

# Forensic Science Center



*The Forensic Science Center houses a variety of state-of-the-art analytical tools ranging from gas chromatograph/mass spectrometers to ultratrace DNA detection techniques. The Center's multidisciplinary staff provides expertise in organic and inorganic analytical chemistry, nuclear science, biochemistry, and genetics useful for supporting law enforcement and for verifying compliance with international treaties and agreements.*

**S**INCE 1991, the Laboratory's Forensic Science Center has focused a comprehensive range of analytical expertise on issues related to nonproliferation, counterterrorism, and domestic law enforcement. During this short period, LLNL's singular combination of human and technological resources has made the Center among the best of its kind in the world. The Center has already demonstrated impressive analytical capabilities in organic, inorganic, and biological chemistry as well as in other disciplines.

What is forensic science? Traditionally, the term has been applied to the scientific analysis of evidence in the context of civil or criminal law. More recently, forensic science is increasingly being used to

monitor or verify compliance with international treaties and agreements, especially those dealing with weapons of mass destruction. This new concern reflects the substantial changes in the international environment brought about by the end of the Cold War. For example, clandestine attempts by nations to manufacture or acquire weapons of mass destruction have stimulated efforts to develop the most up-to-date technologies to ensure that intelligence information is analyzed as accurately and reliably as possible. International terrorism is another concern. Identifying terrorists by tracing their activities, as in the recent bombing of the World Trade Center in New York City, is a challenge that can require all the resources of forensic science.

The extension of traditional forensic science to these and other areas calls for facilities that are able to support comprehensive, multidisciplinary efforts. It is this challenge that the Laboratory's Forensic Science Center was designed to meet. The Center houses a variety of state-of-the-art analytical tools ranging from gas-chromatograph/mass spectrometers to ultratrace DNA detection techniques. In the hands of an experienced staff of specialists, these and other technologies deliver a full range of forensic science capabilities.

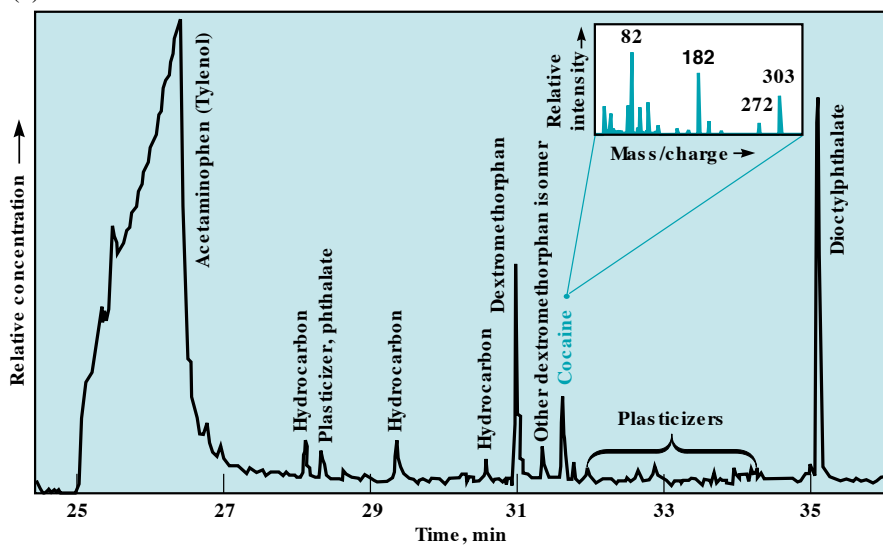
## **Nuclear Proliferation**

As the threat of clandestine nuclear proliferation grows, the task of

(a)



(b)



**Figure 1.** (a) Our portable gas chromatograph/mass spectrometer (GC/MS) can be used to analyze any liquid, solid, or gas chemical sample in the field. Results from a typical GC/MS analysis (b) show the separation of compounds in the sample. Shown here is a sample of confiscated cocaine, which had been adulterated with Tylenol and other compounds. Identification of the various compounds is achieved through analysis of the ion fragmentation patterns of some 2000 individual mass spectra. The inset graph is one such mass spectral fragmentation pattern, which reveals enough structural information to identify the compound as cocaine.

acquiring definitive information about a suspect nation's present and future nuclear capabilities becomes more demanding and complex. Such information includes activities related to the processing, procurement, diversion, or dispersion of special nuclear material.

Nuclear-related activities produce a variety of indicators. High-explosive implosion tests, for example, leave tell-tale chemical residues in the environment. The detection of distinctive radionuclides or enriched isotopic species is another sign of a clandestine nuclear program.

To strengthen the Center's analytical capabilities, it has teamed with Laboratory experts in nuclear, radiochemical, isotopic, and inorganic chemistry. This partnership expands the Center's technology base to include many varieties of sensitive equipment for detecting and discriminating all forms of nuclear radiation.

### New Approaches to Identifying Chemical Samples

Unknown samples arrive at the Center in many different forms and states of stabilization. Some are water, vegetation, or soil samples; others are "wipes" of substances that may be related to clandestine weapons-production activities. Many such substances are present only in minute quantities whose characteristic chemical "signatures" may be masked by a host of background chemicals also present in the sample. Others have deteriorated into decomposition products. Many are contaminated by extraneous chemicals. To analyze and interpret such samples accurately, we must be able to isolate and identify all their component chemical species as well as their relative concentrations.

Chemical weapons of mass destruction are a focus of monitoring

under the provisions of international treaty, including the Chemical Weapons Convention of 1989. The demands of analyzing unknown chemical samples obtained from a variety of sites around the world have stimulated the development of new technologies. In addition, inspections in support of the Chemical Weapons Convention may not allow suspect samples to be removed to a laboratory; rather, they must be analyzed on-site. We have designed field-analysis kits that can analyze a sample shortly after it is taken, an advantage when dealing with substances that may be unstable, highly reactive, or otherwise perishable.

### Remote Chemical Monitoring System

One of these technologies under development is a portable chemistry analyzer known as an ion cyclotron resonance mass spectrometer (ICR-MS). The design of the ICR-MS is based on the principle of the so-called Penning ion trap. Ions of the target compound are injected into a small chamber containing electrodes that generate a static axial electric field. A magnetic field produced by a permanent magnet radially confines the ions within the trap. The high homogeneity of the magnetic field enables us to make extremely accurate mass-spectroscopic measurements.

The ICR-MS can be configured to detect specific chemicals at very low levels of concentration. The low power requirements of the instrument and its simple electronic circuitry, together with the compactness of the spectrometer, the vacuum system, and the computer, permit a very small package. (The ICR-MS has the potential to fit within an enclosure no larger than a coffee can.) We are also working to develop a version that can be left

unattended in the field to perform diagnostic chemical analyses.

### Chemistry Lab in a Suitcase

Another technology we have developed is a miniature gas chromatograph/mass spectrometer (GC/MS). Completely self-contained in a 28-kg (~61-lb), suitcase-sized package (Figure 1a), this instrument is optimized to detect ultratrace (microgram or less) quantities of narcotics and compounds related to chemical-warfare agents, including their precursors and decomposition products. This instrumentation is ideally suited to support most nonproliferation efforts and investigations related to chemical pollutants released into the environment.

Gas chromatography is a technique widely used to separate mixtures of compounds. In our GC/MS, a solid, liquid, or air sample is injected into the end of a long and very small, hollow quartz column through which hydrogen gas is flowing continually. With heat, the sample is rapidly vaporized into an aerosol and carried into the column, where each component in the mixture is separated. Because chemicals all have different vapor pressures and polarities, each will migrate down the heated, gas-filled column at a different rate. The various chemical species, therefore, are completely separated from the initial mixture and reach the mass spectrometer at different times (typically, over a period of 2 to 45 min) in a relatively pure form.

As each chemical enters the mass spectrometer, it is bombarded with an electron beam, which causes the molecules to break apart into fragment ions. These fragments are sorted and displayed for the operator to study (see Figure 1b). Each chemical produces a unique

fingerprint of fragment ions that is used to identify the compound. GC/MS can analyze samples as small as a grain of salt, and total unknowns can be conclusively identified very quickly. The miniature GC/MS is now carried inside a suitcase, and we are working to reduce its size further so that it can fit into a briefcase.

### Airborne Mass Spectrometer

A new mass spectrometer that we are currently building and testing allows extremely low levels (a few parts per trillion) of chemicals in air to be collected and detected very rapidly. Having the sensory capability of a German Shepherd guard dog, this mass spectrometer has a combined ion-storage trap and time-of-flight (IT/TOF) configuration (Figure 2a).

The new instrument draws air into an inlet port where any trace chemicals are ionized. The molecular ions are then drawn into an ion-storage trap, where they are contained by radiofrequency-driven electrodes. After a sufficient amount (about 10 pg; 1 pg =  $10^{-12}$ g) of target chemicals have been stored (every 10–100 ms), they are pulsed down a flight tube, detected, and displayed for the operator (see Figure 2b). This new instrument is unique in that it can acquire data on the order of thousands of spectra per second, making it suitable for high-speed aircraft sampling of air samples. Potential applications include identifying hazardous and chemical spills, monitoring industrial stacks and materials for volatile compounds, detecting concealed contraband, and surveying the environment. This instrument is particularly useful for sampling a released plume of smoke or airborne chemical that is only available for an instant in time.

We have completed a laboratory-size IT/TOF instrument (Figure 3). Work is now in progress to design and build a smaller transportable instrument that can be placed under the wing of a surveillance aircraft.

### Detecting Low Levels of Chemiluminescent Compounds

Researchers, intelligence agencies, and environmental scientists must sometimes identify minute quantities of compounds that emit light when

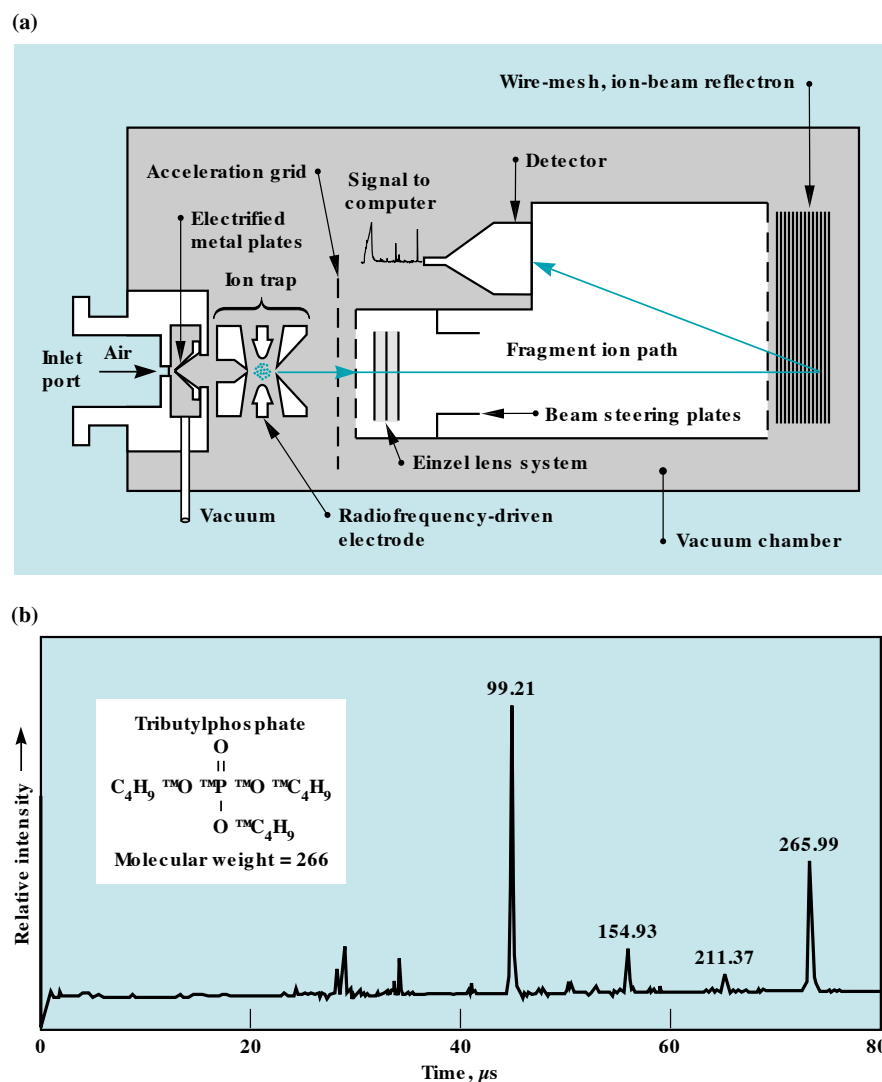
exposed to certain chemicals. We are using a technique called high-performance liquid chromatography to probe the lower limits of detection for a family of such compounds, called polycyclic aromatic hydrocarbons.

Liquid chromatographic instruments mix the substance to be analyzed with hydrogen peroxide ( $H_2O_2$ ) and another activating chemical, bis(2-4,6-trichlorophenyl) oxalate (TCPO). The target compound emits light of a characteristic frequency—a phenomenon known as chemiluminescence—that can be recorded and used to identify it at extremely low levels. Such compounds also give off light when illuminated with certain short frequencies of light, a process known as fluorescence.

We use liquid chromatography with tandem ultraviolet absorption, fluorescence, and chemiluminescence detectors and compare the results of the three techniques for different concentrations of a target compound. Our goal is to determine the relative sensitivities of the three detection methods for extremely small (nanogram to picogram) concentrations of compounds against backgrounds that may include a variety of contaminants.

### An International Effort

Activities in support of the Chemical Weapons Convention and the Nuclear Non-Proliferation Treaty are international in scope. In cooperation with about a dozen other countries, the Forensic Science Center is participating in a series of "round-robin" exercises designed to probe the capabilities of analytical chemistry facilities around the world. In these continuing exercises, realistic samples of a variety of substances, whose identities are known only to the preparing agency, are sent out to the participants, who analyze them and report their results. Target compounds



**Figure 2.** The ion-storage trap/time-of-flight (IT/TOF) mass spectrometer can detect and analyze trace chemicals in any air sample. The instrument (a) is divided into three regions: an ionization source, an ion-storage trap, and a mass spectrometer. The mass spectrometer separates the ions according to their mass, which usually takes 10–100  $\mu s$ . Shown in (b) is an ion fragmentation pattern that reveals predominant mass-to-charge ratios of 99.21, 154.93, 211.37, and 265.99. These species are consistent with the presence of tributylphosphate, a chemical indicative of uranium reprocessing for the recovery of plutonium.



are often present at extremely low concentrations.

Seventeen laboratories in fourteen countries participated in Round Robin III (1992). A laboratory in The Netherlands prepared the samples, which consisted of concrete, paint, and rubber matrices spiked with chemical-warfare-related compounds (e.g., precursors and degradation products). Among the analytical methods used by participating laboratories were GC/MS, Fourier-transform infrared spectrometry, and nuclear magnetic-resonance spectrometry.

The Laboratory's Forensic Science Center did very well in the exercise, one of the few facilities to do so. Participation in these round-robin exercises has deepened our understanding of the operational parameters needed to detect key chemical signatures in "real-world" environmental samples.

### Domestic Activities

Not all of the Forensic Science Center's activities are in support of international investigations. We have performed some domestic investigations to learn more about the value of our technologies and to gain practical experience with real-world samples. We have had excellent success in applying our resources to cases involving extraordinary circumstances or demanding unusually high-quality forensic analyses. Some of these studies have been undertaken at the request of local regulatory or law-enforcement agencies.

### What Ever Happened to Baby Jane?

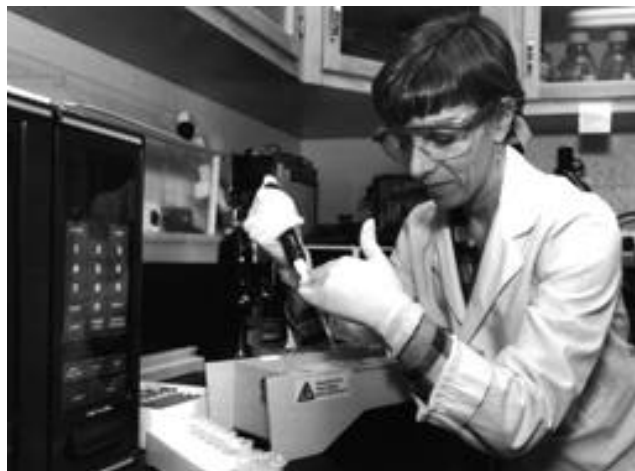
One such recent case began in October 1988, when a shallow, unmarked grave in a remote area of northern California yielded the decomposed body of a woman. A

lengthy search of dental records identified her to be a Berkeley artist reported missing two years earlier, together with her 18-month-old baby. A detective's hunch led the police to suspect a link between the case and the body of an infant that had washed

ashore in Tiberon several months before the woman's body was found. The media dubbed the unidentified infant "Baby Jane Doe." To establish whether the two were related, in the summer of 1992, the Forensic Science Center was requested to do supporting



**Figure 3.** Our laboratory-size ion-storage trap/time-of-flight (IT/TOF) instrument. We are currently designing a smaller portable instrument.



**Figure 4.** To determine whether an unidentified baby and woman were related, the Center was called upon to analyze cell samples from both bodies. Using the polymerase chain reaction, LLNL researchers replicated the gene samples. Here, biomedical scientist Marge Segraves is loading a gene segment suspended in a gel before the samples are separated for comparison.

DNA analyses of cell samples from both bodies (Figure 4).

DNA analysis requires only a few intact body cells, which can be taken from minute traces of blood or tissue. In this case, the analysis was complicated by the fact that both bodies were badly decomposed. Researchers used a technique called the polymerase chain reaction (see the box on p. 7) to amplify and then compare the DNA samples. The same DNA segment taken from different individuals can be of different lengths. The more closely two individuals are related, the more likely are their genes to match in length. After using the polymerase chain reaction to amplify the DNA samples, the replicated segments are suspended in a gel. An electric current applied to the gel then separates the DNA fragments by size for comparison.

Although not conclusive, our analysis suggested it was highly likely that the woman was the mother

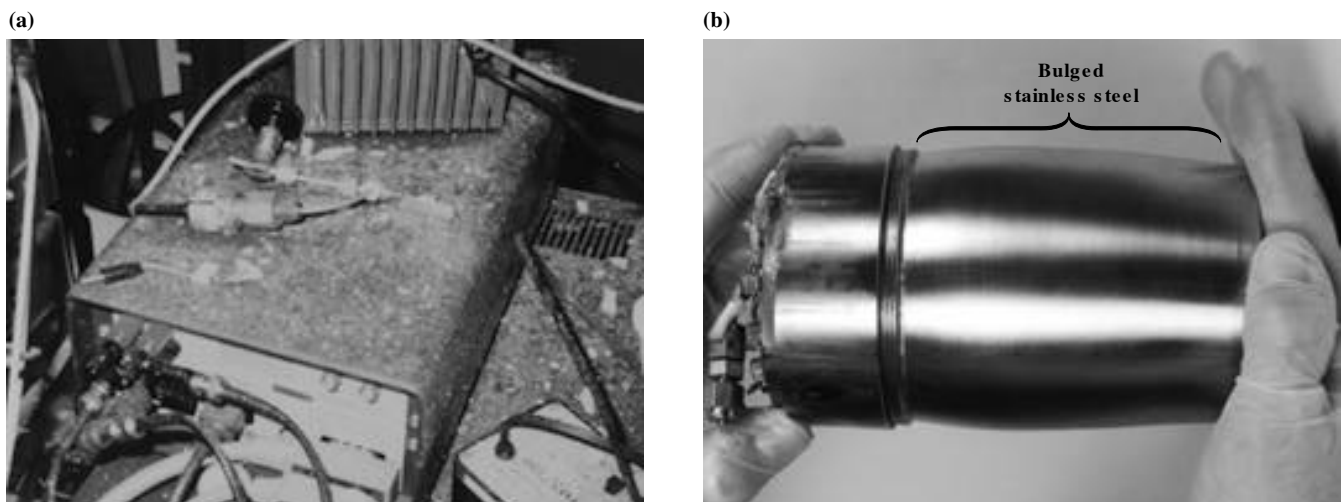
of Baby Jane. An eerie aspect of the case was that the infant appeared to be about the same age as when it had disappeared two years earlier. This circumstance led authorities to conclude that both bodies had been frozen for some time before being disposed. The woman's husband was sought as the prime suspect in the killings. Although he never confessed, he committed suicide in 1992, leaving a note in which he asked for forgiveness. The case was subsequently closed by the Marin County District Attorney's Office.

### Cold Fusion Heats Up

In another case, we were asked by California's Occupational Safety and Health Administration (OSHA) to analyze debris from an explosion that occurred during a 1992 "cold-fusion" experiment at a research laboratory in California (Figure 5). Such experiments began in 1989, when two University of Utah scientists conducting electrochemistry

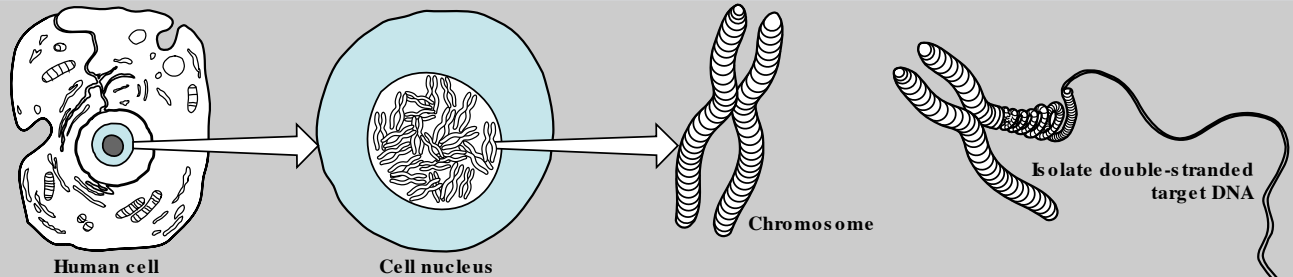
experiments reported the production of excess heat that they attributed to hitherto unknown thermonuclear fusion processes. For a brief time, the cold-fusion phenomenon captured public attention as a new and possibly revolutionary energy source. Since then, however, the majority of the scientific community has rejected these claims, attributing positive experimental results to various sources of error.

In the explosion, one researcher was killed and several others were injured. Drawing upon the Laboratory's scientific expertise in many different fields—about 65 professionals in all—we performed an extensive suite of analytical tests on the debris. We eliminated any nuclear reactions, high explosives, or illegal tampering as possible causes of the explosion. An unanticipated result of our investigation was that machine-shop lubricating oil could have been a potential contributor to the incident,



**Figure 5.** In 1992, an explosion occurred during a "cold-fusion" experiment at a research laboratory in California. About 65 LLNL scientists were called upon to analyze the debris (a) to shed light on potential causes of the explosion. The distended cold-fusion cell after the explosion is shown in (b). Our most unanticipated result was that machine-shop lubricating oil could have been a potential contributor to the incident.<sup>1</sup>

## The Polymerase Chain Reaction: A Printing Press for DNA

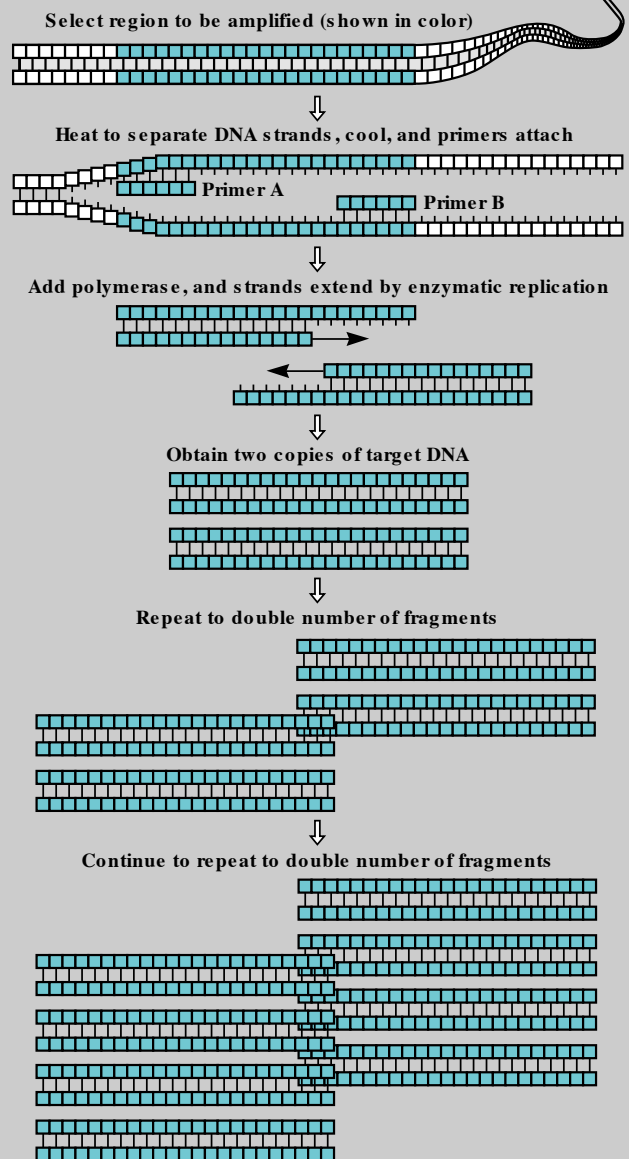


To analyze DNA, investigators need many copies of a particular targeted DNA segment. A relatively quick and highly efficient way to copy DNA—or as researchers say, to “amplify” DNA molecules in a test tube without needing a host cell—is the polymerase chain reaction (PCR). The PCR is to genes what Gutenberg’s printing press was to the written word. With it, researchers can amplify any DNA sequence regardless of its origin (virus, bacteria, plant, or any human cell) hundreds of millions of times in a matter of hours.

The PCR is especially valuable because the reaction is easily automated and can amplify extremely small amounts of starting material. Thus, it has had a major impact on clinical medicine, genetic research and diagnosis, and evolutionary biology as well as forensic science.

The process is based on a special polymerase enzyme (a protein acting as a catalyst) that can synthesize a new strand of DNA complementary to a target strand. The starting mixture contains the DNA sample of interest, the four building blocks of DNA (called DNA bases), and two DNA fragments (called primers) that flank the target sequence. As shown in the illustration, the mixture is first heated to separate the double strands of DNA. Cooling allows the primers to find and bind to their complementary sequences on the separated strands. The primers define the ends of the DNA to be duplicated. Then, the DNA polymerizing enzyme catalyzes the synthesis of two new strands of DNA that are complementary to the original two.

Repeated heating and cooling cycles multiply the target DNA exponentially because each new double strand separates to become two new DNA templates for further synthesis. Some 20 cycles of the PCR can amplify the target DNA by a factor of a million in about an hour.



and we recommended to OSHA that all cold-fusion containers should be scrupulously cleaned in the future. We have submitted a paper describing the most important of these results to a peer-reviewed journal.<sup>1</sup>

### The Japanese Connection

During a San Francisco conference last year, a Japanese criminologist described new imaging materials that could have important applications in fighting international and domestic crime. The materials enable investigators to visualize latent fingerprints on the gummed surface of duct tape, often used in bombings and kidnappings. The Japanese

demonstrated a black powder that—when mixed with a liquid, applied to the desired surface, and then rinsed off—leaves a small residue that highlights any latent fingerprints.

Although American law enforcement officials showed considerable interest in the materials, there was a problem. According to U.S. law, any such material must be accompanied by a Material Safety Data Sheet, which was lacking in this case. Language barriers and differing safety regulations prevented the U.S. from learning the composition directly. A forensic specialist at the Livermore Police Department contacted the Laboratory, and the Forensic Science Center agreed to analyze the materials (Figure 6).

The liquid turned out to be 90% water and 10% propylene glycol, with a trace of detergent. Scanning electron microscopy established that the extremely fine black powder was alumina coated with iron, similar to that used in toners for copying machines or printers.

Last spring, the Center received a letter from the California Criminalistics Institute describing the language and legal difficulties that had earlier frustrated the preparation of a Material Safety Data Sheet. The letter thanked the Laboratory for its analyses, which had provided the necessary information, and announced that the Japanese materials would “soon be made available to forensic print specialists throughout the country.”

### Summary

Using the comprehensive array of sophisticated technologies from across the entire Laboratory, our Forensic

Science Center is able to quickly characterize evidentiary materials of importance both to national security and to forensic aspects of domestic law enforcement. The Center’s analytical capabilities feature state-of-the-art sensitivities for detecting virtually any target compound contained in any sample. Our approach maximizes the information returned from limited samples collected by a variety of verification, inspection, monitoring, and law-enforcement agencies. As the pertinent technologies develop, we will continue to enhance these analytical tools.

**Key Words:** Chemical Weapons Convention; cold fusion; fingerprint imaging materials; Forensic Science Center; Fourier-transform infrared spectrometry; fragmentation ions; gas chromatograph/mass spectrometry (GC/MS); high-performance liquid chromatograph; ion cyclotron resonance/mass spectrometry (ICR/MS); ion storage trap/time-of-flight (IT/TOF); Non-Proliferation Treaty; nuclear magnetic resonance; polymerase chain reaction (PCR); Round Robin III.

### Reference

1. P. M. Grant, R. E. Whipple, A. Alcaraz, J. S. Haas, and B. D. Andresen, “Hydrocarbon Oil Found in the Interior of a ‘Cold Fusion’ Electrolysis Cell After Fatal Explosion,” *Fusion Technology* 25(2), (1994).



**Figure 6.** The Forensic Science Center analyzed and determined the composition of new fingerprint imaging materials from Japan. These materials now allow investigators nationwide to visualize latent prints from previously intractable surfaces, such as the sticky side of adhesive tape.



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# Melanoma at LLNL: An Update



*In recent years, the rate of diagnosis of the more lethal form of melanoma among LLNL workers, which was previously elevated, has returned to that of the surrounding geographical area where most employees live. If our program of employee awareness about melanoma, enhanced surveillance, and early diagnosis continues to lead to decreased mortality from this disease, then such an approach may have important public health implications for the broader community.*

**F**ROM 1972 to 1977, the Laboratory experienced a diagnosis rate of malignant melanoma among its employees that was three to four times higher than expected based on rates for the surrounding Alameda and Contra Costa counties in the Bay Area.<sup>1</sup> In 1984, Austin and Reynolds from the California Department of Health Services reported the results of their study comparing individuals diagnosed with melanoma and

otherwise healthy controls from the Laboratory. These researchers concluded that five occupational factors were “causally associated” with melanoma risk at LLNL.<sup>2</sup> The factors were exposure to radioactive materials, exposure to volatile photographic chemicals, work at Site 300, visits to the Pacific Test Site, and duties as a chemist.

Later external reviews of the report were conducted by experts in epidemiology and biostatistics at the

University of North Carolina.

These reviewers concluded that the methods Austin and Reynolds had used were appropriate and correctly carried out. However, the conclusion concerning a causal relation between occupational factors and melanoma among employees was overstated, according to the panel of reviewers.

This article summarizes the main results of studies carried out since 1980 and the recent outcome of an extensive, new investigation of the