tiny cracks appear in the crystal that grow with each laser shot. Eventually the damage significantly disrupts the laser beam quality by distorting the laser light and reducing its energy.

Future areas of study also include growing several crystallized proteins, among them human insulin, whose use depends on a better understanding of how they grow and dissolve in solution. The crystal development team is also planning to study biomineralization in more detail, in particular the growth characteristics of the essential calcium carbonate mineral that forms the skeletal tissue of most organisms. The study should shed light on how living organisms produce crystalline materials, thereby pointing the way for new, nanostructured materials for industry.

By understanding and then controlling the crystallization process at the molecular level, complex microstructures can be synthesized that will affect many disciplines and technologies, says De Yoreo. "There's a revolution on the horizon in materials and materials processing, but to get there we need to acquire the scientific underpinnings of crystal growth," he says. Thanks to the AFM, that day is rapidly approaching.

Key Words: atomic-force microscope (AFM), protein crystallography, crystals, KDP (potassium dihydrogen phosphate), National Ignition Facility (NIF), scanning tunneling microscope (STM), stockpile stewardship.

References

1. "Growing High-Quality KDP Crystals Quickly," *Energy & Technology Review*, UCRL-52000-94-11 (November 1994), pp. 3–5.
2. The December 1994 issue of *Energy & Technology Review*, UCRL-52000-94-12, is dedicated to a complete description of NIF and its planned uses.

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About the Scientists

JAMES DE YOREO joined Lawrence Livermore National Laboratory in 1989 as a physicist in the Chemistry and Materials Science Directorate. He received his B.S. from Colby College and his M.S. and Ph.D. from Cornell University. He is currently the leader of the Laboratory's crystal development team and has done extensive research in crystal growth physics and applications. In 1994, he shared an R&D 100 Award for the development of a rapid growth process for KDP (potassium dihydrogen phosphate) laser crystals with colleagues at the Laboratory and at Moscow State University in Russia. He has written numerous articles on organic and inorganic crystal growth and is co-holder of one existing and one pending U.S. patent related to crystal growth.

TERRY LAND received both her B.S. in chemistry (1988) and her Ph.D. in physical chemistry (1992) from the University of California, Irvine. She joined the Laboratory's Chemistry and Materials Science Directorate in 1992. Her primary area of academic and professional research has been the fundamental growth mechanisms of solution-grown inorganic and macromolecular biological crystals using advanced techniques such as scanning tunneling and atomic-force microscopy. She has co-written over 20 scholarly articles and has been a presenter and invited speaker at meetings and conferences in the U.S. and Europe on the mechanisms and techniques of crystal growth.

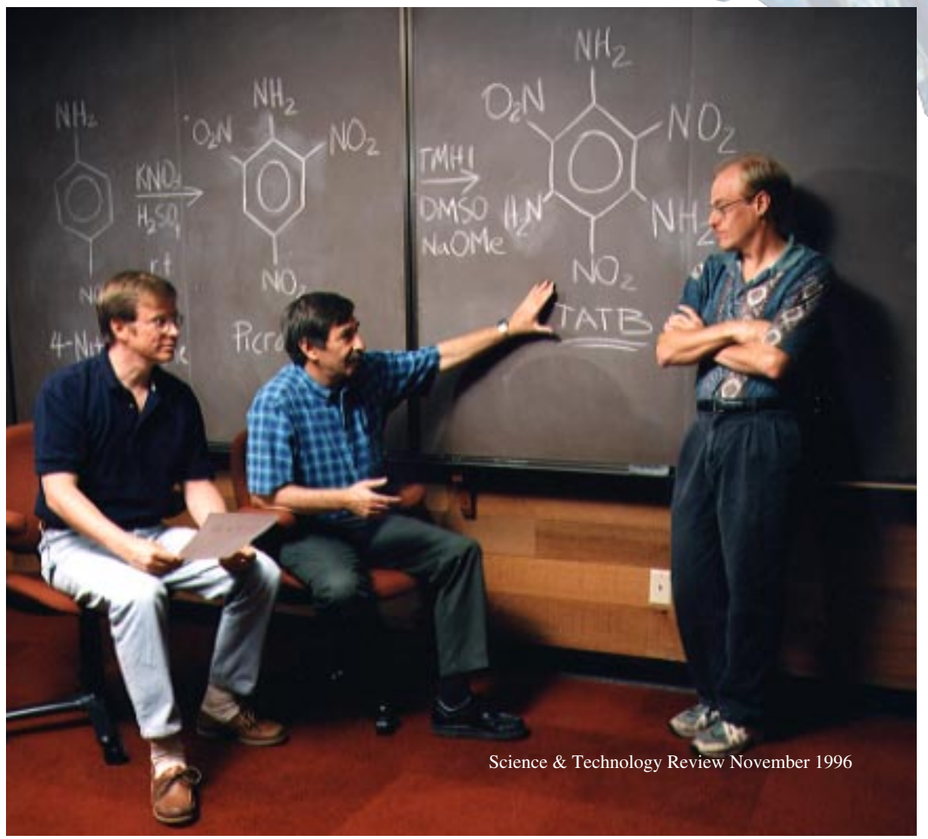
Addressing a Cold War Legacy with a New Way to Produce TATB

ONE of the most important accomplishments made by weapons laboratories' chemists in the past two decades has been the formulation of powerful conventional high explosives that are remarkably insensitive to high temperatures, shock, and impact. These insensitive high explosives (IHEs) significantly improve the safety and survivability of munitions, weapons, and personnel. The Department of Energy's most important IHE for use in modern nuclear warheads is TATB (triamino-trinitrobenzene) because its resistance to heat and physical shock is greater than that of any other known material of comparable energy.

The Department of Energy currently maintains an estimated five-year supply of TATB for its Stockpile Stewardship and Management Program (see the August 1996 *Science & Technology Review*, pp. 6–15), which is designed to ensure the safety, security, and reliability of the U.S. nuclear stockpile. The Department of Defense is also studying the possible use of TATB as an insensitive booster material, because even with its safety characteristics, a given amount of that explosive has more power than an equivalent volume of TNT.

In addition to its military uses, TATB has been proposed for use as a reagent in the manufacturing of components for liquid crystal computer displays. There is also interest in employing the explosive in the civilian sector for deep oil well explorations where heat-insensitive explosives are required.

Despite its broad potential, the high cost of manufacturing TATB has limited its use. Several years ago, TATB produced on an industrial scale in the U.S. was priced at $90 to $250 per kilogram. Today it is available to customers outside DOE for



Rob Schmidt (left), Alex Mitchell, and Phil Pagoria discuss the chemistry of the method for synthesizing TATB (triamino-trinitrobenzene) developed at Livermore. Their method lowers the cost and production time of this insensitive high explosive and increases the environmental friendliness of the manufacturing process. (The reaction scheme on the board appears also in the figure on p. 23.)

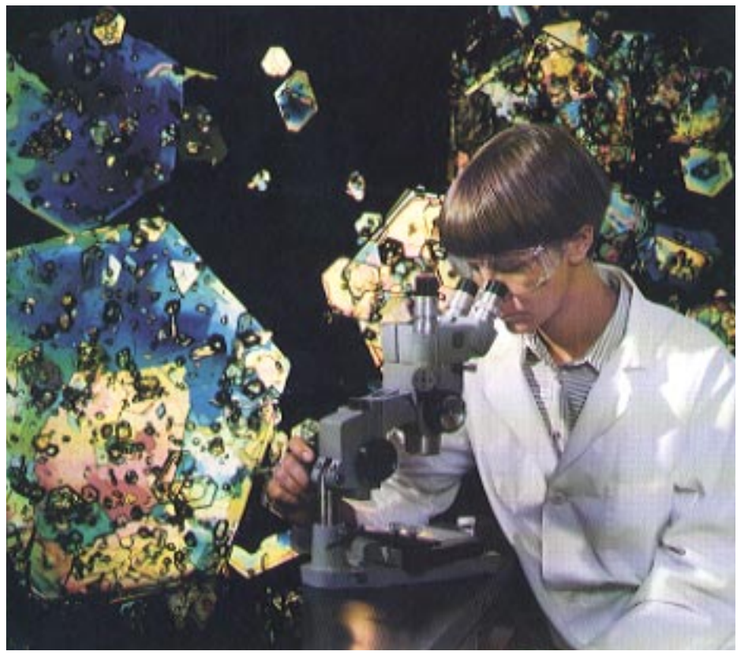
about $200 per kilogram. In response to a need for a more economical product, chemists at Lawrence Livermore have developed a flexible and convenient means of synthesizing TATB as well as DATB (diamino-trinitrobenzene), a closely related but less well known IHE developed by the U.S. Navy. The initial phase of this work was funded by the Department of Defense (U.S. Navy) to explore the chemical conversion of surplus energetic materials to higher value products as an alternative to detonation.

The Lawrence Livermore process—also called the VNS (vicarious nucleophilic substitution) process—should be able to produce TATB for less than $90 a kilogram on an industrial scale in about 40% less manufacturing time. The process also offers significant advantages over the current method of synthesis in environmental friendliness, for example, by avoiding chlorinated starting materials. What's more, the process uses either inexpensive, commercially available chemicals or surplus energetic materials from both the former Soviet Union (UDMH, a rocket propellant) and the U.S. (Explosive D, a high explosive).

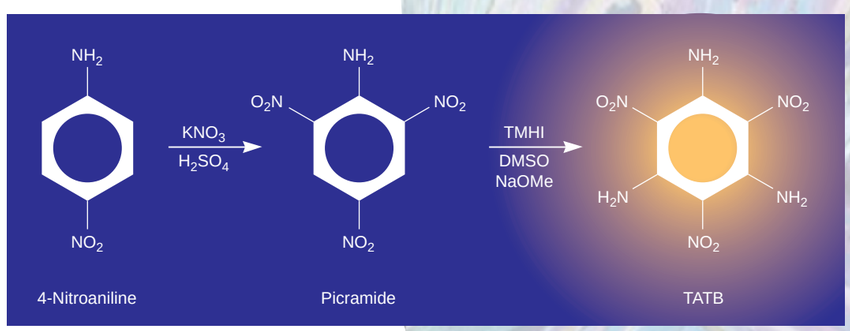
By using UDMH (*uns*-dimethylhydrazine) and Explosive D (ammonium picrate), this process disposes of energetic materials left over as a legacy of the Cold War in an environmentally responsible manner. It allows the use of surplus energetic materials as unique feedstocks to make more valuable materials such as higher value explosives or other products. Indeed, the new chemistry is also applicable to the synthesis of chemicals that are important intermediates in the preparation of numerous pharmaceutical and agricultural chemicals.

Current Process Produces Impurities

The currently accepted method for manufacturing TATB in the U.S. involves a reaction sequence that starts with the relatively expensive and domestically unavailable chlorinated compound TCB (trichlorobenzene). Elevated temperatures of 150°C are required for two of the reaction steps leading to TATB. The major impurity produced is ammonium chloride; in addition there are low levels of chlorinated reaction side-products.



Fran Foltz examines crystals of TATB (triamino-trinitrobenzene) under a microscope. The background photograph shows TATB crystals at high magnification.



The process of synthesizing TATB (triamino-trinitrobenzene) from picramide using TMHI (trimethylhydrazinium iodide) as expressed in this reaction scheme may result in a large decrease in the cost of TATB.

The VNS process is more environmentally friendly than the current synthesis. It employs mild reaction conditions and eliminates the need for chlorinated starting materials. The latter characteristic is especially important in light of the growing movement to eliminate chlorinated compounds from the industrial sector altogether because of their possible adverse environmental effects.

The VNS process depends on two key materials, TMHI (trimethylhydrazinium iodide) and picramide (trinitroaniline), which can be obtained from either inexpensive starting compounds or surplus energetic materials available from demilitarization activities. TMHI can be prepared directly from hydrazine and methyl iodide, or it can be synthesized by reacting UDMH with methyl iodide. Some 30,000 metric tons of UDMH rocket propellant are located in the former Soviet Union, where they await disposal in a safe and environmentally responsible manner.

Two U.S. companies have received congressional funding to demilitarize UDMH in Russia using a chemical process that produces lower value products (ammonia and dimethylamine). In contrast, the VNS process converts UVMH to TMHI, which will be used for the production of higher value products such as TATB.

TMHI reacts with picramide in the presence of a strong base to give TATB at a yield of over 95%. Picramide may be obtained from low-cost, domestically available nitroaniline. Or, as in the synthesis of TMHI, picramide may be synthesized from a surplus munition, in this case, Explosive D. Several million kilograms of Explosive D are available for disposal in the U.S.

New Process to Increase TATB Availability

The availability of relatively inexpensive TATB using the improved synthesis will facilitate its use, both for military and civilian applications. At the same time, the VNS process provides a new avenue for disposing of large quantities of energetic materials that are a legacy of the Cold War. The process reflects a new perspective within both the Department of Defense and the Department of Energy—treating surplus energetic materials as assets to be recycled whenever possible.

This new approach to the synthesis of TATB and other insensitive energetic materials is still in the development stage. Over the next year, the synthesis will progress from the 10-gram scale at the Laboratory's state-of-the-art High Explosives Applications Facility to the kilogram-pilot-plant scale at Site 300. During this stage, the necessary performance and sensitivity tests will be conducted to qualify the synthesis in terms of ease of use, purity, particle size, and cost. The process will also be evaluated for environmental friendliness and waste reduction. At the conclusion of the study, the technology will be ready for transfer to an industrial partner for commercial scale-up.

Key Words: insensitive high explosives (IHE), stockpile stewardship, TATB (triamino-trinitrobenzene).

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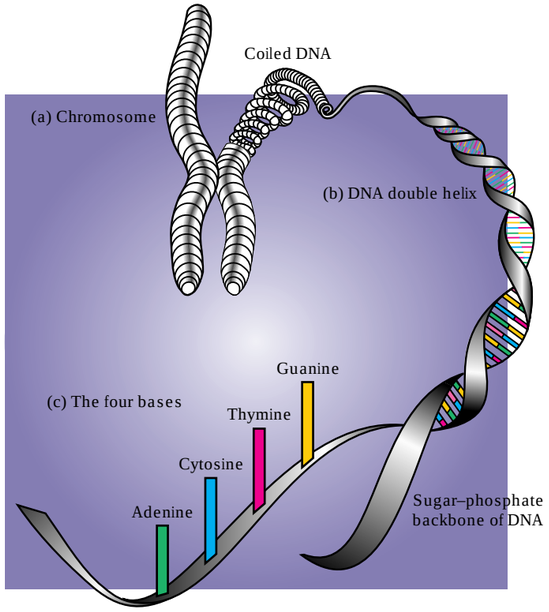
DNA Sequencing
The Next Step in the Search for Genes

FOR Lawrence Livermore researchers involved in the Human Genome Project, gene hunting is like standing in front of a mountain, shovel in hand, and knowing somewhere, amongst tons of rock, is the motherlode.

The search has been going on for years, but it has accelerated recently to a new level, noted Linda Ashworth, a Lawrence Livermore biomedical scientist working in the Laboratory's Human Genome Center. "In 1992, about 80% of our effort was devoted to generating road maps for specific chromosomes or regions on a chromosome.[1] Now, about 70% of our effort goes towards sequencing DNA and furthering sequencing technology."

Sequencing involves determining the exact order of the individual chemical building blocks, or bases, that form DNA. The four chemical bases—commonly abbreviated as A, G, C, and T—bind together to create base pairs that are the "business end" of the DNA molecule. (See figure at right.)

After researchers sequence a piece of DNA, they search for the special strings of sequence that form genes. The ultimate goal of the worldwide Human Genome Project is to find all the genes in the DNA sequence and develop tools for using this information in the study of human biology and medicine. Major benefits will be a better understanding of and treatments for genetic diseases.



The basics of genetics. Each cell in the human body (except red blood cells) contains 23 pairs of chromosomes. Chromosomes are inherited: each parent contributes one chromosome per pair to their children. (a) Each chromosome is made up of a tightly coiled strand of DNA. The current research lies in the details of the DNA structure, which, in its uncoiled state reveals (b) the familiar "double helix" shape. If we picture DNA as a twisted ladder, the sides, made of sugar and phosphate molecules, are connected by (c) rungs made of chemicals called "bases." DNA has four bases—adenine (A), thymine (T), guanine (G), and cytosine (C)—that form interlocking pairs. The order of the bases along the length of the ladder is called the DNA sequence. The hunt for genes is focused on reading the order of the bases for each DNA strand and determining which parts of the sequence constitute genes.

To the Hunt

It is a hunt of gigantic magnitude, a bit like chopping away at Mount Everest with a pick and shovel. Genes range in size from 1,000 base pairs (bp) to over 1,000,000 bp. The smallest human chromosome (21) contains approximately 45 million bp; the largest (chromosome 1) has approximately 250 million. The entire human genome contains about 3 billion bp. As of mid-August 1996, about half of one percent of the human genome had been sequenced worldwide in 15,000 bp chunks or longer. "Maybe six times that amount has been sequenced in smaller pieces, which are useful for diagnostic purposes," according to Jane Lamerdin, one of the Center's researchers.

To put things in perspective, there are perhaps 100,000 human genes scattered throughout the chromosomes, interspersed with non-gene material. "Chromosome 19, the one we're focusing on here at Livermore, has about 2% of the total DNA, so we're estimating as many as 2,000 genes," said Ashworth. "We have a handle on about 400, so there are a lot left to find." (See the box, next page.)

High Technology to the Rescue

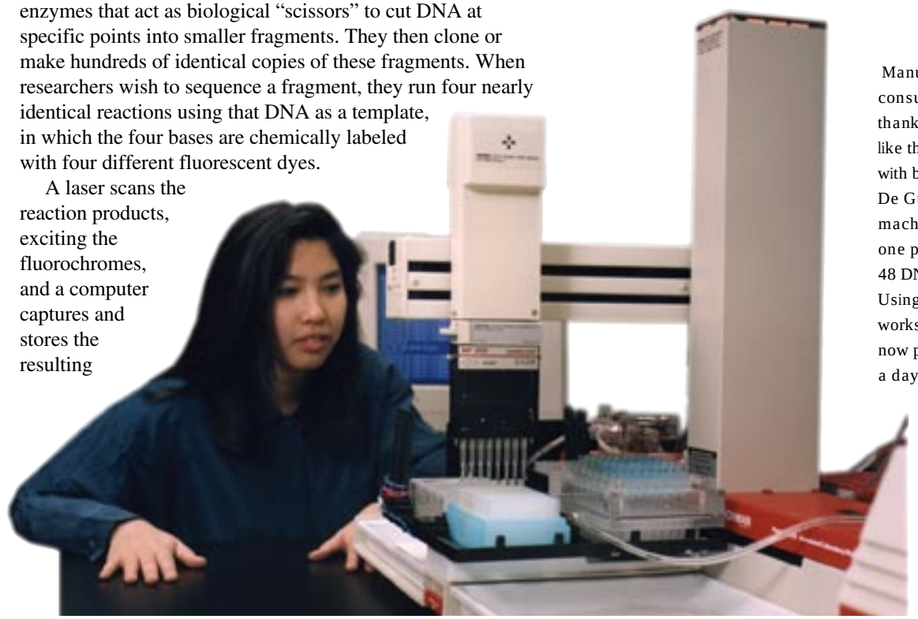
What has made it possible to even contemplate sequencing the entire genome are advances in genetic-engineering technologies in the past decade.

Not so long ago, sequencing 40,000 bp was considered a worthy multiyear thesis project for a Ph.D. student. Livermore's Center now sequences this amount in less than a week using the Center's integrated system that sequences and tracks the DNA fragments being studied.

The best of current technology allows researchers to sequence about 1,000 bp along a stretch of a piece of DNA. Most facilities only sequence in 300-bp chunks. The Laboratory's Genome Center currently sequences about 700 to 800 bp along the DNA. "We're entering the era of production sequencing," said Lamerdin. "A lot of the up-front work has been automated. There's no more manual pipetting, for instance. We have robots to do that." (See the photo below.)

To sequence a section of DNA, researchers first use special enzymes that act as biological "scissors" to cut DNA at specific points into smaller fragments. They then clone or make hundreds of identical copies of these fragments. When researchers wish to sequence a fragment, they run four nearly identical reactions using that DNA as a template, in which the four bases are chemically labeled with four different fluorescent dyes.

A laser scans the reaction products, exciting the fluorochromes, and a computer captures and stores the resulting fluorescent signals. (See the photo on p. 26.) Software automatically determines the order of bases from the four-color data. The Center has 13 of these sequencing machines, each capable of reading more than 25,000 bases a day. Additional software actually hunts for particular A, G, C, and T combinations that mark the beginnings and endings of genes.



Manual pipetting is a time-consuming task of the past, thanks to automated workstations like the one pictured at the left with biomedical scientist Maria De Guzman. Before these machines were available, it took one person all day to process 48 DNA samples for sequencing. Using the Genome Center's three workstations, one person can now process up to 1,000 samples a day.

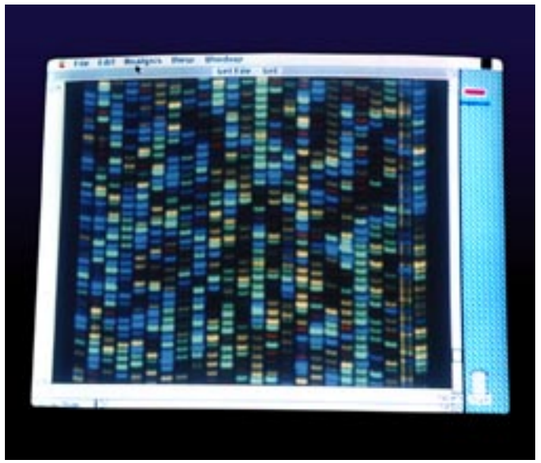
Genes at Livermore

The difference between weapons testing and gene hunting may seem enormous, but their connection relates to how the study of biology became an integral part of the Laboratory's work. Livermore's first biomedical program was chartered in 1963 to study the radiation dose to humans of isotopes in the environment; a natural extension was to explore how radiation and chemicals interact with human genetic material to produce cancers, mutations, and other adverse biological effects.

In the last 20 years, advances in microbiology, biochemistry, genetics, and bioengineering gave rise to the field of biotechnology. Recent advances in genetic-engineering technologies then made it possible to examine and sequence DNA faster and more efficiently than ever imagined. The Laboratory was well positioned to take advantage of this new field, which combines the disciplines of biology, genetics, engineering, and computer science. Pulling from other Laboratory organizations, the Biology and Biotechnology Research Program called on engineers, physicists, and computer scientists to join biologists to help solve the mystery of the human genome.

In 1987, researchers at Lawrence Livermore began studying all of chromosome 19. This project grew out of research on three genes, each involved in the repair of DNA damaged by radiation or chemicals. As the Laboratory became known worldwide for its work on this chromosome, other researchers, hunting for genes thought to be somewhere on this chromosome, contacted the Laboratory and international collaborations were formed. Such collaborations discovered, for example, the genes for myotonic dystrophy (a late-onset genetic disease causing muscle atrophy) and a form of dwarfism called pseudoachondroplasia.

In 1990, the Department of Energy and the National Institutes of Health formed the joint Human Genome Project. The long-term goal of this 15-year project is to decipher the DNA of the entire human genome. Three DOE national laboratories—Lawrence Livermore, Los Alamos, and Lawrence Berkeley—are DOE centers for this project, while NIH supports eight facilities involved in this work.



Livermore's Human Genome Center has 13 sequencing machines, each capable of reading more than 25,000 bases a day. This image shows one form of their output, where each color in the vertical bands or "sequencing ladders" corresponds to one of the four DNA bases. Using the following translator, blue = C; green = A; yellow = G; red = T, you can trace a sequence ladder vertically and read a small portion of the genetic code.

A relational database, developed by Lawrence Livermore computer scientists, keeps track of where each clone is, what has been done to it, who did it, when they did it, and what has yet to be done. "When the sequencing was someone's thesis project, the individual usually kept track of progress in a notebook," explained Lamerdin. "But in this kind of high-throughput environment, we need computers to track the progress of all these pieces and also to help us make decisions. Computational support is a critical element in the success of this project."

After Sequencing

Determining the human genome sequence and finding the genes is really just a first step. "Knowing the bases that make up a gene and where it's located on a chromosome doesn't tell you what the gene *does*," noted Ashworth. "After sequencing, we still need to determine what proteins the genes produce, and what those proteins do in the cell."

Why bother? First and foremost, genes and their proteins hold the key to unlocking the mysteries of inherited diseases. Once the genetic code for a disease is broken, gene and drug therapies can follow. For example, the gene for cystic fibrosis was discovered four years ago, and while we are still a long way off from "fixing" the gene defect that causes this disease, unraveling the gene's secrets has allowed private industry to deal with one of the major symptoms of cystic fibrosis.

"So, the sequence is really a starting point," said Ashworth. "We still need to know the structure and function of the protein produced by the gene, and how that protein interacts in the environment of the cell. The sequence, you might say, is the detailed map we need to help us find the buried treasure."

Future *S&TR* highlights will discuss the Center's work on the next-generation sequencing machine and a collaboration to uncover the gene involved in one form of inherited kidney disease.

Key Words: chromosome, DNA sequencing, gene, Human Genome Project.

Reference

1. "The Human Genome Project," *Energy & Technology Review*, UCRL-52000-92-4/5 (April/May 1992), pp. 29–62.

For further information contact Linda Ashworth (510) 422-5665 (ashworth1@llnl.gov).
The Human Genome Center's Internet home page is available at http://www.llnl.gov/bbrp/genome/genome.html. The Department of Energy's "Primer on Molecular Genetics" is available on the Internet at http://www.gdb.org/Dan/DOE/intro.html.

Patents and Awards

Each month in this space we report on the patents issued to and/or the awards received by Laboratory employees. Our goal is to showcase the distinguished scientific and technical achievements of our employees as well as to indicate the scale and scope of the work done at the Laboratory.

Patents

Patent issued to	Patent title, number, and date of issue	Summary of disclosure
John W. Elmer Dennis W. O'Brien	**Electron Beam Machining Using Rotating and Shaped Beam Power Distribution** U.S. Patent 5,534,677 July 9, 1996	An apparatus and method for electron beam (EB) machining (drilling, cutting, and welding) that uses conventional EB guns, power supplies, and welding machine technology without the need for fast bias pulsing technology. A magnetic lensing (EB optics) system and electronic controls are used to concurrently bend, focus, shape, scan, and rotate the beam to protect the EB gun, to create a desired effective power-density distribution, and to rotate or scan this shaped beam in a controlled way.
Mark Bowers Allen Hankla	**Phase and Birefringence Aberration Correction** U.S. Patent 5,535,049 July 9, 1996	A four-wave mixing phase conjugate mirror that corrects phase aberrations of a coherent electromagnetic beam and birefringence induced upon that beam. The stimulated Brillouin scattering phase conjugation technique is augmented to include Brillouin-enhanced four-wave mixing. A seed beam is generated by a main oscillator that arrives at the phase conjugate cell before the signal beams in order to initiate the Brillouin effect. The signal beam being amplified through the amplifier chain is split into two perpendicularly polarized beams.
Craig J. Rivers Roanne A. Lee Glenn E. Jones	**Electrically Shielded Enclosure with Magnetically Retained Removable Cover** U.S. Patent 5,534,663 July 9, 1996	An enclosure having electrical components and an easily removable shielded cover with magnetic securement means to secure the cover to the enclosure in a manner that provides an electrical seal between the cover and the enclosure to prevent the passage of electromagnetic radiation through the joint between the cover and the enclosure. The magnetic securement means are provided on the surface of the enclosure surrounding the opening and facing the cover, and ferromagnetic means are provided on the surface of the cover facing the magnetic securement means.
Troy W. Barbee, Jr. Timothy Weihs	**Ignitable Heterogeneous Stratified Structure for the Propagation of an Internal Exothermic Chemical Reaction along an Expanding Wavefront and Method of Making Same** U.S. Patent 5,538,795 July 23, 1996	A multilayer structure with a selectable propagating reaction front velocity (V), a reaction initiation temperature attained by application of external energy, and an amount of energy delivered by a reaction of alternating unreacted layers of the multilayer structure. Because V is selectable and controllable, a variety of different applications for the multilayer structures are possible, including their use as ignitors, in joining applications, in fabrication of new materials, as smart materials, and in medical applications and devices.
Chi Y. Fu	**Process for Forming Synapses in Neural Networks and Resistor Therefor** U.S. Patent 5,538,915 July 23, 1996	A customizable neural network where one or more resistors form each synapse. All the resistors in the synaptic array are identical, thus simplifying the processing issues. Doped, amorphous silicon is used as the resistor material to create extremely high resistances occupying very small spaces. Connected in series with each resistor in the array is at least one severable conductor whose uppermost layer has a lower reflectivity of laser energy than typical metal conductors at a desired laser wavelength.

Awards

James "Buddy" Swingle recently received the **Intelligence Community Seal Medallion** from Central Intelligence Agency Director John Deutch during a ceremony at CIA headquarters in Langley, Virginia. Swingle, who is executive secretary and acting chairman of the Joint Atomic Energy Intelligence Committee, was cited for his "sustained superior performance" in producing foreign nuclear intelligence reports that "provided significant assistance to the intelligence and policy communities." Swingle has been at Livermore since 1972, working on a variety of high-energy laser and nuclear weapons programs. He joined the Nonproliferation, Arms Control, and International Security Directorate in 1990 and was recently named leader of Z Division.

Lawrence Livermore's **Storm Water Management Program** has received the U.S. Environmental Protection Agency's 1996 **National Storm Water Control Program Excellence Award** in the industrial category. The award recognizes the Laboratory's efforts to curb water pollution through improved storm water control. Representatives from the Storm Water Management Program, a part of the Environmental Protection Department in the Laboratory's Plant Operations Directorate traveled to Dallas, Texas, in early October to receive the award at the Annual Water Environment Federation Conference.

Three teams of Laboratory employees are recipients of **Hammer Awards** for the Department of Energy. They are: the **Life Cycle Asset Management** (LCAM) team from the Plant Engineering Department, a part of the Plant Operations Directorate; the **Directives Reengineering Group**, out of the Office of Scientific and Technical Information in the Director's Office; and the **Performance Management Team**, working out of the Office of Policy, also in the Director's Office. The Hammer Awards were created by Vice President Al Gore to recognize special achievements in the efforts to reinvent government by improving customer service, cutting red tape, empowering employees, or getting back to basics.