in 2000.

Researchers using ThermoSequenase for DNA sequencing now obtain 10 to 25 percent more information from each sequencing experiment. The availability of ThermoSequenase has effectively reduced the cost of sequencing substantially. It has also enabled greater use of advanced automated sequencing machines that can...

² At the start of the project, Amersham was producing and marketing a DNA polymerase known as Sequenase that could produce accurate, uniform sequencing. However, this product was not thermally stable and could not compete with Taq when used in cycle sequencing machines.

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now operate without the need for constant monitoring of enzyme amounts. Customers of services using ThermoSequenase benefit from more accurate and more efficient sequencing. Development of ThermoSequenase also has stimulated competition in the enzyme market and has improved the quality of enzymes in biotechnology applications.

A New Field of Research Bears Fruit in Unexpected Ways

ATP’s cofunded project with Amersham has been praised as one of the first federally supported efforts to explore the potential of newly discovered deep-sea life. This has opened up a new field of research that was completely unknown two decades ago.⁴

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The Amersham researchers developed methods and applied them to search deep-sea life for an enzyme that offered a thermally stable and more accurate means of DNA sequencing. It did not find the hoped-for sequencing enzyme in the deep sea; in fact, during the course of the project, Amersham was able to conclude that hyperthermophiles were not the answer to the search for a better polymerase enzyme for DNA sequencing. The company quickly took a different approach to solving the problem. The project helped to position Amersham and its academic collaborators so that they could take advantage of new emerging techniques in enzyme reengineering. Pioneering use of these techniques led to accelerated development of ThermoSequenase. Hence, the project achieved its goal, but not in the expected way. And, it did find a useful enzyme in the deep sea, though not the one of central focus.

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Amersham has effectively diffused knowledge gained through the project by issuing 16 journal publications and a number of patents. The company filed for seven U.S. patents, five of which had been granted at the time of this study. In turn, the development of ThermoSequenase, and the release of information about it, have led to greater market competition, and encouraged the development of competing enzymes.