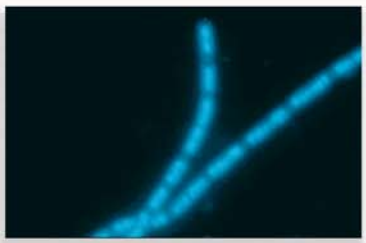
Tracing Biothreats

with Molecular Signatures

by Amy Reeves

Fragments of anthrax DNA in gel

Biological agents have long been used as tools of war and terrorism. Unfortunately, today's biological weapons are far more sophisticated than the plague-infested corpses catapulted over city walls or the dead livestock used to poison medieval water supplies. A few nations have devoted considerable resources to "weaponizing" and stockpiling infectious microbes.



Stained *Bacillus anthracis*

Bacillus spores

Photos by Kevin N. Roark

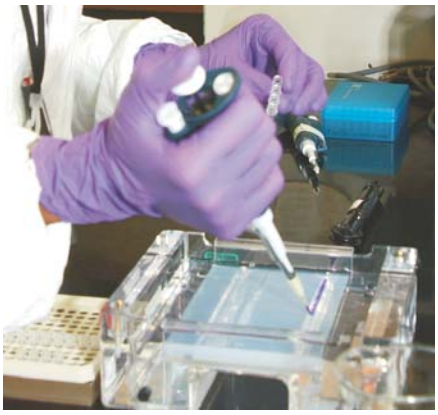
For over a decade, a team of researchers in the Bioscience Division has been working to prevent the proliferation of biological weapons. The team has developed a powerful set of tools and techniques for deciphering molecular signatures—genetic patterns that distinguish bacterial species and strains. These signatures are key to detecting, identifying, and tracing potential biothreat agents, including the microbes that cause anthrax, plague, and botulism.

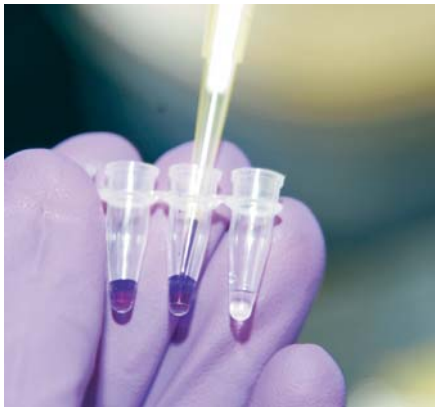
DNA Extraction and Analysis Tools

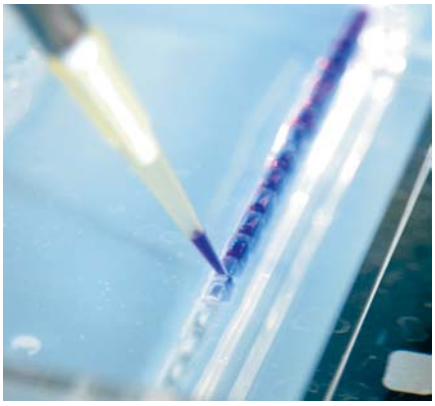
Some of the team's tools, such as rapid DNA-sequencing and bioinformatics techniques, were originally developed as part of the Human Genome Project. Others were developed to solve specific problems encountered during the team's early efforts to devise ways to detect DNA from biothreat agents in environmental and forensic samples.

The first problem was how to extract the DNA to be analyzed. The team devised new methods and instruments to wrest enough quality DNA from preserved tissue or complex environmental samples (such as from soil, ventilation filters, or liquid waste streams) to allow detailed molecular analyses.

The researchers then had to develop a method to determine whether the extracted DNA included DNA from any known biothreat agents. The method had to be sensitive enough to detect trace amounts of biothreat DNA and, to avoid "false positive" results, specific enough to detect DNA only from such







Photos by Kevin N. Roark

(Top) Samples of anthrax DNA are loaded into a gel. Gel electrophoresis is used to separate DNA fragments for analysis. (Middle) Close-up of fluorescent dye being added to DNA samples before they are loaded. (Bottom) Close-up of the gel during loading. The separated fragments (shown in the gel on page 15) provide information about the anthrax strain and its likely sources.

agents and no others, including any close relatives.

Most samples, whether medical or environmental, contain a "mishmash" of DNA from all the organisms or tissue collected, such as human DNA in medical samples or benign soil bacteria and decomposed leaf remnants in environmental samples. The researchers realized that to identify specific DNA from this mishmash, they had to make the target DNA "stand out." The detection method they developed, which is now used by the FBI and the Centers for Disease Control, uses PCR, or polymerase chain reaction, to amplify (make more copies of) the target DNA.

PCR is a laboratory technique that mimics nature's method for replicating DNA. During natural DNA replication, after enzymes unzip the complementary strands of DNA, other enzymes, called polymerases, use the single strands of DNA as templates for producing new complementary strands. The result is two DNA helixes formed from one. The PCR technique uses short DNA fragments, called primers, that bind to part of a strand of target DNA and provide a starting point for the DNA polymerase. After the target DNA has been copied, the strands are separated, and the process

Anthrax Investigations

In 1979, an outbreak of anthrax in the Soviet Union killed more than sixty people. Soviet officials attributed the outbreak to consumption of contaminated meat. However, Western scientists suspected the real cause of the outbreak was inhalation of spores accidentally released from a nearby military research facility.

When the Bioscience Division team analyzed the DNA in preserved tissue samples from eleven victims, it found that the victims were infected with at least five separate strains of *Bacillus anthracis*—in contrast with all known natural outbreaks, which involve a single strain. The multistrain infections suggested that the Soviets were intentionally mixing strains, possibly to complicate initial identification or to experiment with multiple-drug or vaccine resistance.

During an investigation of Iraq's suspected biological weapons program, agents from the United Nations Special Commission (UNSCOM) collected samples from several research and production facilities, including one at Al Hakam. Iraq claimed this facility produced only animal feed and pesticides for agricultural crops. However, the Los Alamos team's molecular analyses detected genetic signatures from an organism that was closely related to *B. anthracis* and somewhat related to the biopesticide *B. thuringiensis,* though it lacked any pesticide properties. The large quantity found in samples collected from an industrial-scale drying unit raised the possibility that the organism was being used as a surrogate for *B. anthracis*.

is repeated until there are enough copies of the target DNA for analysis.

The PCR primers developed by the Los Alamos team are key to the specificity of its detection method. To bind to the template strand of target DNA—a prerequisite for PCR amplification—the primer must be complementary to part of the target DNA. By creating a set of primers with DNA sequences specific to each biothreat agent, the team developed a way to unequivocally amplify only DNA from specific pathogens.

Beyond Species: Identifying Strains

Because all potential biothreat agents are derived from natural sources, determining where a microbe came from and whether or not it was introduced by human activities requires more-detailed analysis. The approach pioneered by the Los Alamos team and their collaborators at Northern Arizona University has been to study the molecular genetics of different pathogens beyond the species to the strain level. The team has developed a battery of methods for distinguishing strains based on tiny differences in their DNA sequences. These methods can even reveal whether a strain has been genetically engineered to complicate identification or enhance drug resistance.

One widely used method relies on enzymes to break the microbe's DNA at specific places. The pattern of the sizes of fragments generated—the fragment profile—is then compared with profiles in a database. If it exactly matches a profile in the database, the microbe's identity can be determined, as well as where the microbe may have originated and how it might be treated. If the profile does not exactly match another but shows significant similarities, scientists can at least determine the microbe's relationship to other species and strains.

Other methods use strain-specific PCR primers to magnify subtle differences in the DNA sequences of related strains. Such primers are already available for the microbes that cause anthrax and plague, and the team is working to design strain-specific primers for other species.

And still other methods involve determining and comparing DNA sequences. Sequence data enables scientists to verify the identification of a species or strain, and it provides clues about how the microbe works—for example, why one strain might be more infectious or more resistant to an antibiotic than another.

Given its expertise and diagnostic tools, the team has been asked to provide technical support to several international investigations involving suspected biological weapon programs (see the sidebar on page 16). "Our strong foundation in biology and genetics has enabled us to respond rapidly when called upon," explained Jill Trewhella, Director of the Bioscience Division.

Los Alamos is also providing technical support to several ongoing investigations, including the response to last fall's anthrax attacks. These attacks caused five deaths, as well as widespread fear, and had significant economic repercussions. A larger attack—whether directed at humans or key agricultural crops—could be even more devastating. "Our hope is that our detection, identification, and attribution expertise will deter the use of such weapons," Trewhella continued, "and that anyone tempted to use such weapons will realize that if they do, we will identify them and they will be caught." ■

Photo courtesy of The White House

During a recent DOE demonstration on homeland security, Bioscience Division Director Jill Trewhella briefed President George W. Bush on Lab technologies for analyzing DNA from biothreat agents. Trewhella showcased the Lab's "dirt to data" portable DNA analysis system, which extracts and quantifies DNA from environmental samples, amplifies target DNA through the use of PCR, and analyzes the results. She explained how the division's research supports national security: division scientists have quickly leveraged technologies developed for programs such as the Human Genome Project by adapting them to analyzing biothreat agents.

The Researchers

Over its history, the **Bioscience Division team** has included scientists from a variety of disciplines across the Laboratory. From the beginning, the team has been led by **Paul Jackson**, who joined the Laboratory in 1981 after earning his Ph.D. in molecular biology at the University of Utah. Jackson holds six patents and is a Laboratory Fellow.