AMERSHAM PHARMACIA BIOTECH
(formerly U.S. Biochemical Corporation)

Searching for New Enzymes in Deep-Sea Microorganisms

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.

COMPOSITE PERFORMANCE SCORE
(Based on a four star rating.)
★ ★ ★

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

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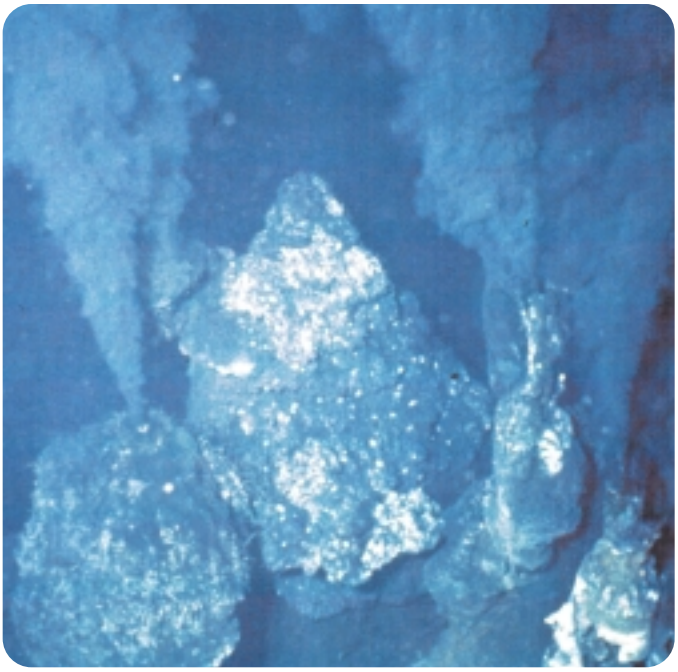

Deep Sea Vent "Black Smoker."

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.

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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.

At the time, USB, a small company located in Cleveland, Ohio, was a recognized leader in the development of DNA sequencing products and had more than 20 years of experience in the biochemical industry. Shortly 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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As the work developed, researchers found that the DNA polymerases from deep-sea hyperthermophiles, like Pyrococcus furiosus*, did not outperform Taq.*

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

[1] S. Tabor and C. Richardson, *Proceedings of the National Academy of Sciences*, 92, pp. 6339-6343, 1995.

PROJECT HIGHLIGHTS

PROJECT:

To study deep-sea microorganisms in an effort to identify, isolate, and characterize their commercially important enzymes for use in life sciences research, including a suitable enzyme for DNA sequencing, and industrial applications.

Duration: 2/15/1994 – 2/14/1997

ATP Number: 93-01-0113

FUNDING (in thousands):

ATP	$1,558	65%
Company	839	35%
Total	$2,397	

ACCOMPLISHMENTS:

Recent advances in molecular biology and discoveries of exotic new life forms have created commercial opportunities in the fields of genomics and enzyme development. The ATP-funded projeect led by Amersham explored the properties of newly discovered, heat-loving microorganisms with the objective of advancing the scientific knowledge of their genetic makeup and identifying the commercial potential of their expressed enzymes. Key accomplishments for this project include:

- isolation and genomic characterization of hyperthermophiles obtained from deep-sea thermal vent fluids;
- successful partial DNA sequencing of hyperthermophiles using directed cDNA cloning and other advanced sequencing techniques;
- successful cloning of genes encoding enzymes that have unique applications in life science research and are of significant commercial interest. These enzymes include DNA polymerases used in DNA cycle sequencing and several modifying enzymes used to improve the detection, selection and manipulation of DNA sequences;
- publication of sequencing results as "A Survey of the Genome of the Hyperthermophilic Archaeon Pyrococcus Furiosus" in the first volume of *Genome Science and Technology*, one of 16 publications which helped to diffuse knowledge gained through the project;
- applied for 7 patents and, by the time of this study, had been granted 5 patents:

  "Thermostable alkaline phosphatase of thermus thermophilus" (No. 5,633,138: filed 5/30/1995, granted 5/27/97);

  "Thermostable DNA polymerase from thermoanaerobacter thermohydrosulfuricus" (No. 5,744,312: filed 12/13/1996, granted 4/28/1998);

  "Thermostable DNA polymerases" (No. 5,885,813: filed 5/14/1996, granted 5/23/1999);

  "Modified Pol-II type DNA polymerases" (No. 5,827,716: filed 7/30/1996, granted 10/27/1998);

  "Proteins from pyrococcus furiosus" (No. 5,719,056: filed 4/26/1996, granted 2/17/1998).

CITATIONS BY OTHERS OF PROJECT'S PATENTS:

See Figure 3.1.

COMMERCIALIZATION STATUS:

From its research on hyperthermophiles, Amersham developed a thermally stable alkaline phosphatase with applications in the detection of genetic diseases. This product has reached sales of $1.2 million per year. With the application of newly discovered enzyme reengineering techniques, Amersham accelerated development of ThermoSequenase, a DNA polymerase that is both thermostable and accurate for DNA sequencing. Sales of ThermoSequenase are $15 million per year and are expected to reach $60 million by 2000. Enzymes to replace chemical catalysts in large-scale industrial applications have yet to be developed.

OUTLOOK:

Amersham completed many of the technical objectives of this project and accelerated research and development of new enzymes for use in DNA diagnostics. Although it successfully isolated at least one commercially useful enzyme, it did not achieve the ultimate goal of finding a superior hyperthermophilic enzyme. Nevertheless, the project is at least partially responsible for accelerated development of a thermally stable DNA polymerase, ThermoSequenase. This enzyme has helped to revolutionize automated DNA cycle sequencing by providing improved accuracy in fluorescent readouts of sequence information. Benefits are accruing from a growing number of health care and diagnostic applications that rely on accurate and timely DNA sequence information. The potential application areas are numerous, including medical diagnostics, gene therapy, drug discovery, human therapeutics, cell cloning, cancer genetics, agricultural biotechnology, forensic identification, toxicology, and environmental monitoring.

The development of competing enzymes is underway and there is substantial market competition in this area. The outlook is promising that the considerable knowledge gained from this project may yet lead to the development of new classes of industrial enzymes.

Composite Performance Score: ★ ★ ★

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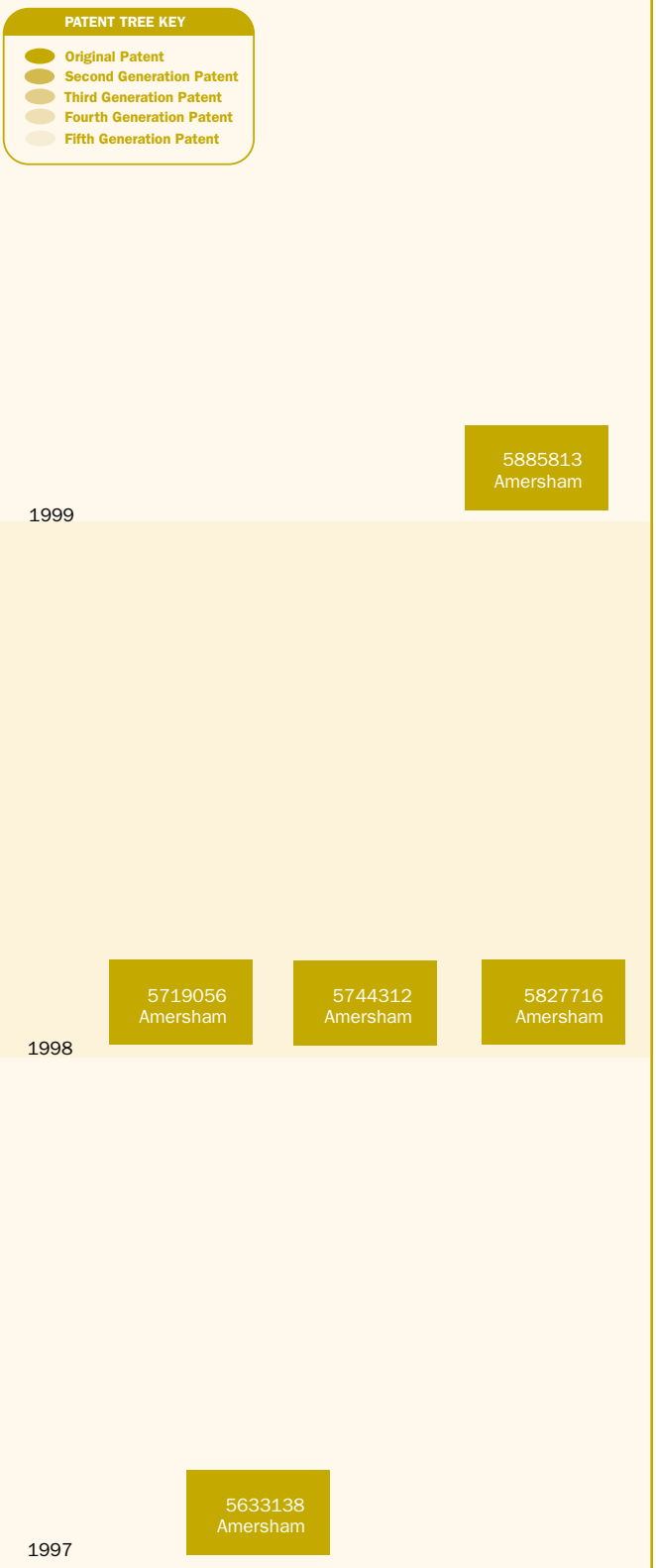
in a polymerase, Tabor and Richardson showed that they could affect the enzyme's ability to discriminate among nucleotides, and thereby produce uniform sequence signals. This important discovery meant that the structure of an enzyme such as Taq could be reengineered to provide the discrimination functions 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

Figure 3.1 Patent Tree for Project Led by Amersham: Citations by Others of Amersham Patents



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

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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, stating, "We would be in deep trouble if [such technologies] were at a less mature stage of development."[3]

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.

[3] The Seventh International Genome Sequencing and Analysis Conference, September 1995, available on the Internet < www.ol-nl.cov/TecllResources/Hulllall Genome/publicat/97pr/evolve.html>.

Researchers using ThermoSequenase for DNA sequencing now obtain 10 to 25 percent more information from each sequencing experiment.

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.

[4] William J. Brode, The Universe Below: *Discovering the Secrets of the Deep Sea*, Touchstone: Simon & Schuster, 1997, p. 283.