

Third Wave Technologies, Inc.

Cleavase Technology Reduces Cost and Shortens Time of Genetic Analysis

By 1994, scientists were generating vast amounts of genetic sequence data, but they lacked a low-cost, user-friendly system to analyze that data and translate it into a format useful for research and diagnostic work. The analysis systems that were available for determining genetic differences between individuals required expensive and extended procedures under highly controlled conditions. Prior to 1994, scientists from Third Wave Technologies, Inc., a company of three researchers, had discovered a promising new enzyme technology that had the potential to facilitate the more rapid, inexpensive, and simplified analysis of individual genetic variabilities. Third Wave's Cleavase technology held the potential to reduce the cost of genetic analysis by up to 90 percent and shorten the analysis time from days to hours.

Private capital sources viewed the technology, and the company, as too high risk for investment. However, the proposed innovation, cost savings, and potential for creating a new tool for genetic analysis met the criteria to receive cost-shared funds for a two-year research project as part of the Advanced Technology Program's (ATP) "Tools for DNA Diagnostics" focused program. By the end of the ATP-funded project, three generic genetic analysis tools had been created. The company had also presented its findings at a dozen conferences and had devoted substantial additional funds to continue the research and development of its Cleavase-based products. In 2001, Third Wave earned more than \$34 million in revenues and conducted a successful initial public offering.

COMPOSITE PERFORMANCE SCORE

(based on a four star rating)

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Research and data for Status Report 94-05-0012 were collected during December 2001 and December 2002 - January 2003.

Scientists Lack Methods To Analyze Genetic Data

The genetic revolution was well underway in 1994. Researchers were looking for ways to identify new genes and genetic mismatches between those genes. Genetic mismatches are a basis of genetic diversity and carry significant information with regard to the cause of disease and normal development. Mismatches are common, and most of the differences between individuals are differences in single nucleotide polymorphisms (more commonly known as SNPs). Researchers in the public and private sectors spent considerable effort determining how to analyze SNPs and other types of genetic mismatches in order to accelerate the rate of research and discovery within the diagnostic and therapeutics fields.

In 1994, no commercially viable method existed for analyzing genetic mismatches. In order to determine if these genetic mismatches were present, scientists had to perform time-consuming and expensive analyses, which involved either resequencing the DNA using polymerase chain reaction (PCR, which is a process by which a specific region of DNA is amplified by DNA synthesis enzymes working from the two "primer" ends banding the area to be amplified and working inwards) or using a process known as restriction fragment length polymorphism (RFLP) determination. RFLP is a technique in which organisms may be differentiated by an analysis of the patterns derived from cleaving their DNA. In order to perform PCR, scientists still needed to perform extensive testing to detect and separate the desired DNA strands. RFLP analysis also required

many time-consuming and complicated steps. As the Human Genome Project advanced and continued to generate volumes of genetic data, scientists needed a faster process for analyzing gene sequences.

Cleavase Technology Could Potentially Bridge the Gap

Nucleic acid sequence data for genes were accumulating through the Human Genome Project and other sources. Scientists needed fast, cost-effective, and easy-to-use tests for the detection of mutations. In 1993, Third Wave developed a system that included novel methods and enzymes for the targeted detection and cleavage of DNA and RNA. The basis for Third Wave's proposed toolbox derived from a set of newly discovered bacterial enzymes that fell under the company's trade name Cleavase, but that had yet to be fully developed. The key principle behind the Cleavase enzymes is that they can easily be molecularly tuned to cleave any predetermined sequence of nucleic acids. Successful exploitation of the unique features of Third Wave's Cleavase enzymes could potentially lead to a major breakthrough in nucleic acid detection capability, but further research was required.

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Third Wave scientists believed that a fully developed Cleavase-based DNA analysis system would provide a fast and inexpensive means of obtaining biological and human genetic information. Research and diagnostic answers could be available in a few minutes instead of hours or days. Moreover, the cost of Cleavase-based diagnostics was expected to be 10 to 20 percent of the cost of the systems on the market in 1994. As a result, Cleavase-based products could offer effective new ways to facilitate therapeutic decisions based on individual genetics, could help accelerate the rate of research and discovery in general, and could help reduce U.S. healthcare costs by making widespread, preventive diagnostic screening less costly.

ATP Funding Necessary To Explore Cleavase's Properties

Third Wave was founded in October 1992 to develop new methods of analyzing genetic material. Less than one year later, the company created its basic Cleavase technology, but as an early-stage company, Third Wave did not have ready access to capital that could be used to fully research Cleavase's capabilities. In its proposal to ATP, Third Wave's CEO, Dr. Lance Fors, commented that without ATP funding, Third Wave would have to abandon development of the potentially powerful generic Cleavase technology in favor of focusing on developing one or two DNA diagnostic kits for specific applications. With ATP support, however, Third Wave would be in a position to increase the rate of research by at least a factor of four. At the time of Third Wave's 1994 application, sources of private capital viewed the company's technology as too high risk for private funding. Because Third Wave was the only company pursuing this technology, it would not advance without ATP support.

Enormous Potential Market Existed for Cleavase-Based Products

The Cleavase technology, if developed successfully, would allow any target DNA or RNA sequence to be detected and cleaved at any desired position in a fast, automated, user-friendly, low-cost fashion that would make large-scale screening possible for the first time.

Research and diagnostic answers could be available in a few minutes instead of hours or days.

In 1994, there was strong and immediate demand for this technology in the research marketplace from more than 400,000 life science researchers in 55,000 research laboratories worldwide. Cleavase-based diagnostic products would also find strong demand in hospital testing laboratories, clinical laboratories, and doctors' offices. Applications included low-cost, kit-based diagnostic products for DNA typing; military and

criminal identification; forensic science; paternity testing; food processing test applications like monitoring for E.coli in beef, agriculture, and animal husbandry; and changes in DNA and RNA sequences or RNA expression levels in response to environmental changes.

Cleavase Technology Resulted in Two Specific Applications

Third Wave used part of the ATP funds to create a process that was at least as effective as RFLP. The new process, known as Cleavase Fragment Length Polymorphism (CFLP), generated a distinct bar code for every unique DNA sequence. Thus, genetic mismatches could be detected by comparing a sample to a normal coding pattern. The method was expected to have a broad range of applications for research, the diagnosis and treatment of infections and hereditary diseases, and the acceleration of drug development. For example, CFLP could distinguish between different strands of the bacterium that causes tuberculosis. This meant that doctors would be able to treat specific patients with tailored drug treatments that would minimize drug-resistance problems.

The rest of the ATP funds were used to develop a preliminary assay that would later become the "gold standard" for non-polymerase chain reaction-based (non-PCR-based) SNP detection due to its speed, accuracy, and relative ease of use. Using this assay, Third Wave developed a rapid non-PCR-based SNP detection system that works by invading the normal DNA duplex with a third piece of single-stranded DNA. Hence, the system became known as the Invader system. In the Invader process, two short DNA probes hybridize to the target to form the structure recognized by the Cleavase enzyme. When the proper structure is formed, the enzyme then cuts one of the probes to produce a target-specific fluorescent signal. Each target generates thousands of signals per hour, yielding millions of detectable signals per target. In short, it brings detection levels in line with those of PCR without requiring the post-amplification tests to separate the genetic material that PCR requires.

After this ATP-funded project was complete, Third Wave sought and received additional research money from public and private sources to further develop the CFLP and Invader technology. In 1997, Third Wave

received another ATP award to build genetic detection tools for healthcare applications. This research ultimately led to a robust product line. The enhanced Invader assay began to generate revenues in 2000. That year, Third Wave earned \$11.4 million in revenues. In 2001, revenues increased to \$34.1 million, and with cost of goods sold at \$32.9 million that year, Third Wave finally became profitable. On the strength of these revenues, the company held an initial public offering (IPO) in 2001, which raised \$82.5 million for Third Wave. With the economic downturn, however, Third Wave found it difficult to maintain profitability. Nevertheless, the Invader assay continues to be the gold standard for anyone engaged in non-PCR analysis.

Third Wave Shares Project Knowledge

Third Wave shared a significant amount of the knowledge it gained through the ATP-funded project. The company has published more than 20 papers and has presented more than 30 posters. Third Wave representatives also made presentations at 12 conferences during 1995 and 1996 to showcase the information learned from the ATP-funded research. In addition, various newspapers, magazines, and trade press published six articles about Third Wave's ATP-funded technology in 1995 and 1996 and numerous additional articles since then. Third Wave has 10 issued U.S. patents flowing from technology developed during the project. Moreover, the successful project substantially improved Third Wave's credibility, which assisted company executives in obtaining additional capital and in conducting the 2001 IPO.

Conclusion

In 1994, scientists sought to shorten the time required to analyze genetic data. Third Wave applied to ATP for award money to fund their research into this new method of genetic analysis. In their research plan, they proposed to step outside traditional thinking and develop an entirely new method of analyzing segments of genetic material. By the close of the ATP-funded project, Third Wave had created a new method of analyzing DNA and had disseminated that knowledge through publications and patent applications. Third Wave conducted an initial public offering and continues to produce DNA analysis machinery.

PROJECT HIGHLIGHTS

Third Wave Technologies, Inc.

Project Title: Cleavase Technology Reduces Cost and Shortens Time of Genetic Analysis (Development of a Generic Technology for the Targeted Detection and Cleavage of DNA and RNA)

Project: To develop simple-to-use, low-cost diagnostic tools that rapidly detect specific DNA and RNA sequences for broad-based medical diagnosis and for tracking treatments.

Duration: 1/1/1995-12/31/1996

ATP Number: 94-05-0012

Funding (in thousands):

ATP Final Cost	\$1,998	72%
Participant Final Cost	<u>771</u>	28%
Total	\$2,769	

Accomplishments: During this research project, Third Wave grew from a company of 3 researchers to a staff of 195. The ATP-funded program brought Cleavase technology to the point where it could be developed into prototypes and specialized into different products.

Technology developed during this ATP-funded project also led Third Wave to apply for 11 patents (of which 10 have been issued) for its Cleavase Fragment Length Polymorphism (CFLP) and Invader technology, as well as for enzyme improvements. The following patents resulted from this project:

- "Rapid detection of mutations in the p53 gene" (5,843,654: filed June 7, 1995, granted December 1, 1998)
- "Rapid detection and identification of pathogens" (6,372,424: filed August 30, 1995, granted April 16, 2002)
- "Detection of nucleic acid sequences by invader-directed cleavage" (5,846,717: filed January 24, 1996, granted December 8, 1998)
- "Invasive cleavage of nucleic acids" (6,348,314: filed July 9, 1996, granted February 19, 2002)
- "Detection of nucleic acid sequences by invader-directed cleavage" (6,001,567: filed July 12, 1996, granted December 14, 1999)

- "Invasive cleavage of nucleic acids" (5,985,557: filed November 26, 1996, granted November 16, 1999)
- "Cleavage of nucleic acid using thermostable *Methanococcus jannaschii* FEN-1 endonucleases" (5,843,669: filed November 29, 1996, granted December 1, 1998)
- "Cleavage of nucleic acids" (6,090,543: filed December 2, 1996, granted July 18, 2000)
- "Cleavage agents" (6,090,606: filed December 2, 1996, granted July 18, 2000)
- "Rapid detection and identification of nucleic acid variants" (5,888,780: filed February 19, 1997, granted March 30, 1999)

Third Wave has published more than 20 papers and has presented more than 30 posters. Third Wave representatives made presentations at 12 conferences during 1995 and 1996 to showcase the information learned from the ATP-funded research. In addition, in 1995 and 1996, various newspapers, magazines, and trade press published six articles about the technology and numerous additional articles have been published since then. The successful ATP project substantially improved Third Wave's credibility, which assisted company executives in obtaining additional capital and in conducting its 2001 initial public offering.

Commercialization Status: By 1997, the ATP-funded research brought the Cleavase system to the point where Third Wave-funded research and development activities could develop products. From 1997 to 2001, Third Wave commercialized several CFLPs.

Outlook: At the close of this project, the commercial outlook for Third Wave's Cleavase technology was uncertain because no products had reached the commercialization phase. Between 1997 and 2001, the outlook improved significantly as numerous products became available. As of January 2002, Third Wave and the entire biotechnology industry have experienced a major downturn, which has led to a significant contraction in the market for the company's products. Third Wave has since reorganized and now focuses on the clinical molecular diagnostic market. Until the current volatility in the biotechnology market ends, the outlook for

Third Wave will be uncertain. However, CFLP-based DNA analysis will continue to be used in laboratories, making the outlook for the technology very good.

Composite Performance Score: * * * *

Number of Employees: Three employees at project start, 195 as of December 2002.

Focused Program: Tools for DNA Diagnostics, 1994

Company:

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