chapter 15

DNA: the indispensable forensic science tool

MIDDELL, TIFFANY
ISC8TS

Learning Objectives

After studying this chapter you should be able to:

- Name the parts of a nucleotide and explain how they are linked together to form DNA
- Understand the concept of base pairing as it relates to the double-helix structure of DNA
- Contrast DNA strands that code for the production of proteins with strands that contain repeating base sequences
- Explain the technology of polymerase chain reaction (PCR) and how it applies to forensic DNA typing
- Understand the concept of electrophoresis
- Understand the structure of an STR
- Describe the difference between nuclear and mitochondrial DNA
- Understand the use of DNA computerized databases in criminal investigation
- List the necessary procedures for the proper preservation of bloodstained evidence for laboratory DNA analysis

KEY TERMS

amelogenin gene
amino acids
buccal cells
chromosome
complementary base pairing
deoxyribonucleic acid (DNA)
electrophoresis
epithelial cells
human genome
hybridization
low copy number
mitochondria
multiplexing
nucleotide
picogram
polymer
polymerase chain reaction (PCR)
primer
proteins
replication
restriction fragment length polymorphisms (RFLPs)
sequencing
short tandem repeat (STR)
substrate control
tandem repeat
touch DNA
Y-STRs

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deoxyribonucleic acid (DNA)
The molecules carrying the body's genetic information; DNA is double stranded in the shape of a double helix.

chromosome
A rodlike structure in the cell nucleus, along which the genes are located; it is composed of DNA surrounded by other material, mainly proteins.

polymer
A substance composed of a large number of atoms; these atoms are usually arranged in repeating units, or monomers.

nucleotide
The unit of DNA consisting of one of four bases—adenine, guanine, cytosine, or thymine—attached to a phosphate–sugar group.

The discovery of **deoxyribonucleic acid (DNA)**, the deciphering of its structure, and the decoding of its genetic information were turning points in our understanding of the underlying concepts of inheritance. Now, with incredible speed, as molecular biologists unravel the basic structure of genes, we can create new products through genetic engineering and develop diagnostic tools and treatments for genetic disorders.

For a number of years, these developments were of seemingly peripheral interest to forensic scientists. All that changed when, in 1985, what started out as a more or less routine investigation into the structure of a human gene led to the discovery that portions of the DNA structure of certain genes are as unique to each individual as fingerprints. Alec Jeffreys and his colleagues at Leicester University, England, who were responsible for these revelations, named the process for isolating and reading these DNA markers *DNA fingerprinting*. As researchers uncovered new approaches and variations to the original Jeffreys technique, the terms *DNA profiling* and *DNA typing* came to be applied to describe this relatively new technology.

This discovery caught the imagination of the forensic science community because forensic scientists have long desired to link with certainty biological evidence such as blood, semen, hair, or tissue to a single individual. Although conventional testing procedures had gone a long way toward narrowing the source of biological materials, individualization remained an elusive goal. Now DNA typing has allowed forensic scientists to accomplish this goal. The technique is still relatively new, but in the few years since its introduction, DNA typing has become routine in public crime laboratories and has been made available to interested parties through the services of a number of skilled private laboratories. In the United States, courts have overwhelmingly admitted DNA evidence and accepted the reliability of its scientific underpinnings.

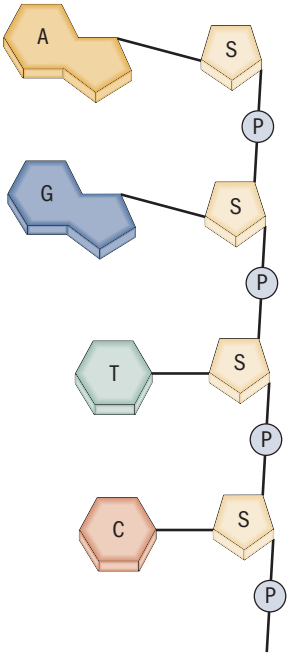

FIGURE 15–1
How nucleotides can be linked to form a DNA strand. S designates the sugar component, which is joined with phosphate groups (P) to form the backbone of DNA. Projecting from the backbone are four bases: *A*, adenine; *G*, guanine; *T*, thymine; and *C*, cytosine.

What Is DNA?

Inside each of 60 trillion cells in the human body are strands of genetic material called **chromosomes**. Arranged along the chromosomes, like beads on a thread, are nearly 25,000 genes. The gene is the fundamental unit of heredity. It instructs the body cells to make proteins that determine everything from hair color to our susceptibility to diseases. Each gene is actually composed of DNA specifically designed to carry out a single body function.

Interestingly, although DNA was first discovered in 1868, scientists were slow to understand and appreciate its fundamental role in inheritance. Painstakingly, researchers developed evidence that DNA was probably the substance by which genetic instructions are passed from one generation to the next. But the major breakthrough in comprehending how DNA works did not occur until the early 1950s, when two researchers, James Watson and Francis Crick, deduced the structure of DNA. It turns out that DNA is an extraordinary molecule skillfully designed to carry out the task of controlling the genetic traits of all living cells, plant and animal.

Structure of DNA

Before examining the implications of Watson and Crick's discovery, let's see how DNA is constructed. DNA is a **polymer**. As we will learn in Chapter 12, a polymer is a very large molecule made by linking a series of repeating units.

NUCLEOTIDES In the case of DNA, the repeating units are known as **nucleotides**. A nucleotide is composed of a sugar molecule, a phosphorus-containing group, and a nitrogen-containing molecule called a *base*. Figure 15–1 shows how nucleotides can be strung together to form a DNA strand. In this figure, S designates the sugar component, which is joined with a phosphate group to form the backbone of the DNA strand. Projecting from the backbone are the bases.

The key to understanding how DNA works is to appreciate the fact that only four types of bases are associated with DNA: adenine, cytosine, guanine, and thymine. To simplify our discussion of DNA, we will designate each of these bases by the first letter of their names. Hence, *A* will stand for adenine, *C* will stand for cytosine, *G* will stand for guanine, and *T* will represent thymine.

Again, notice in Figure 15–1 how the bases project from the backbone of DNA. Also, although this figure shows a DNA strand of four bases, keep in mind that in theory there is no limit to the length of the DNA strand; in fact, a DNA strand can be composed of a long chain with millions of bases. The information just discussed was well known to Watson and Crick by

the time they set about detailing the structure of DNA. Their efforts led to the discovery that the DNA molecule is actually composed of two DNA strands coiled into a *double helix*. This can be thought of as resembling two wires twisted around each other.

As these researchers manipulated scale models of DNA strands, they realized that the only way the bases on each strand could be properly aligned with each other in a double-helix configuration was to place base *A* opposite *T* and *G* opposite *C*. Watson and Crick had solved the puzzle of the double helix and presented the world with a simple but elegant picture of DNA (see Figure 15–2).

COMPLEMENTARY BASE PAIRING The only arrangement possible in the double-helix configuration was the pairing of bases *A* to *T* and *G* to *C*, a concept that has become known as **complementary base pairing**. Although *A*–*T* and *G*–*C* pairs are always required, there are no restrictions on how the bases are to be sequenced on a DNA strand. Thus, one can observe the sequences *T*–*A*–*T*–*T* or *G*–*T*–*A*–*A* or *G*–*T*–*C*–*A*. When these sequences are joined with their complements in a double-helix configuration, they pair as follows:

complementary base pairing
The specific pairing of base *A* with *T* and base *G* with *C* in double-stranded DNA.

```
T A T T     G T A A     G T C A
| | | |     | | | |     | | | |
A T A A     C A T T     C A G T
```

Any base can follow another on a DNA strand, which means that the possible number of different sequence combinations is staggering! Consider that the average human chromosome has DNA containing 100 million base pairs. All of the human chromosomes taken together contain about 3 billion base pairs. From these numbers, we can begin to appreciate the diversity of DNA

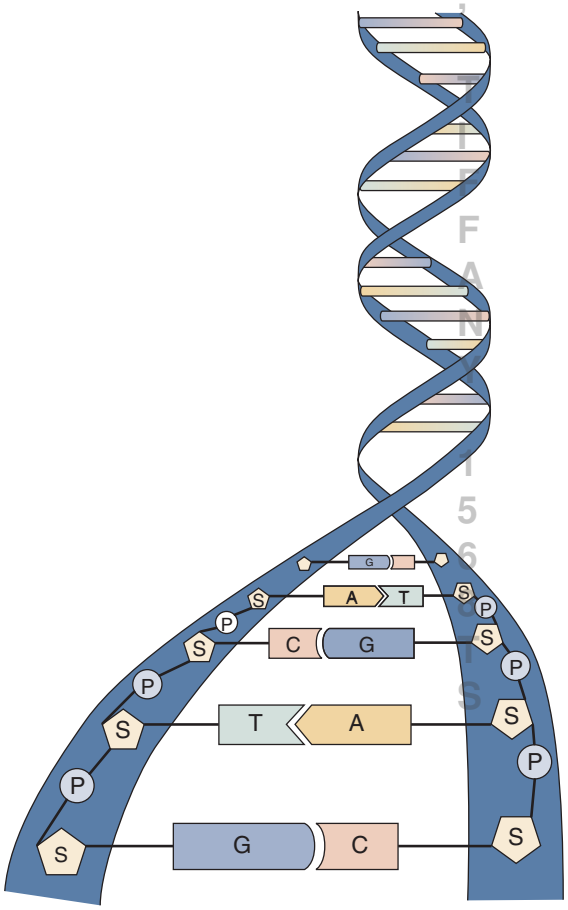

FIGURE 15–2
A representation of a DNA double helix. Notice how bases *G* and *C* pair with each other, as do bases *A* and *T*. This is the only arrangement in which two DNA strands can align with each other in a double-helix configuration.

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WEBEXTRA 15.1
What Is DNA?

and hence the diversity of living organisms. DNA is like a book of instructions. The alphabet used to create the book is simple enough: *A*, *T*, *G*, and *C*. The order in which these letters are arranged defines the role and function of a DNA molecule.

DNA at Work

proteins
Polymers of amino acids that play basic roles in the structures and functions of living things.

amino acids
The building blocks of proteins; there are twenty common amino acids; amino acids are linked to form a protein; the types of amino acids and the order in which they're linked determine the character of each protein.

The inheritable traits that are controlled by DNA arise out of its ability to direct the production of complex molecules called **proteins**. Proteins are actually made by linking a combination of **amino acids**. Although thousands of proteins exist, they can all be derived from a combination of up to 20 known amino acids. The sequence of amino acids in a protein chain determines the shape and function of the protein. Let's look at one example: The protein hemoglobin is found in our red blood cells. It carries oxygen to our body cells and removes carbon dioxide from these cells. One of the four amino acid chains of "normal" hemoglobin is shown in Figure 15–3(a). Studies of individuals with sickle-cell anemia show that this inheritable disorder arises from the presence of "abnormal" hemoglobin in their red blood cells. An amino acid chain for "abnormal" hemoglobin is shown in Figure 15–3(b). Note that the sole difference between "normal" and "abnormal" or sickle-cell hemoglobin arises from the substitution of one amino acid for another in the protein chain.

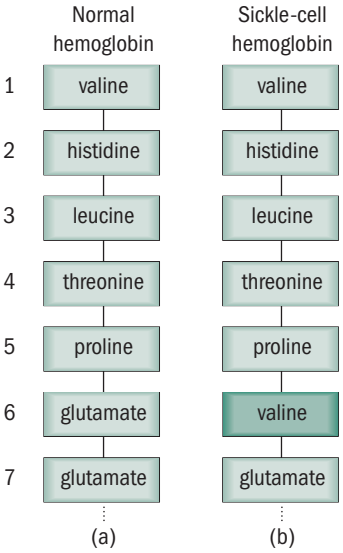

FIGURE 15–3
(a) A string of amino acids composes one of the protein chains of hemoglobin. (b) Substitution of just one amino acid for another in the protein chain results in sickle-cell hemoglobin.

The genetic information that determines the amino acid sequence for every protein manufactured in the human body is stored in DNA in a genetic code that relies on the sequence of bases along the DNA strand. The alphabet of DNA is simple—*A*, *T*, *G*, and *C*—but the key to deciphering the genetic code is to know that each amino acid is coded by a sequence of three bases. Thus, the amino acid alanine is coded by the combination *C–G–T*; the amino acid aspartate is coded by the combination *C–T–A*; and the amino acid phenylalanine is coded by the combination *A–A–A*. With this code in hand, we can now see how the amino acid sequence in a protein chain is determined by the structure of DNA. Consider the DNA segment

$$–C–G–T–C–T–A–A–A–A–C–G–T–$$

The triplet code contained within this segment translates into

$$[C–G–T] – [C–T–A] – [A–A–T] – [C–G–T]$$
alanine aspartate phenylalanine alanine

or the protein chain

alanine — *aspartate* — *phenylalanine* — *alanine*

Interestingly, this code is not restricted to humans. Almost all living cells studied to date use the same genetic code as the language of protein synthesis.[1]

If we look at the difference between "normal" and sickle-cell hemoglobin (see Figure 15–3), we see that the latter is formed by substituting one amino acid (valine) for another (glutamate). Within the DNA segment that codes for the production of normal hemoglobin, the letter sequence is

$$–[C–C–T]–[G–A–G]–[G–A–G]–$$
proline glutamate glutamate

Individuals with sickle-cell disease carry the sequence

$$–[C–C–T]–[G–T–G]–[G–A–G]–$$
proline valine glutamate

Thus, we see that a single base or letter change (*T* has been substituted for *A* in valine) is the underlying cause of sickle-cell anemia, demonstrating the delicate chemical balance between health and disease in the human body.

As scientists unravel the base sequences of DNA, they obtain a greater appreciation for the roles that proteins play in the chemistry of life. Already the genes responsible for hemophilia,

[1] Instructions for assembling proteins are actually carried from DNA to another region of the cell by ribonucleic acid (RNA). RNA is directly involved in the assembly of the protein using the genetic code it received from DNA.

Duchenne muscular dystrophy, and Huntington's disease have been located. Once scientists have isolated a disease-causing gene, they can determine the protein that the gene has directed the cell to manufacture. By studying these proteins—or the absence of them—scientists will be able to devise a treatment for genetic disorders.

A 13-year project to determine the order of bases on all 23 pairs of human chromosomes (also called the **human genome**) is now complete. Knowing where on a specific chromosome DNA codes for the production of a particular protein is useful for diagnosing and treating genetic diseases. This information is crucial for understanding the underlying causes of cancer. Also, comparing the human genome with that of other organisms will help us understand the role and implications of evolution.

human genome
The total DNA content found within the nucleus of a human cell; it is composed of approximately three billion base pairs of genetic information.

replication
The synthesis of new DNA from existing DNA.

polymerase chain reaction (PCR)
A technique for replicating or copying a portion of a DNA strand outside a living cell; this technique leads to millions of copies of the DNA strand.

Replication of DNA

Once the double-helix structure of DNA was discovered, how DNA duplicated itself before cell division became apparent. The concept of base pairing in DNA suggests the analogy of positive and negative photographic film. Each strand of DNA in the double helix has the same information; one can make a positive print from a negative or a negative from a positive.

The Process of Replication

The synthesis of new DNA from existing DNA begins with the unwinding of the DNA strands in the double helix. Each strand is then exposed to a collection of free nucleotides. Letter by letter, the double helix is re-created as the nucleotides are assembled in the proper order, as dictated by the principle of base pairing (*A* with *T* and *G* with *C*). The result is the emergence of two identical copies of DNA where before there was only one (see Figure 15–4). A cell can now pass on its genetic identity when it divides.

Many enzymes and proteins are involved in unwinding the DNA strands, keeping the two DNA strands apart, and assembling the new DNA strands. For example, DNA *polymerases* are enzymes that assemble a new DNA strand in the proper base sequence determined by the original, or parent, DNA strand. DNA polymerases also "proofread" the growing DNA double helices for mismatched base pairs, which are replaced with correct bases.

Until recently, the phenomenon of DNA **replication** appeared to be of only academic interest to forensic scientists interested in DNA for identification. However, this changed when researchers perfected the technology of using DNA polymerases to copy a DNA strand located outside a living cell. This laboratory technique is known as **polymerase chain reaction (PCR)**. Put simply, PCR is a technique designed to copy or multiply DNA strands in a laboratory test tube.

In PCR, small quantities of DNA or broken pieces of DNA found in crime-scene evidence can be copied with the aid of a DNA polymerase. The copying process is highly temperature dependent and can be accomplished in an automated fashion using a DNA thermal cycler (see Figure 15–5). Each cycle of the PCR technique results in a doubling of the DNA, as shown in Figure 15–4. Within a few hours, 30 cycles can multiply DNA a billionfold. Once DNA copies are in hand, they can be analyzed by any of the methods of modern molecular biology. The ability to multiply small bits of DNA opens new and exciting avenues for forensic scientists to explore. It means that sample size is no longer a limitation in characterizing DNA recovered from crime-scene evidence.

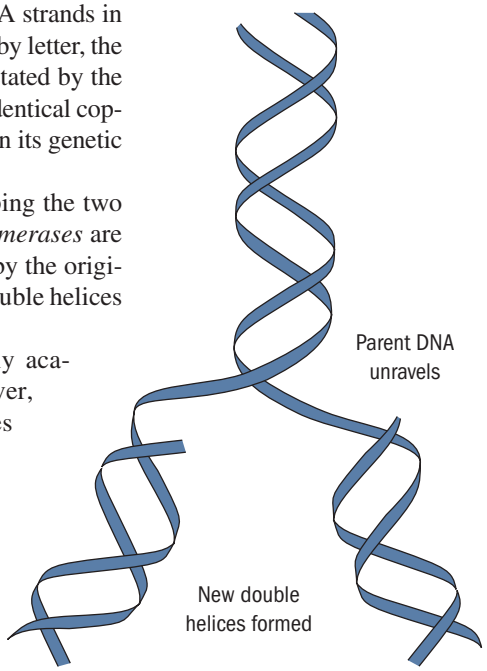

FIGURE 15–4
Replication of DNA. The strands of the original DNA molecule are separated, and two new strands are assembled.

DNA Typing with Short Tandem Repeats

Tandem Repeats

Geneticists have discovered that portions of the DNA molecule contain sequences of letters that are repeated numerous times. In fact, more than 30 percent of the human genome is composed of repeating segments of DNA. These repeating sequences, or **tandem repeats**, seem to act as filler or spacers between the coding regions of DNA. Although these repeating segments do not seem

tandem repeat
A region of a chromosome that contains multiple copies of a core DNA sequence that are arranged in a repeating fashion.

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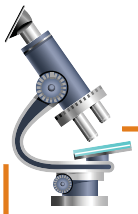
FIGURE 15–5
The DNA Thermal Cycler, an instrument that automates the rapid and precise temperature changes required to copy a DNA strand. Within a matter of hours, DNA can be multiplied a millionfold.



inside the science

Polymerase Chain Reaction

The most important feature of PCR is the knowledge that an enzyme called *DNA polymerase* can be directed to synthesize a specific region of DNA. In a relatively straightforward manner, PCR can be used to repeatedly duplicate or amplify a strand of DNA millions of times. As an example, let's consider a segment of DNA that we want to duplicate by PCR:

*–G–T–C–T–C–A–G–C–T–T–**C–C–A–G–***

*–**C–A–G–A**–G–T–C–G–A–A–G–G–T–C–*

To perform PCR on this DNA segment, short sequences of DNA on each side of the region of interest must be identified. In the example shown here, the short sequences are designated by boldface letters in the DNA segment. These short DNA segments must be available in a pure form known as a **primer** if the PCR technique is going to work.

The first step in PCR is to heat the DNA strands to about 94°C. At this temperature, the double-stranded DNA molecules separate completely:

–G–T–C–T–C–A–G–C–T–T–C–C–A–G–

–C–A–G–A–G–T–C–G–A–A–G–G–T–C–

The second step is to add the primers to the separated strands and allow the primers to combine, or **hybridize**, with the strands by lowering the test-tube temperature to about 60°C.

–G–T–C–T–C–A–G–C–T–T–C–C–A–G–

–C–A–G–A

C–C–A–G

–C–A–G–A–G–T–C–G–A–A–G–G–T–C–

The third step is to add the DNA polymerase and a mixture of free nucleotides *(A, C, G, T)* to the separated strands. When the test tube is heated to 72°C, the polymerase enzyme directs the rebuilding of a double-stranded DNA molecule, extending the primers by adding the appropriate bases, one at a time, resulting in the production of two complete pairs of double-stranded DNA segments:

–G–T–C–T–C–A–G–C–T–T–C–C–A–G–

C–A–G–A–G–T–C–G–A–A–G–G–T–C–

G–T–C–T–C–A–G–C–T–T–C–C–A–G

–C–A–G–A–G–T–C–G–A–A–G–G–T–C–

This completes the first cycle of the PCR technique, which results in a doubling of the number of DNA molecules from one to two. The cycle of heating, cooling, and strand rebuilding is then repeated, resulting in a further doubling of the DNA molecules. On completion of the second cycle, four double-stranded DNA molecules have been created from the original double-stranded DNA sample. Typically, 28 to 32 cycles are carried out to yield more than one billion copies of the original DNA molecule. Each cycle takes less than two minutes.

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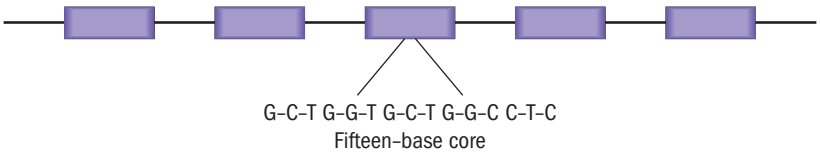

FIGURE 15–6

A DNA segment consisting of a series of repeating DNA units. In this illustration, the 15-base core can repeat itself hundreds of times. The entire DNA segment is typically hundreds to thousands of bases long.

primer
A short strand of DNA used to target a region of DNA for replication by PCR.

hybridization
The process of joining two complementary strands of DNA to form a double-stranded molecule.

WEBEXTRA 15.2
Polymerase Chain Reaction

to affect our outward appearance or control any other basic genetic function, they are nevertheless part of our genetic makeup, inherited from our parents in the manner illustrated by the Punnett square (page 366). The origin and significance of these tandem repeats is a mystery, but to forensic scientists they offer a means of distinguishing one individual from another through DNA typing.

Forensic scientists first began applying DNA technology to human identity in 1985. From the beginning, attention has focused on the tandem repeats of the genome. These repeats can be visualized as a string of connected boxes with each box having the same core sequence of DNA bases (see Figure 15–6). All humans have the same type of repeats, but there is tremendous variation in the number of repeats that each of us has.

Up until the mid-1990s, the forensic community aimed its efforts at characterizing repeat segments known as **restriction fragment length polymorphisms (RFLPs)**. A number of different RFLPs were selected by the forensic science community for performing DNA typing. Typically a core sequence is 15 to 35 bases long and repeats itself up to one thousand times. These repeats are cut out of the DNA double helix by a restriction enzyme that acts like a pair of scissors. Once the DNA molecules have been cut up by the restriction enzyme, the resulting fragments were sorted out by separating the fragments by a technique known as **electrophoresis**.

RFLP DNA typing has the distinction of being the first scientifically accepted protocol in the United States used for the forensic characterization of DNA. However, its utility has been short lived. New technology incorporating PCR has supplanted RFLP. In its short history, perhaps RFLP's most startling impact related to the impeachment trial of President Bill Clinton. The whole complexion of the investigation regarding the relationship of the president with a White House intern, Monica Lewinsky, changed when it was revealed that Ms. Lewinsky possessed a dress that she claimed was stained with the president's semen. The FBI Laboratory was asked to compare the DNA extracted from the dress stain with that of the president. An RFLP match was obtained between the president's DNA and the stain. The combined frequency of occurrence for the seven DNA types found was nearly one in eight trillion, an undeniable link. The dress and a copy of the FBI DNA report are shown in Figure 15–7.

Why couldn't the PCR technology be applied to RFLP DNA typing? Simply put, the RFLP strands are too long, often containing thousands of bases. PCR is best used with DNA strands that are no longer than a couple of hundred bases. The obvious solution to this problem is to characterize DNA strands that are much shorter than RFLPs. Another advantage in moving to shorter DNA strands is that they would be expected to be more stable and less subject to degradation brought about by adverse environmental conditions. The long RFLP strands tend to break apart under adverse conditions not uncommon at crime scenes.

restriction fragment length polymorphisms (RFLPs)
Different fragment lengths of base pairs that result from cutting a DNA molecule with restriction enzymes.

electrophoresis
A technique for separating molecules through their migration on a support medium while under the influence of an electrical potential.

Short Tandem Repeats (STRs)

Currently, **short tandem repeat (STR)** analysis has emerged as the most successful and widely used DNA-profiling procedure. STRs are locations (loci) on the chromosome that contain short sequence elements that repeat themselves within the DNA molecule. They serve as helpful markers for identification because they are found in great abundance throughout the human genome.

STRs normally consist of repeating sequences of three to seven bases; the entire strand of an STR is also very short, less than 450 bases long. These strands are significantly shorter than those encountered in other DNA typing procedures. This means that STRs are much less susceptible to degradation and are often recovered from bodies or stains that have been subject to extreme decomposition. Also, because of their shortness, STRs are an ideal candidate for multiplication by PCR, thus overcoming the limited-sample-size problem often associated with crime-scene

WEBEXTRA 15.3
An Animated Demonstration of Gel Electrophoresis

short tandem repeat (STR)
A region of a DNA molecule that contains short segments consisting of three to seven repeating base pairs.

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FEDERAL BUREAU OF INVESTIGATION
WASHINGTON, D. C. 20535

Report of Examination

Examiner Name:	[redacted]	Date:	08/17/98
Unit:	DNA Analysis 1	Phone No.:	202-324-4409
FBI File No.:	29D-OIC-LR-35063	Lab No.:	980730002 S BO 980803100 S BO

Results of Examinations:

Deoxyribonucleic acid (DNA) profiles for the genetic loci D2S44, D17S79, D1S7, D4S139, D10S28, D5S110 and D7S467 were developed from HaeIII-digested high molecular weight DNA extracted from specimens K39 and Q3243-1(a semen stain removed from specimen Q3243). Based on the results of these seven genetic loci, specimen K39 (CLINTON) is the source of the DNA obtained from specimen Q3243-1, to a reasonable degree of scientific certainty.

No DNA-RFLP examinations were conducted on specimen Q3243-2 (a semen stain removed from specimen Q3243).

BLACK – 1,440,000,000,000
CAUC – 7,870,000,000,000
SEH – 3,140,000,000,000
S&H – 943,000,000,000

DNAU1 - Page 1 of 1

This Report Is Furnished For Official Use Only

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Q3243

Courtesy of Federal Bureau of Investigation

FIGURE 15–7
The dress and the FBI Report of Examination for a semen stain located on the dress.

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Electrophoresis

Electrophoresis is somewhat related to thin-layer chromatography (discussed in Chapter 9) in that it separates materials according to their migration rates on a stationary solid phase. However, electrophoresis does not use a moving liquid phase to move the material; instead, an electrical potential is placed across the stationary medium.

The nature of the medium can vary; most forensic applications call for a starch or agar gel coated onto a glass plate. Under these conditions, only substances that possess an electrical charge migrate across the stationary phase (see Figure 1 [a–c]). Because many substances in blood carry an electrical charge, they can be separated and identified by electrophoresis. The technique is particularly useful for separating and identifying complex biochemical mixtures. In forensic science, electrophoresis is most useful for characterizing proteins and DNA in dried blood.

Forensic serologists have developed several electrophoretic procedures for characterizing DNA in dried blood. Mixtures of DNA fragments can be separated by gel electrophoresis by taking advantage of the fact that the rate of movement of DNA across a gel-coated plate depends on the molecule's size. Smaller DNA fragments move faster along the plate than larger DNA fragments. After completing the electrophoresis run, the separated DNA is stained with a suitable developing agent for visual observation (see Figure 2).

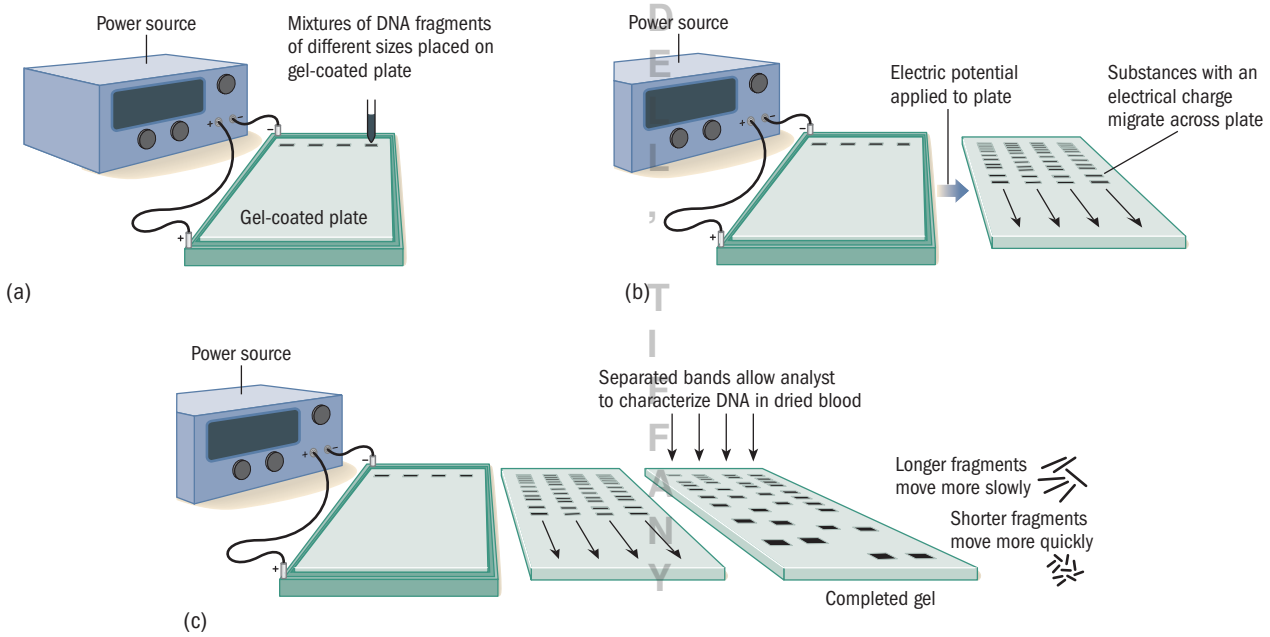

FIGURE 1
The technique of gel electrophoresis. (a) Applying samples to the plate. (b) Applying electric potential to the plate to cause the fragments to migrate. (c) Separation of the fragments on the gel allows for analysis.

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FIGURE 2
DNA fragments separated by gel electrophoresis are visualized under a UV light.

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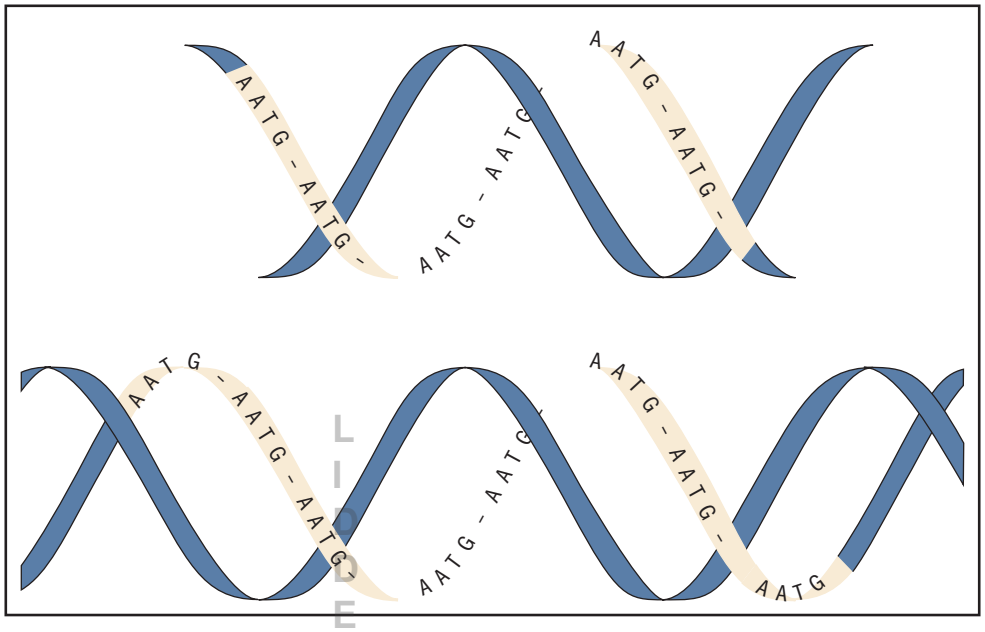

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FIGURE 15–8

Variants of the short tandem repeat TH01. The upper DNA strand contains six repeats of the sequence *A-A-T-G*; the lower DNA strand contains eight repeats of the sequence *A-A-T-G*. This DNA type is designated as TH01 6,8.

evidence. Only the equivalent of 18 DNA-containing cells is needed to obtain a DNA profile. For instance, STR profiles have been used to identify the origin of saliva residue on envelopes, stamps, soda cans, and cigarette butts.

To understand the utility of STRs in forensic science, let's look at one commonly used STR known as TH01. This DNA segment contains the repeating sequence *A–A–T–G*. Seven TH01 variants have been identified in the human genome. These variants contain 5 to 11 repeats of *A–A–T–G*. Figure 15–8 illustrates two such TH01 variants, one containing 6 repeats and the other containing 8 repeats of *A–A–T–G*.

During a forensic examination, TH01 is extracted from biological materials and amplified by PCR as described earlier. The ability to copy an STR means that extremely small amounts of the molecule can be detected and analyzed. Once the STRs have been copied or amplified, they are separated by electrophoresis. Here, the STRs are forced to move across a gel-coated plate under the influence of an electrical potential. Smaller DNA fragments move along the plate faster than do larger DNA fragments. By examining the distance the STR has migrated on the electrophoretic plate, one can determine the number of *A–A–T–G* repeats in the STR. Every person has two STR types for TH01, one inherited from each parent. Thus, for example, one may find in a semen stain TH01 with six repeats and eight repeats. This combination of TH01 is found in approximately 3.5 percent of the population. It is important to understand that all humans have the same type of repeats, but there is tremendous variation in the number of repeats each of us has.

When examining an STR DNA pattern, one merely needs to look for a match between band sets. For example, in Figure 15–9 DNA extracted from a crime-scene stain matches the DNA recovered from one of three suspects. When comparing only one STR, a limited number of people in a population would have the same STR fragment pattern as the suspect. However, by using additional STRs, a high degree of discrimination or complete individualization can be achieved.

Multiplexing

multiplexing
A technique that simultaneously detects more than one DNA marker in a single analysis.

What makes STRs so attractive to forensic scientists is that hundreds of types of STRs are found in human genes. The more STRs one can characterize, the smaller the percentage of the population from which these STRs can emanate. This gives rise to the concept of **multiplexing**. Using PCR technology, one can simultaneously extract and amplify a combination of different STRs.

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FIGURE 15–9
A DNA profile pattern of a suspect and its match to crime-scene DNA. From left to right, lane 1 is a DNA standard marker; lane 2 is the crime-scene DNA; and lanes 3 to 5 are control samples from suspects 1, 2, and 3, respectively. Crime-scene DNA matches suspect #2.

One STR system on the commercial market is the STR Blue Kit. This kit provides the necessary materials for amplifying and detecting three STRs (a process called *triplexing*): D3S1358, vWA, and FGA. The design of the system ensures that the size of the STRs does not overlap, thereby allowing each marker to be viewed clearly on an electrophoretic gel, as shown in Figure 15–10. In the United States, the forensic science community has standardized 13 STRs for entry into a national database known as the Combined DNA Index System (CODIS).

When an STR is selected for analysis, not only must the identity and number of core repeats be defined, but the sequence of bases flanking the repeats must also be known. This knowledge allows commercial manufacturers of STR typing kits to prepare the correct primers to delineate the STR segment to be amplified by PCR. Also, a mix of different primers aimed at different STRs will be used to simultaneously amplify a multitude of STRs (i.e., to multiplex). In fact, one STR kit on the commercial market can simultaneously make copies of 15 different STRs (see Figure 15–11).

DNA Typing with STRs

The 13 CODIS STRs are listed in Table 15–1 along with their probabilities of identity. The probability of identity is a measure of the likelihood that two individuals selected at random will have an identical STR type. The smaller the value of this probability, the more discriminating the STR. A high degree of discrimination and even individualization can be attained by analyzing a combination of STRs (multiplexing). Because STRs occur independently of each other, the probability of biological evidence having a particular combination of STR types is determined by the product of their frequency of occurrence in a population. This combination is referred to as the *product rule* (see page 64). Hence, the greater the number of STRs characterized, the smaller the frequency of occurrence of the analyzed sample in the general population.

The combination of the first 3 STRs shown in Table 15–1 typically produces a frequency of occurrence of about 1 in 5,000. A combination of the first 6 STRs typically yields a frequency of occurrence in the range of one in two million for the Caucasian population, and if the top 9 STRs are determined in combination, this frequency declines to about one in one billion. The combination of all 13 STRs shown in Table 15–1 typically produces frequencies of occurrence that measure in the

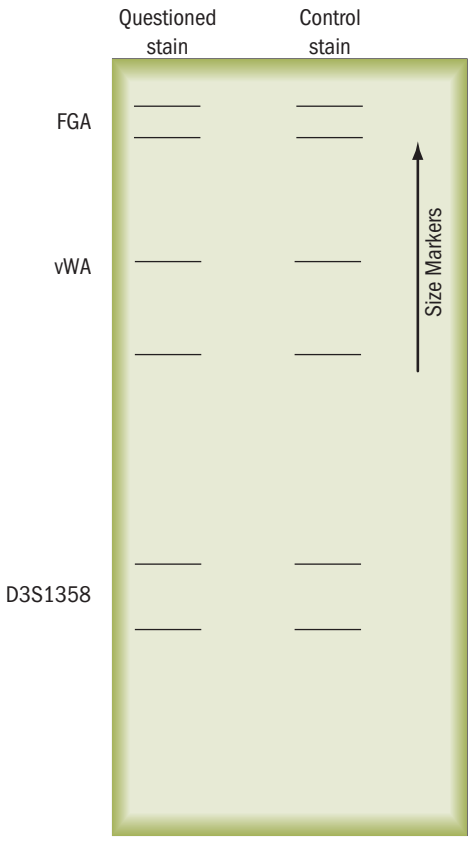

FIGURE 15–10
A triplex system containing three loci: FGA, vWA, and D3S1358, indicating a match between the questioned and the standard/reference stains.

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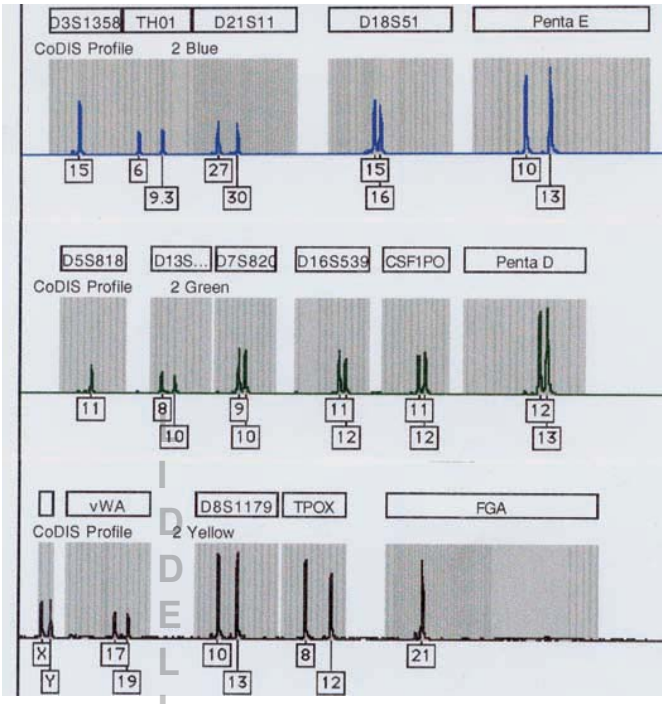

FIGURE 15–11
STR profile for 15 loci.

TABLE 15–1
The 13 CODIS STRs and Their Probability of Identities

STR	African American	U.S. Caucasian
D3S1358	0.094	0.075
vWA	0.063	0.062
FGA	0.033	0.036
TH01	0.109	0.081
TPOX	0.090	0.195
CSF1PO	0.081	0.112
D5S818	0.112	0.158
D13S317	0.136	0.085
D7S820	0.080	0.065
D8S1179	0.082	0.067
D21S11	0.034	0.039
D18S51	0.029	0.028
D16S539	0.070	0.089

Source: The Future of Forensic DNA Testing: Predictions of the Research and Development Working Group. Washington, D.C.: National Institute of Justice, Department of Justice, 2000, p. 41.

WEBEXTRA 15.4
Understand the Operational Principles of Capillary Electrophoresis

range of 1 in 575 trillion for Caucasian Americans and 1 in 900 trillion for African Americans. Importantly, several commercially available kits allow forensic scientists to profile STRs in the kinds of combinations cited here.

Sex Identification Using STRs

amelogenin gene
A genetic locus useful for determining gender.

Manufacturers of commercial STR kits typically used by crime laboratories provide one additional piece of useful information along with STR types: the sex of the DNA contributor. The focus of attention here is the **amelogenin gene** located on both the X and Y chromosomes. This

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gene, which is actually the gene for tooth pulp, has an interesting characteristic in that it is shorter by six bases in the X chromosome than in the Y chromosome. Hence, when the amelogenin gene is amplified by PCR and separated by electrophoresis, males, who have an X and a Y chromosome, show two bands; females, who have two X chromosomes, have just one band. Typically, these results are obtained in conjunction with STR types.

Another tool in the arsenal of the DNA analyst is the ability to type STRs located on the Y chromosome. The Y chromosome is male specific and is always paired with the X chromosome. Although more than 400 **Y-STRs** have been identified, only a small number of them are being used for forensic applications. One commercial kit allows for the characterization of 17 Y chromosome STRs. When can it be advantageous to seek out Y-STR types? Generally, Y-STRs are useful for analyzing blood, saliva, or a vaginal swab that is a mix originating from more than one male. For example, Y-STRs prove useful when multiple males are involved in a sexual assault. Further simplifying the analysis is that any DNA in the mixture that originates from a female will not show.

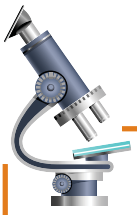
Y-STRs
Short tandem repeats located on the human Y chromosome.

Keep in mind that STR types derived from the Y chromosome originate only from this single male chromosome. A female subject, or one with an XX chromosome pattern, does not contribute any DNA information. Also, unlike a conventional STR type that is derived from two chromosomes and typically shows two bands or peaks, a Y-STR has only one band or peak for each STR type.

For example, the traditional STR DNA pattern may prove to be overly complex in the case of a vaginal swab containing the semen of two males. Each STR type would be expected to show four bands, two from each male. Also complicating the appearance of the DNA profile may be the presence of DNA from skin cells emanating from the walls of the vagina. In this circumstance, homing in on the Y chromosome greatly simplifies the appearance and interpretation of the DNA profile. Thus, when presented with a DNA mixture of two males and one female, Y-STR analysis would show only two bands (one band for each male) for each Y-STR type.

When gauging the significance of a Y-STR match between questioned and known specimens, one should take into consideration that all male paternal relatives (e.g., brothers, father, male offspring, and uncles) would be expected to have the same Y-STR profile.

Another advantage of employing STR technology is to extend the success of detecting evidential DNA from vaginal swabs collected from rape victims. Casework experience has demonstrated significant difficulties in obtaining traditional STR DNA profiles for the male donor from vaginal swabs collected after three to four days after intercourse. However, the application of Y-STR technology often extends the routine postcoital detection time to five days for the male donor.



inside the science

Capillary Electrophoresis

The separation of STRs can typically be carried out on a flat gel-coated electrophoretic plate, as described earlier. However, the need to reduce analysis time and to automate sampling and data collection has led to the emergence of *capillary electrophoresis* as the preferred technology for characterization of STRs. Capillary electrophoresis is carried out in a thin glass column rather than on the surface of a coated-glass plate.

As illustrated in the figure, each end of the column is immersed in a reservoir of buffer liquid that also holds electrodes (coated with platinum) to supply high-voltage energy. The column is coated with a gel polymer, and the DNA-containing sample solution is injected into one end of the column by applying a high voltage to an electrode immersed in the DNA solution. The STR fragments then move through the column under the influence of an electrical potential at a speed that is related to the length of the STR fragments. The other end of the column is connected to a detector that tracks the separated STRs as they emerge from the column. As the DNA peaks pass through the detector, they are recorded on a display known as an *electropherogram*, as shown in the figure.

(*continued*)

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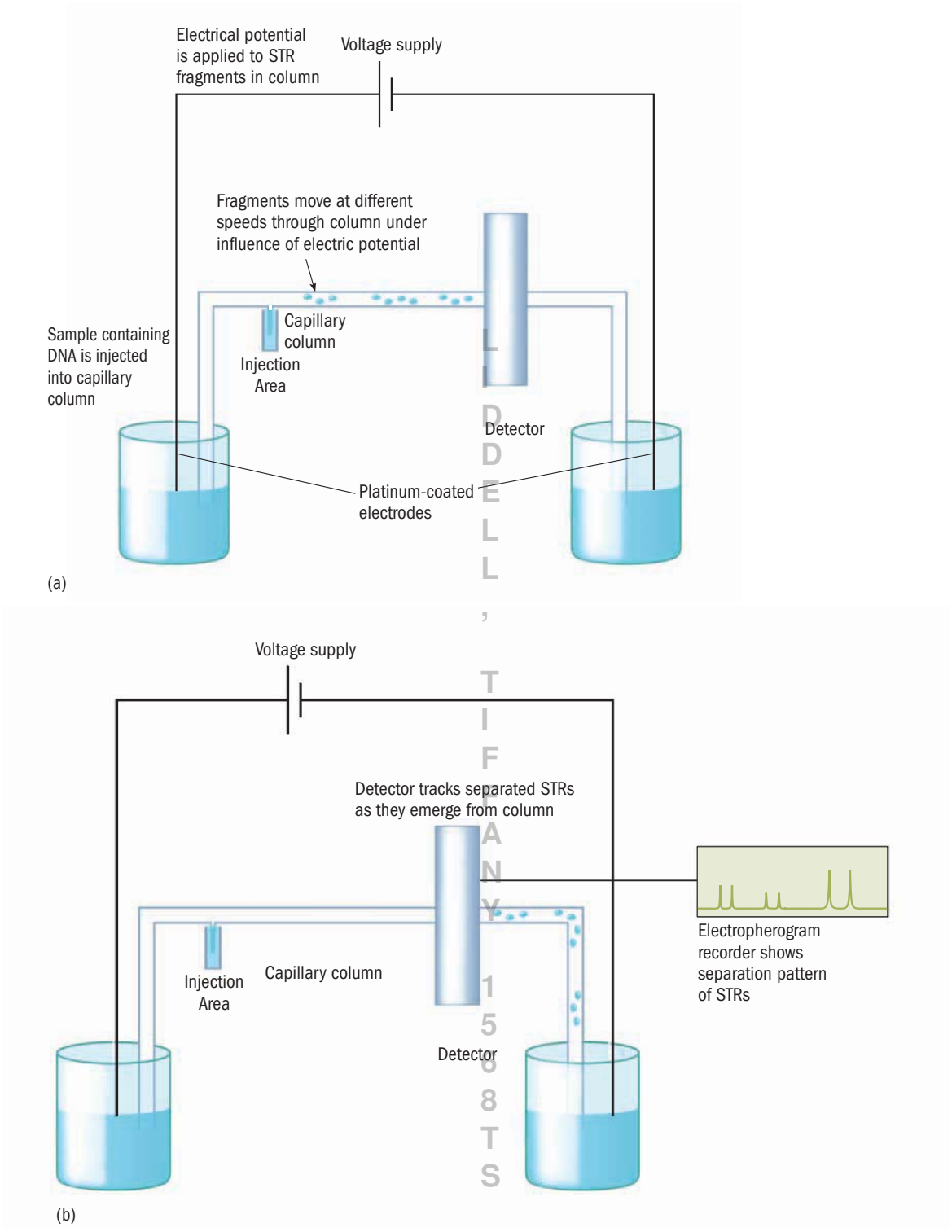

Capillary electrophoresis technology has evolved from the traditional flat gel electrophoresis approach. The separation of DNA segments is carried out on the interior wall of a glass capillary tube that is kept at a constant voltage. The size of the DNA fragments determines the speed at which they move through the column. This figure illustrates the separation of three sets of STRs (triplexing).

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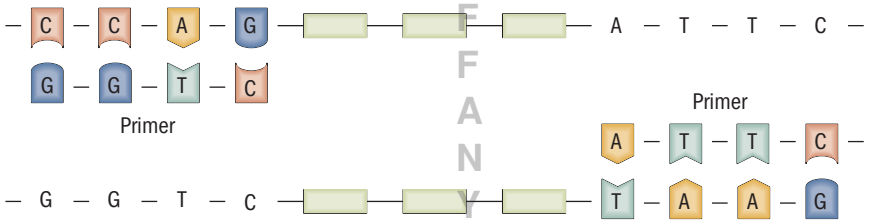
MiniSTRs

The forensic science community turned to STRs when it became apparent that short segments of DNA would be required to meet the requirements of PCR. Commercial manufacturers of DNA-typing kits prepared a series of 13 STRs for compatibility with the CODIS DNA database that ranged in length from 100 to 450 bases. One obvious benefit in working with short DNA segments was the likelihood that useful information could be extracted even from fragmented DNA. This often proved to be the case, but not always. On occasion, degraded DNA is encountered that is so badly damaged that traditional STR analysis is not possible. Prolonged exposure of DNA to extreme environmental elements, such as temperature extremes, humidity, or microbial activity, can lead to such degradation. An approach to dealing with this problem is to further shorten the STR strands that emerge from the PCR process.

The approach that has been taken to accomplish this task is to create new primers that can be positioned closer to the STR repeat region (see the figure). The shorter STR products (called *amplicons*) that now emerge from PCR increase the chances of characterizing badly fragmented strands of DNA. These smaller amplicons are called "miniSTRs." One manufacturer of STR kits has produced a miniSTR kit designed to amplify eight miniSTRs, seven of which are totally compatible with the CODIS database. The miniSTRs range in size from 71 to 250 bases. A DNA analyst suspecting a degraded sample now has the option, if sample size permits, of running both traditional STR and miniSTR determinations, or just the latter.

The advent of miniSTRs means that forensic scientists can now analyze samples that were once thought to be of no value. One of the first benefactors of miniSTR technology was the identification of a number of victims from the Waco Branch Davidian fire. Also, a number of World Trade Center victims were identified by miniSTR technology. Another focus of attention has been human hair. In the past, extracting nuclear DNA out of the hair shaft has been enormously difficult; the number of STRs in hair has been found to be very low as well as highly degraded. However, one study has demonstrated that miniSTRs may overcome some of the difficulties in obtaining partial profiles from the degraded DNA present in shed hair.[2]



Appropriate primers are positioned close to the repeat units of a DNA segment in order to initiate the PCR process that will create short or mini STRs.

Significance of DNA Typing

STR DNA typing has become an essential and basic investigative tool in the law enforcement community. The technology has progressed at a rapid rate and in only a few years has surmounted numerous legal challenges to become vital evidence for resolving violent crimes and sex offenses. DNA evidence is impartial, implicating the guilty and exonerating the innocent.

In a number of well-publicized cases, DNA evidence has exonerated individuals who have been wrongly convicted and imprisoned. The importance of DNA analyses in criminal investigations has also placed added burdens on crime laboratories to improve their quality-assurance procedures and to ensure the correctness of their results. In several well-publicized instances, the accuracy of DNA tests conducted by government-funded laboratories has been called into question.

WEBEXTRA 15.5
See the 13 CODIS STRs and Their Chromosomal Positions

WEBEXTRA 15.6
See How to Calculate the Frequency of Occurrence of a DNA Profile

WEBEXTRA 15.7
See the Electropherogram Record from One Individual's DNA

WEBEXTRA 15.8
An Animation Depicting Y-STRs

[2] K. E. Opel et al., "Evaluation and Quantification of Nuclear DNA from Human Telogen Hairs," *Journal of Forensic Sciences* 53 (2008): 853.

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The Combined DNA Index System (CODIS)

Perhaps the most significant investigative tool to arise from a DNA-typing program is CODIS (Combined DNA Index System), a computer software program developed by the FBI that maintains local, state, and national databases of DNA profiles from convicted offenders, unsolved crime-scene evidence, and profiles of missing people. CODIS allows crime laboratories to compare DNA types recovered from crime-scene evidence to those of convicted sex offenders and other convicted criminals.

Thousands of CODIS matches have linked serial crimes to each other and have solved crimes by allowing investigators to match crime-scene evidence to known convicted offenders. This capability is of tremendous value to investigators in cases in which the police have not been able to identify a suspect. The CODIS concept has already had a significant impact on police investigations in various states, as shown in the Case Files feature on page 70.

Mitochondrial DNA

Typically, when one describes DNA in the context of a criminal investigation, the subject is assumed to be the DNA in the nucleus of a cell. Actually, a human cell contains two types of DNA—nuclear and mitochondrial. The first constitutes the 23 pairs of chromosomes in the nuclei of our cells. Each parent contributes to the genetic makeup of these chromosomes. Mitochondrial DNA (mtDNA), on the other hand, is found outside the nucleus of the cell and is inherited solely from the mother.

mitochondria
Small structures located outside the nucleus of a cell; these structures supply energy to the cell; maternally inherited DNA is found in each mitochondrion.

Mitochondria are cell structures found in all human cells. They are the power plants of the body, providing about 90 percent of the energy that the body needs to function. A single mitochondrion contains several loops of DNA, all of which are involved in energy generation. Further, because each cell in our bodies contains hundreds to thousands of mitochondria, there are hundreds to thousands of mtDNA copies in a human cell. This compares to just one set of

case files

Cold Case Hit

In 1990, a series of attacks on elderly victims was committed in Goldsboro, North Carolina, by an unknown individual dubbed the Night Stalker. During one such attack in March, an elderly woman was brutally sexually assaulted and almost murdered. Her daughter's early arrival home saved the woman's life. The suspect fled, leaving behind materials intended to burn the residence and the victim in an attempt to conceal the crime.

In July 1990, another elderly woman was sexually assaulted and murdered in her home. Three months later, a third elderly woman was sexually assaulted and stabbed to death. Her husband was also murdered. Although their house was set alight in an attempt to cover up the crime, fire and rescue personnel pulled the bodies from the house before it was engulfed in flames. DNA analysis of biological evidence collected from vaginal swabs from the three sexual assault victims enabled authorities to conclude that the same perpetrator had committed all three crimes. However, there was no suspect.

More than ten years after these crimes were committed, law enforcement authorities retested the biological evidence from all three cases using newer DNA technology and entered the DNA profiles into North Carolina's DNA database. The DNA profile developed from the crime-scene evidence was compared to thousands of convicted-offender profiles already in the database.

In April 2001, a "cold hit" was made: The DNA profiles was matched to that of an individual in the convicted-offender DNA database. The perpetrator had been convicted of shooting into an occupied dwelling, an offense that requires inclusion of the convict's DNA in the North Carolina DNA database. The suspect was brought into custody for questioning and was served with a search warrant to obtain a sample of his blood. That sample was analyzed and compared to the crime-scene evidence, confirming the DNA database match. When confronted with the DNA evidence, the suspect confessed to all three crimes.

Source: National Institute of Justice, "Using DNA to Solve Cold Cases" (NIJ Special Report), July 2002.

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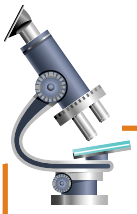

inside the science

Familial DNA—Expanding the DNA Database

In 1984, Deborah Sykes was raped and stabbed to death as she walked to work in Winston-Salem, North Carolina. A month later, Darryl Hunt, then 19 years old, was arrested and eventually convicted of the crime. Hunt insisted that he was innocent, and by 1990, DNA testing of semen found on Sykes proved that he was not its source. Nevertheless, North Carolina prosecutors ignored this new evidence and he remained in jail. Finally, a search against Darryl Hunt's STR profile in the North Carolina DNA database revealed a close but not perfect match to a genetic profile already in the database, that of his brother. Upon further investigation, that man, Willard Brown, confessed to Sykes's murder in 2003, and Hunt was finally freed from prison.

In this case, DNA profiling exonerated an innocent man and helped lead the police to the real culprit. The Sykes case illustrates how the contents of a criminal DNA database can be dramatically expanded to aid the police in identifying criminals by searching the database for near matches.

Typically, the CODIS database is used to find exact matches with crime-scene DNA. However, taking into account the facts that the 13 STR loci that constitute U.S. offender DNA databases are genetically inherited and that each individual's DNA profile is genetically determined by one's parents creates opportunities to use the database's raw data to search out close relatives. DNA profiles of related individuals are likely to show a higher proportion of shared STR loci as compared to unrelated individuals. Hence, searching the database for profiles that have a high degree of commonality may lead to the identification of a close relative of the perpetrator. Interestingly, studies have shown that a person's chances of committing a crime increase if a parent or sibling had previously done so. A 1999 U.S. Department of Justice survey found that 46 percent of jail inmates had at least one close relative who had also been incarcerated.

The potential for improving the effectiveness of DNA database searches is considerable. Familial searches of a DNA database would dramatically increase the size of the database by three or more times because every profile that is entered would, in effect, contain genetic information about the STR alleles of the donor's parents, siblings, and children. One study estimates that using familial DNA searches could increase the "cold hit" rates by 40 percent. Considering the fact that there have been about 95,000 cold hits in the United States, familial DNA has the potential for identifying thousands of additional criminal suspects.

The concept of familial DNA searching has been routinely adopted in the United Kingdom. In the United States, the FBI notifies investigators about close matches it finds using its current software, but the agency has no current plans to modify its search algorithms to optimize the database's capacity to ferret out near or close matches. This leaves it up to the states to decide whether to release identifying information about an offender whose DNA closely matches a crime-scene sample from another state. Challengers to familial database searching have cited it as a violation of constitutional protections against unreasonable search and seizure. A number of mixed state court decisions have failed to produce a consensus on the constitutionality of familial DNA database searches.

nuclear DNA located in that same cell. Thus, forensic scientists are offered enhanced sensitivity and the opportunity to characterize mtDNA when nuclear DNA is significantly degraded, such as in charred remains, or when nuclear DNA may be present in a small quantity (such as in a hair shaft). Interestingly, when authorities cannot obtain a reference sample from an individual who may be long deceased or missing, an mtDNA reference sample can be obtained from any maternally related relative. However, all individuals of the same maternal lineage will be indistinguishable by mtDNA analysis.

Although mtDNA analysis is significantly more sensitive than nuclear DNA profiling, forensic analysis of mtDNA is more rigorous, time consuming, and costly than nuclear DNA profiling. For this reason, only a handful of public and private forensic laboratories receive evidence for this type of determination. The FBI Laboratory has imposed strict limitations on the types of cases in which it will apply mtDNA technology.

sequencing
A procedure used to determine the order of the base pairs that constitute DNA.

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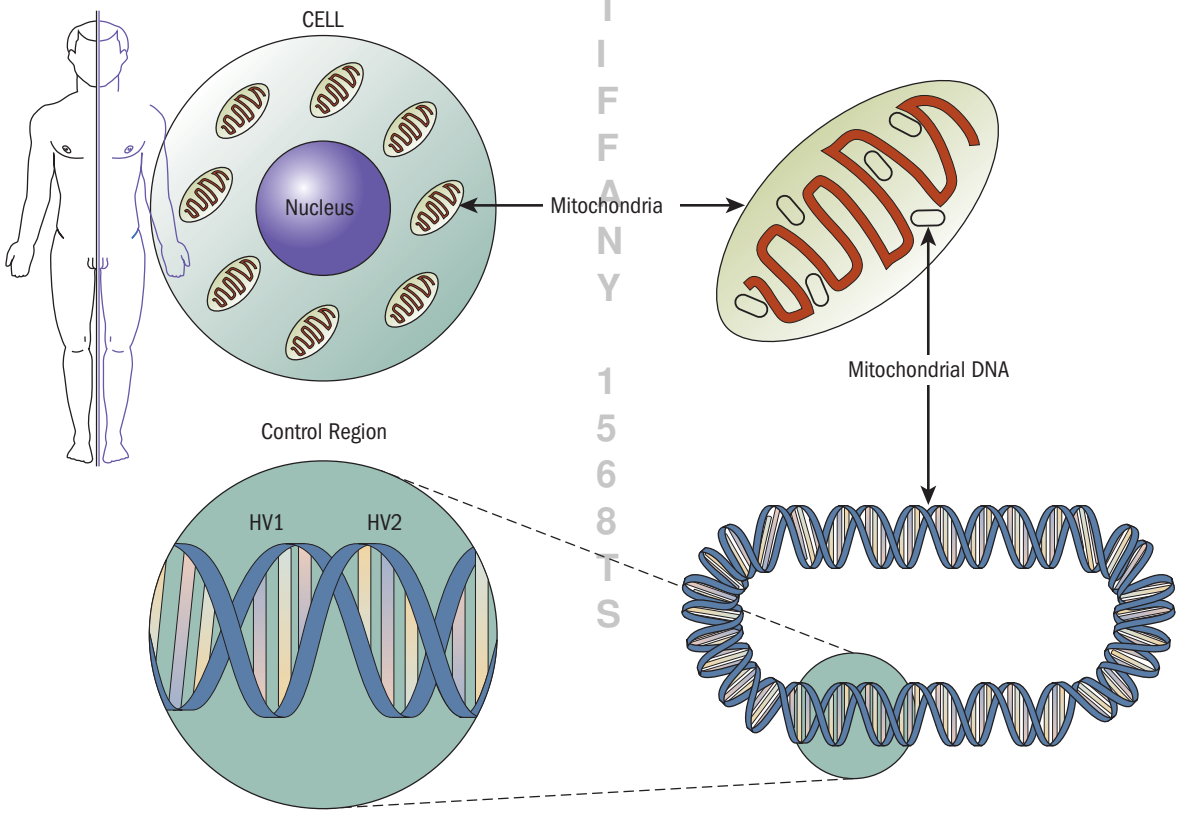
inside the science

Forensic Aspects of Mitochondrial DNA

As was previously discussed, nuclear DNA is composed of a continuous linear strand of nucleotides (*A*, *T*, *G*, and *C*). On the other hand, mtDNA is constructed in a circular or loop configuration. Each loop contains enough *A*, *T*, *G*, and *C* (approximately 16,569 units) to make up 37 genes involved in mitochondrial energy generation. Two regions of mtDNA have been found to be highly variable in the human population. These two regions have been designated hypervariable region I (HV1) and hypervariable region II (HV2), as shown in the figure. As indicated previously, the process for analyzing HV1 and HV2 is tedious. It involves generating many copies of these DNA hypervariable regions by PCR and then determining the order of the *A*–*T*–*G*–*C* bases constituting the hypervariable regions. This process is known as **sequencing**. The FBI Laboratory, the Armed Forces DNA Identification Laboratory, and other laboratories have collaborated to compile an mtDNA population database containing the base sequences from HV1 and HV2.

Once the sequences of the hypervariable regions from a case sample are obtained, most laboratories simply report the number of times these sequences appear in the mtDNA database maintained by the FBI. The mtDNA database contains about five thousand sequences. This approach permits an assessment of how common or rare an observed mtDNA sequence is in the database. Interestingly, many of the sequences that have been determined in casework are unique to the existing database, and many types are present at frequencies no greater than 1 percent in the database. Thus it is often possible to demonstrate how uncommon a particular mitochondrial DNA sequence is. However, even under the best circumstances, mtDNA typing does not approach STR analysis in its discrimination power. Thus, mtDNA analysis is best reserved for samples for which nuclear DNA typing is simply not possible.



Every cell in the body contains hundreds of mitochondria, which provide energy to the cell. Each mitochondrion contains numerous copies of DNA shaped in the form of a loop. Distinctive differences between individuals in their mitochondrial DNA makeup are found in two specific segments of the control region on the DNA loop known as HV1 and HV2.

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Collection and Preservation of Biological Evidence for DNA Analysis

WEBEXTRA 15.9
See How We Inherit Our Mitochondrial DNA

WEBEXTRA 15.10
Look into the Structure of Mitochondrial DNA and See How It Is Used for DNA Typing

Since the early 1990s, the advent of DNA profiling has vaulted biological crime-scene evidence to a stature of importance that is eclipsed only by the fingerprint. In fact, the high sensitivity of DNA determinations has even changed the way police investigators define biological evidence.

Just how sensitive is STR profiling? Forensic analysts using currently accepted protocols can reach sensitivity levels as low as 125 **picograms**. Interestingly, a human cell has an estimated 7 picograms of DNA, which means that only 18 DNA-bearing cells are needed to obtain an STR profile. However, modifications in the technology can readily extend the level of detection down to 9 cells. A quantity of DNA that is below the normal level of detection is defined as a **low copy number**. (However, analysts must take extraordinary care in analyzing low-copy-number DNA and often may find that courts will not allow this data to be admissible in a criminal trial.) With this technology in hand, the horizon of the criminal investigator extends beyond the traditional dried blood or semen stain to include stamps and envelopes licked with saliva, a cup or can that has touched a person's lips, chewing gum, the sweat band of a hat, or a bedsheet containing dead skin cells. Likewise, skin or **epithelial cells** transferred onto the surface of a weapon, the interior of a glove, or a pen have yielded DNA results.[3]

picogram
One-trillionth of a gram, or 0.000000000001 gram.

low copy number
Fewer than 18 DNA-bearing cells.

epithelial cells
The outer layer of skin cells; these DNA-bearing cells often fall off or are rubbed off onto objects retrieved from crime scenes.

The phenomenon of transferring DNA via skin cells onto the surface of an object has come to be called **touch DNA**. Again, keep in mind that, in theory, only 18 skin cells deposited on an object are required to obtain a DNA profile.

touch DNA
DNA from skin cells transferred onto the surface of an object by simple contact.

Collection of Biological Evidence

However, before investigators become enamored with the wonders of DNA, they should first realize that the crime scene must be treated in the traditional manner. Before the collection of evidence begins, biological evidence should be photographed close up and its location relative to the entire crime scene recorded through notes, sketches, and photographs. If the shape and position of bloodstains may provide information about the circumstances of the crime, an expert must immediately conduct an on-the-spot evaluation of the blood evidence. The significance of the position and shape of bloodstains can best be ascertained when the expert has an on-site overview of the entire crime scene and can better reconstruct the movement of the individuals involved. No attempt should be made to disturb the blood pattern before this phase of the investigation is completed.

The evidence collector must handle all body fluids and biologically stained materials with a minimum amount of personal contact. All body fluids must be assumed to be infectious; hence, wearing disposable latex gloves while handling the evidence is required. Latex gloves also significantly reduce the possibility that the evidence collector will contaminate the evidence. These gloves should be changed frequently during the evidence-collection phase of the investigation. Safety considerations and avoidance of contamination also call for the wearing of face masks, a lab coat, eye protection, shoe covers, and possibly coveralls.

The deposition of DNA onto crime-scene objects via saliva, sweat, skin, blood, and semen has created a vast array of forensic evidence that is quite different from the traditional evidence collected at crime scenes prior to the DNA era (see Table 15–2).

Packaging of Biological Evidence

Biological evidence should not be packaged in plastic or airtight containers because accumulation of residual moisture could contribute to the growth of DNA-destroying bacteria and fungi. **Each stained article should be packaged separately in a paper bag or a well-ventilated box.** A red-bio-hazard label must be attached to each container. If feasible, the entire stained article

[3] R. A. Wickenheiser, "Trace DNA: A Review, Discussion of Theory, and Application of the Transfer of Trace Qualities through Skin Contact," *Journal of Forensic Sciences* 47 (2002): 442.

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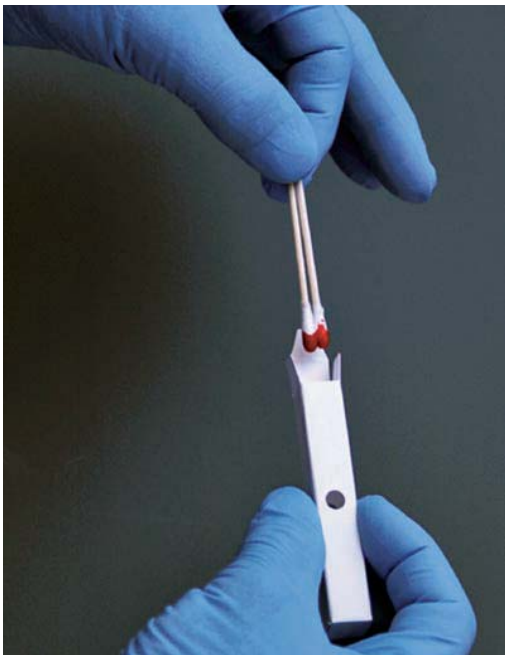
TABLE 15–2

Location and Sources of DNA at Crime Scenes

Evidence	Possible Location of DNA on the Evidence	Source of DNA
Baseball bat or similar weapon	Handle, end	Sweat, skin, blood, tissue
Hat, bandanna, mask	Inside	Sweat, hair, dandruff
Eyeglasses	Nose or ear pieces, lens	Sweat, skin
Facial tissue, cotton swab	Surface area	Mucus, blood, sweat, semen, ear wax
Dirty laundry	Surface area	Blood, sweat, semen
Toothpick	Tips	Saliva
Used cigarette	Cigarette butt	Saliva
Stamp or envelope	Licked area	Saliva
Tape or ligature	Inside/outside surface	Skin, sweat
Bottle, can, glass	Sides, mouthpiece	Saliva, sweat
Used condom	Inside/outside surface	Semen, vaginal or rectal cells
Blanket, pillow, sheet	Surface area	Sweat, hair, semen, urine, saliva
"Through and through" bullet	Outside surface	Blood, tissue
Bite mark	Person's skin or clothing	Saliva
Fingernail, partial fingernail	Scrapings	Blood, sweat, tissue

Source: National Institute of Justice, U.S. Department of Justice.

substrate control
An unstained object adjacent to an area on which biological material has been deposited.



Courtesy of Tri-Tech Forensics, Inc., Southport, NC

FIGURE 15–12
Air-dried swabs are placed in a swab box for delivery to the forensic laboratory.

buccal cells
Cells derived from the inner cheek lining.

should be packaged and submitted for examination. If this is not possible, dried blood is best removed from a surface with a sterile cotton-tipped swab lightly moistened with distilled water from a dropper bottle. A portion of the unstained surface material near the recovered stain must likewise be removed or swabbed and placed in a separate package. This is known as a **substrate control**. The forensic examiner may use the substrate swab to confirm that the results of the tests performed were brought about by the stain and not by the material on which it was deposited. However, this practice is normally not necessary when DNA determinations are carried out in the laboratory. One point is critical, and that is that the collected swabs must not be packaged in a wet state. After the collection is made, the swab must be air-dried for approximately five to ten minutes. Then it is best to place it in a swab box (see Figure 15–12), which has a circular hole to allow air circulation. The swab box can then be placed in a paper or manila envelope.

All packages containing biological evidence should be refrigerated or stored in a cool location out of direct sunlight until delivery to the laboratory. However, one common exception is blood mixed with soil. Microbes present in soil rapidly degrade DNA. Therefore, blood in soil must be stored in a clean glass or plastic container and immediately frozen.

Obtaining DNA Reference Specimens

Biological evidence attains its full forensic value only when an analyst can compare each of its DNA types to known DNA samples collected from victims and suspects. The least intrusive method for obtaining a DNA standard/reference, one that nonmedical personnel can readily use, is the *buccal swab*. Cotton swabs are placed in the subject's mouth and the inside of the cheek is vigorously swabbed, resulting in the transfer of **buccal cells** onto the swab (see Figure 15–13).

If an individual is not available to give a DNA standard/reference sample, some interesting alternatives are available to evidence collectors, including a toothbrush, combs and hairbrushes,

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a razor, soiled laundry, used cigarette butts, and earplugs. Any of these items may contain a sufficient quantity of DNA for typing purposes. Interestingly, as investigators worked to identify the remains of victims of the World Trade Center attack on September 11, 2001, the families of the missing were requested to supply the New York City DNA Laboratory with these types of items in an effort to match recovered DNA with human remains.

Courtesy of Tri-Tech Forensics, Inc., Southport, NC

FIGURE 15–13

A buccal swab collection kit is designed for use by nonmedical personnel. The cotton-tipped swabs are placed in the subject's mouth and the inside of the cheek is vigorously swabbed, resulting in the transferr of buccal cells onto the cotton bulb of the swab. The kit is then delivered to the forensic laboratory.

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Contamination of DNA Evidence

One key concern during the collection of a DNA-containing specimen is contamination. Contamination can occur by introducing foreign DNA through coughing or sneezing onto a stain during the collection process, or there can be a transfer of DNA when items of evidence are incorrectly placed in contact with each other during packaging. Fortunately, an examination of DNA band patterns in the laboratory readily reveals the presence of contamination. For example, with an STR, one will expect to see a two-band pattern. More than two bands suggest a mixture of DNA from more than one source.

Crime-scene investigators can take some relatively simple steps to minimize contamination of biological evidence:

1. Use disposable gloves.
2. Wear a face mask while collecting evidence, a lab coat, eye protection, as well as shoe covers.
3. Change gloves before handling each new piece of evidence.
4. Collect a substrate control for possible subsequent laboratory examination.
5. Pick up small items of evidence such as cigarette butts and stamps with clean forceps. Disposable forceps are to be used so that they can be discarded after a single evidence collection.
6. Always package each item of evidence in its own well-ventilated container.

WEBEXTRA 15.11
Step into the Role of the First Responding Officer at a Burglary Scene

WEBEXTRA 15.12
Assume the Duties of an Evidence Collection Technician at a Burglary Scene

A common occurrence at crime scenes is to suspect the presence of blood but not be able to observe any with the naked eye. In these situations, the common test of choice is luminol or Bluestar (see page 363). Interestingly, neither luminol nor Bluestar is expected to inhibit the ability to detect and characterize STRs.[4] Therefore, luminol and Bluestar can be used to locate traces of blood and areas that have been washed nearly free of blood without compromising the potential for DNA typing.

case files

Contact Lens Evidence

A woman alleged that she had been held against her will and sexually assaulted by a male friend in an apartment. During the course of the assault, a contact lens was knocked from the victim's eye. After the assault, she escaped, but because she was afraid of the threats made by her attacker, she did not report the assault to the police for three days. When the police examined the apartment, they noted that it had been thoroughly cleaned. A vacuum cleaner bag was seized for examination, and several pieces of material resembling fragments of a contact lens were discovered within the bag.

In the laboratory, approximately 20 nanograms of human DNA was recovered from the contact lens fragments. Cells from both the eyeball and the interior of the eyelids are naturally replaced every 6 to 24 hours. Therefore, both are potential sources for the DNA found. The DNA profile originating from the fragments matched the victim, thus corroborating the victim's account of the crime. The estimated population frequency of occurrence for the nine matching STRs are approximately 1 in 850 million. The suspect subsequently pleaded guilty to the offense.

STR Locus	Victim's DNA Type	Contact Lens
D3S1358	15, 18	15, 18
FGA	24, 25	24, 25
vWA	17, 17	17, 17
TH01	6, 7	6, 7
F13A1	5, 6	5, 6
fes/fps	11, 12	11, 12
D5S818	11, 12	11, 12
D13S317	11, 12	11, 12
D7S820	10, 12	10, 12

Based on information in R. A. Wickenheiser and R. M. Jobin, "Comparison of DNA Recovered from a Contact Lens Using PCR DNA Typing," *Canadian Society of Forensic Science Journal* 32 (1999): 67.

[4] A. M. Gross et al., "The Effect of Luminol on Presumptive Tests and DNA Analysis Using the Polymerase Chain Reaction," *Journal of Forensic Sciences* 44 (1999): 837.

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case files

The JonBenét Ramsey Murder Case

Point–Counterpoint

Point

July 9, 2008

Boulder District Attorney Mary T. Lacy issues the following announcement with regard to the investigation of the murder of JonBenét Ramsey.

On December 25–26, 1996, JonBenét Ramsey was murdered in the home where she lived with her mother, father and brother. Despite a long and intensive investigation, the death of JonBenét remains unsolved.

The murder has received unprecedented publicity and has been shrouded in controversy. That publicity has led to many theories over the years in which suspicion has focused on one family member or another. However, there has been at least one persistent stumbling block to the possibility of prosecuting any Ramsey family members for the death of JonBenét—DNA.

As part of its investigation of the JonBenét Ramsey homicide, the Boulder Police identified genetic material with apparent evidentiary value. Over time, the police continued to investigate DNA, including taking advantage of advances in the science and methodology. One of the results of their efforts was that they identified genetic material and a DNA profile from drops of JonBenét's blood located in the crotch of the underwear she was wearing at the time her body was discovered. That genetic profile belongs to a male and does not belong to anyone in the Ramsey family.

The police department diligently compared that profile to a very large number of people associated with the victim, with her family, and with the investigation, and has not identified the source, innocent or otherwise, of this DNA. The Boulder Police and prosecutors assigned to this investigation in the past also worked conscientiously with laboratory analysts to obtain better results through new approaches and additional tests as they became available. Those efforts ultimately led to the discovery of sufficient genetic markers from this male profile to enter it into the national DNA data bank.

In December of 2002, the Boulder District Attorney's Office, under Mary T. Lacy, assumed responsibility for the investigation of the JonBenét Ramsey homicide. Since then, this office has worked with the Boulder Police Department to continue the investigation of this crime.

In early August of 2007, District Attorney Lacy attended a Continuing Education Program in West Virginia sponsored by the National Institute of Justice on Forensic Biology and DNA. The presenters discussed successful outcomes from a new methodology described as "touch DNA." One method for sampling for touch DNA is the "scraping method." In this process, forensic scientists scrape a surface where there is no observable stain or other indication of possible DNA in an effort to recover for analysis any genetic material that might nonetheless be present. This methodology was not well known in this country until recently and is still used infrequently.

In October of 2007, we decided to pursue the possibility of submitting additional items from the JonBenét Ramsey homicide to be examined using this methodology. We checked with a number of Colorado sources regarding which private laboratory to use for this work. Based upon multiple recommendations, including that of the Boulder Police Department, we contacted the Bode Technology Group located near Washington, D.C., and initiated discussions with the professionals at that laboratory. First Assistant District Attorney Peter Maguire and Investigator Andy Horita spent a full day with staff members at the Bode facility in early December of 2007.

The Bode Technology laboratory applied the "touch DNA" scraping method to both sides of the waist area of the long johns that JonBenét Ramsey was wearing over her underwear when her body was discovered. These sites were chosen because evidence supports the likelihood that the perpetrator removed and/or replaced the long johns, perhaps by handling them on the sides near the waist.

On March 24, 2008, Bode informed us that they had recovered and identified genetic material from both sides of the waist area of the long johns. The unknown male profile previously identified from the inside crotch area of the underwear matched the DNA recovered from the long johns at Bode.

We consulted with a DNA expert from a different laboratory, who recommended additional investigation into the remote possibility that the DNA might have come from sources at the autopsy when this clothing was removed. Additional samples were obtained and then analyzed by the Colorado Bureau of Investigation to assist us in this effort. We received those results on June 27th of this year and are, as a result, confidant that this DNA did not come from innocent sources at the autopsy. As mentioned above, extensive DNA testing had previously excluded people connected to the family and to the investigation as possible innocent sources.

I want to acknowledge my appreciation for the efforts of the Boulder Police Department, Bode Technology Group, the Colorado Bureau of Investigation, and the Denver Police Department Forensic Laboratory for the great work and assistance they have contributed to this investigation.

The unexplained third party DNA on the clothing of the victim is very significant and powerful evidence. It is very unlikely that there would be an innocent explanation for DNA found at three different locations on two separate items of clothing worn by the victim at the time of her murder. This is particularly true in this case because the matching DNA profiles were found on genetic material from inside the crotch of the victim's underwear and near the waist on both sides of her long johns, and because concerted efforts that might identify a source, and perhaps an innocent explanation, were unsuccessful.

It is therefore the position of the Boulder District Attorney's Office that this profile belongs to the perpetrator of the homicide.

DNA is very often the most reliable forensic evidence we can hope to find during a criminal investigation. We rely on it often to bring to justice those who have committed crimes. It can likewise be reliable evidence upon which to remove people from suspicion in appropriate cases.

(*continued*)

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The Boulder District Attorney's Office does not consider any member of the Ramsey family, including John, Patsy, or Burke Ramsey, as suspects in this case. We make this announcement now because we have recently obtained this new scientific evidence that adds significantly to the exculpatory value of the previous scientific evidence. We do so with full appreciation for the other evidence in this case.

Local, national, and even international publicity has focused on the murder of JonBenét Ramsey. Many members of the public came to believe that one or more of the Ramseys, including her mother or her father or even her brother, were responsible for this brutal homicide. Those suspicions were not based on evidence that had been tested in court; rather, they were based on evidence reported by the media.

It is the responsibility of every prosecutor to seek justice. That responsibility includes seeking justice for people whose reputations and lives can be damaged irreparably by the lingering specter of suspicion. In a highly publicized case, the detrimental impact of publicity and suspicion on people's lives can be extreme. The suspicions about the Ramseys in this case created an ongoing living hell for the Ramsey family and their friends, which added to their suffering from the unexplained and devastating loss of JonBenét.

For reasons including those discussed above, we believe that justice dictates that the Ramseys be treated only as victims of this very serious crime. We will accord them all the rights guaranteed to the victims of violent crimes under the law in Colorado and all the respect and sympathy due from one human being to another. To the extent that this office has added to the distress suffered by the Ramsey family at any time or to any degree, I offer my deepest apology.

We prefer that any tips related to this ongoing investigation be submitted in writing or via electronic mail to BoulderDA.org, but they can also be submitted to our tip line at (303) 441–1636.

This office will make no further statements.

Counterpoint

Last year, then–Boulder, Colorado, District Attorney Mary Keenan Lacy, who had been "investigating" the Ramsey case for the last few years, wrote a letter to JonBenét's father John, apologizing for having believed he or his wife, the late Patsy, or their son Burke (then nine) had anything to do with their daughter's 1996 death. Lacy indicated that recent tests from the Bode Laboratory of Virginia revealed that their "new methodology" of touch DNA found a match that proves an intruder was culpable of the slaying in which the six-year-old was molested, strangled and given a fractured skull.

There has always been unmatched, unknown male DNA—likely from saliva—in the inside crotch of the child's underpants. A minute amount, too degraded to get a proper DNA profile, was mixed with her blood when someone stuck her oh-so-slightly with the pointed end of a broken paintbrush on the night of her death. Because the DNA was so insignificant, it was theorized to have come from someone coughing during the manufacturing process, then the blood drops on top rehydrated it. If the DNA and blood were deposited at the same time, they would have degraded at the same rate—but here the blood sample was robust.

Lacy wrote that the lab discovered that sloughed-off skin cells on the waist area of the long johns JonBenét wore over her underpants can be matched to the underpants' DNA.

That was great news for people who want to believe there was an intruder with no link to the family members. No one imagines a parent could harm a child in such a brutal and horrendous fashion. Only problem is, adults—including parents—kill kids all the time. While this murder is unique in its application and renown, from an investigative point of view it's just another homicide that has to be dissected to be understood. Anyone looking rationally at the evidence here, and assessing it as a whole, cannot be pleased with Lacy's letter to Ramsey or the fact that she cleared the most credible suspects in the case. At the least it sets a terrible precedent where other people "under the umbrella of suspicion" in other cases will demand the same treatment if their investigations take a long time to reach a courtroom. Just because someone isn't on trial, or a case has gone cold, doesn't mean that the right people aren't firmly under the microscope of authorities.

Mary Lacy left office in January, 2009, and the new DA, or any in the future, can retract her pronouncement. Frankly it will take a brave person to go against the sea of public opinion by individuals who want to blame a bogeyman. One note of encouragement is the case has been returned to the Boulder police, who are better equipped to investigate than the DA's office ever was. The case had been moved to Lacy's predecessor when the Ramsey family complained that the police spotlight on them was unfair. As we all know, there's no statute of limitations on homicide.

This doesn't take Lacy off the hook, as I see it. Here are some facts of the case which were ignored by her reckless decision. . . .

Touch DNA is nothing new to law enforcement, although Bode has only tested for it for about three years. For some ten years, the FBI lab at Quantico and other labs have used it to capture skin cells from inside masks or gloves, and from guns or knives.

Neither Lacy nor Bode will make available their test results so independent experts can critically review the information. If scientific evidence is to be used to make an argument, proof should be offered. Perhaps some media outlet will file a lawsuit to compel the documents.

What Lacy and Bode have said is that the mystery man's DNA is on the waist area, and that the DNA doesn't match any Ramsey family member. Nothing is stated about where and how much Ramsey DNA was discovered. Patsy dressed the child in those long johns before putting her to bed, and the waistband is precisely what John's two hands touched when he carried his daughter's stiff body in a vertical position upstairs from the basement where she was found deceased. And since DNA can survive multiple launderings, we can't pinpoint when that touch DNA was left on the waistband.

Boulder County Coroner Dr. John Meyer's autopsy report is the Rosetta Stone to this case. It explains the type and order of JonBenét's injuries—asphyxiation by a ligature, then a head blow. It does not reveal who killed the girl. Due to the three-page phony ransom note that many analysts believe was penned by Patsy, the working strategy was to consider Mrs. Ramsey as the perpetrator of all the insults inflicted upon the tot. But while there might have been enough evidence for an arrest, there was not enough for a conviction—and among insiders there was debate about who—if anyone in the household—did what. Without a clear through-line that police and prosecutors could agree upon, what chance would a jury have to find its way to a guilty verdict?

What the autopsy report states without equivocation is that the child suffered vaginal injuries that were "chronic," meaning they predated the murder by days or weeks. We're talking repeated digital penetration that eroded—not ruptured—her

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hymen. Also, the opening of her vagina was twice the size of a similar aged child's. These factors would have been testified to by at least three pediatric gynecological physicians, had the case gone to trial. This unknown pedophile would have needed ongoing intimate access to JonBenét before the night she died. Mary Lacy's early prosecutorial career was as a sex crimes expert, so why didn't she recognize the nature of this little girl's injuries?

If some accident led to JonBenét being strangled, then hit violently in the head, a normal reaction would have been for her caretakers to rush her to a hospital. But that didn't happen, I surmise, because her pre-existing genital injuries would have been noticed. And so, a ridiculous—and sadly, effective—cover-up ensued.

Mary Lacy was responsible for the 2006 debacle where she had arrested and brought back from Thailand a false confessor named John Mark Karr. When the underpants' DNA excluded him from being the perpetrator she let him go and publicly stated: "The DNA could be an artifact. It isn't necessarily the killer's. There's a probability that it's the killer's. But it could be something else."

She added: "No one is really cleared of a homicide until there's a conviction in court, beyond a reasonable doubt. And I don't think you will get any prosecutor, unless they were present with the person at the time of the crime, to clear someone."

What made her change her thinking when she cleared the Ramseys?

More to the point, where are the intruder's skin cells from the rope around the child's neck, the paintbrush, the spoon and bowl of pineapple she ate from just before she died, the white blanket that covered her, the flashlight believed to have hit her head, and the pen and paper used in the bogus ransom note? And where is the intruder's touch DNA on the waistband of JonBenét's underpants? Did the stranger pull down her long johns, then command her to pull down her own panties? Are we to believe he then put on gloves—or maybe a whole scuba suit, since there were no unidentified footprints, finger- or handprints, hairs or fibers?

Woven inside the rope around the neck, which was wrapped around a piece of a broken paintbrush, were fibers from the distinctive jacket Patsy wore that evening—and, allegedly, inside the underpants were fibers from the wool sweater John had on. Patsy's fibers were also in the tote where the paintbrush came from and on the sticky side of the piece of duct tape that covered JonBenét's mouth—a length of tape so small it could have been easily flicked aside by her tongue if it had been placed on her mouth while she was alive.

Lacy wrote that autopsy personnel were swabbed and tested for a DNA match, and thus excluded. But what about crime scene workers or lab technicians? And how many markers are in the touch DNA profile? The underpants' DNA was not enough to get a proper match through CODIS, the federal database. That didn't stop Lacy from sending it through on a regular basis—such busywork has little prospect of ending with a match, but it makes it seem as if something is being done.

Years ago, there was a civil suit in this case wherein a federal judge issued a statement that said, based on her reading of the material submitted to her, there was a higher likelihood of an intruder being the killer than a family member. At that time, Mary Lacy read a statement that suggested the Ramseys were innocent, based on the judicial ruling—though not clearing them. That statement was reportedly dictated to her by a Ramsey associate. What only those close to the case know is that one side of the civil suit completely abandoned its case, never offering paperwork, so the only information the judge had was that which came from the Ramsey camp. Ergo, an easy decision for the judge to make. Since then, Ramsey advisers have pummeled Lacy to clear the family entirely and eventually it happened.

It's egregious when an officer of the court misrepresents scientific evidence to win political favor. Mary Lacy was right to offer up an apology. But it should have been to JonBenét and not her family.

Source: Dawna Kaufmann, investigative journalist. Co-author with Cyril H. Wecht, MD, JD, of *A Question of Murder, Final Exams: True Crime cases from Cyril Wecht, and From Crime Scene to Courtroom.*

chapter summary

Portions of the DNA structure are as unique to each individual as fingerprints. The gene is the fundamental unit of heredity. Each gene is actually composed of DNA specifically designed to control the genetic traits of our cells. DNA is constructed as a very large molecule made by linking a series of repeating units called nucleotides. Four types of bases are associated with the DNA structure: adenine (*A*), guanine (*G*), cytosine (*C*), and thymine (*T*). The bases on each strand are properly aligned in a double-helix configuration. As a result, adenine pairs with thymine and guanine pairs with cytosine. This concept is known as base pairing. The order of the bases is what distinguishes different DNA strands.

Portions of the DNA molecule contain sequences of bases that are repeated numerous times. To a forensic scientist, these tandem repeats offer a means of distinguishing one individual from another through DNA typing. Length differences

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associated with relatively short repeating DNA strands are called short tandem repeats (STRs) and form the basis for the current DNA-typing procedure. They serve as useful markers for identification because they are found in great abundance throughout the human genome. STRs normally consist of repeating sequences 3 to 7 bases long, and the entire strand of an STR is also very short, less than 450 bases long. This means that STRs are much less susceptible to degradation and may often be recovered from bodies or stains that have been subjected to decomposition. Also, because of their shortness, STRs are ideal candidates for multiplication by PCR, in which STR strands are multiplied over a billionfold. PCR is responsible for the ability of STR typing to detect the genetic material of as few as 18 DNA-bearing cells. The more STRs one can characterize, the smaller the percentage of the population from which a particular combination of STRs can emanate. This gives rise to the concept of multiplexing. Using the technology of PCR, one can simultaneously extract and amplify a combination of different STRs. Currently, U.S. crime laboratories have standardized on 13 STRs. With STR analysis, as few as 125 picograms of DNA are required.

Another type of DNA used for individual characterization is mitochondrial DNA. Mitochondrial DNA is located outside the cell's nucleus and is inherited from the mother. However, mitochondrial DNA typing does not approach STR analysis in its discrimination power and thus is best reserved for samples, such as hair, for which STR analysis may not be possible.

Bloodstained evidence should not be packaged in plastic or airtight containers because accumulation of residual moisture could contribute to the growth of blood-destroying bacteria and fungi. Each stained article should be packaged separately in a paper bag or in a well-ventilated box.

review questions

1. The fundamental unit of heredity is the _____.
2. Each gene is actually composed of _____, specifically designed to carry out a single body function.
3. A(n) _____ is a very large molecule made by linking a series of repeating units.
4. A(n) _____ is composed of a sugar molecule, a phosphorus-containing group, and a nitrogen-containing molecule called a base.
5. DNA is actually a very large molecule made by linking a series of _____ to form a natural polymer.
6. _____ different bases are associated with the makeup of DNA.
7. Watson and Crick demonstrated that DNA is composed of two strands coiled into the shape of a(n) _____.
8. The structure of DNA requires the pairing of base *A* to _____ and base *G* to _____.
9. The base sequence *T–G–C–A* can be paired with the base sequence _____ in a double-helix configuration.
10. The inheritable traits that are controlled by DNA arise out of DNA's ability to direct the production of _____.
11. _____ are derived from a combination of up to 20 known amino acids.
12. The production of an amino acid is controlled by a sequence of _____ bases on the DNA molecule.
13. True or False: Enzymes known as DNA polymerase assemble new DNA strands into a proper base sequence during replication. _____
14. True or False: DNA can be copied outside a living cell. _____
15. True or False: All of the letter sequences in DNA code for the production of proteins. _____
16. In STR DNA typing, a typical DNA pattern shows (two, three) bands.
17. True or False: Specimens amenable to DNA typing are blood, semen, body tissues, and hair. _____
18. Short DNA segments containing repeating sequences of three to seven bases are called _____.
19. True or False: The longer the DNA strand, the less susceptible it is to degradation. _____
20. The short length of STRs allows them to be replicated by _____.
21. Used as markers for identification purposes, _____ are locations on the chromosome that contain short sequences that repeat themselves within the DNA molecule and in great abundance throughout the human genome.
22. (CODIS, AFIS) maintains local, state, and national databases of DNA profiles from convicted offenders, unsolved crime-scene evidence, and profiles of missing people.
23. Amazingly, the sensitivity of STR profiling requires only _____ DNA-bearing cells to obtain an STR profile.
24. During evidence collection, all body fluids must be assumed to be _____ and handled with latex-gloved hands.
25. The concept of (CODIS, multiplexing) involves simultaneous detection of more than one DNA marker.

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26. The amelogenin gene shows two bands for a (male, female) and one band for a (male, female).
27. Y-STR typing is useful when one is confronted with a DNA mixture containing more than one (male, female) contributor.
28. Mitochondrial DNA is inherited from the (mother, father).
29. True or False: Mitochondrial DNA is more plentiful in the human cell than is nuclear DNA. _____
30. The national DNA database in the United States has standardized on _____ STRs for entry into the database.
31. True or False: Y-STR data is normally entered into the CODIS database collection. _____.
32. Small amounts of blood are best submitted to a crime laboratory in a (wet, dry) condition.
33. True or False: Airtight packages make the best containers for blood-containing evidence. _____
34. Whole blood collected for DNA-typing purposes must be placed in a vacuum containing the preservative _____.
35. A typical STR DNA type emanating from a single individual shows a (one, two, three)-band pattern.

review questions for inside the science

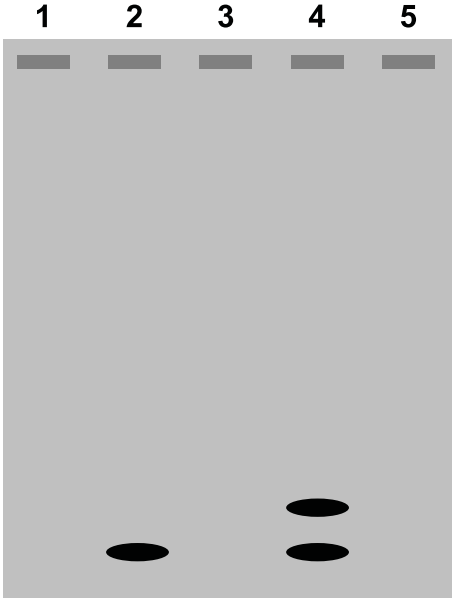
1. True or False: Enzymes known as DNA polymerases assemble new DNA strands into a proper base sequence based off the template strand during replication. _____
2. DNA evidence at a crime scene can be copied by the processes of the _____ with the aid of a DNA polymerase and specific primers.
3. DNA fragments can be separated and identified by (gas chromatography, capillary electrophoresis).
4. (Two, Four) regions of mitochondrial DNA have been found to be highly variable in the human population.
5. True or False: Polymerase chain reaction is a part of the process used in the forensic analysis of RFLP, STRs, and mitochondrial DNA. _____

application and critical thinking

1. The following sequence of bases is located on one strand of a DNA molecule:

   C–G–A–A–T–C–G–C–A–A–T–C–G–A–C–C–T–G

   List the sequence of bases that will form complementary pairs on the other strand of the DNA molecule.
2. Police discover a badly decomposed body buried in an area where a man disappeared some years before. The case was never solved, nor was the victim's body ever recovered. As the lead investigator, you suspect that the newly discovered body is that of the victim. What is your main challenge in using DNA typing to determine whether your suspicion is correct? How would you go about using DNA technology to test your theory?
3. You are a forensic scientist performing DNA typing on a blood sample sent to your laboratory. While performing an STR analysis on the sample, you notice a four-band pattern. What conclusion should you draw? Why?
4. A woman reports being mugged by a masked assailant, whom she scratched on the arm during a brief struggle. The victim is not sure whether the attacker was male or female. DNA analysts extract and amplify the amelogenin gene from the epithelial cells under the victim's fingernails (allegedly belonging to the attacker) and from a buccal swab of the victim. The sample is separated by gel electrophoresis with the result shown here. The victim's amelogenin DNA is in lane 2, and the amelogenin DNA from the fingernail scraping is in lane 4. What conclusion can you draw about the attacker from this result? How did you reach this conclusion?



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5. At a crime scene you encounter each of the following items. For each item, indicate the potential sources of DNA. The five possible choices are saliva, skin cells, sweat, blood, and semen.


donatas1205/Shutterstock

(a) _____


Elena Elisseeva/Shutterstock

(b) _____


somchaij/Shutterstock

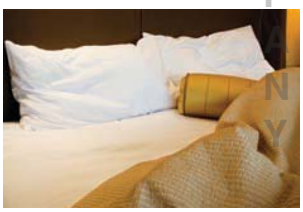
(c) _____


R-O-M-A/Shutterstock

(d) _____


Birgit Reitz-Hofmann/Shutterstock

(e) _____


Lim Yong Hian/Shutterstock

(f) _____


Tarasyuk Igor/ Shutterstock

(g) _____


Africa Studio/Shutterstock

(h) _____

6. The 15-STR locus DNA profile of a missing person, James Dittman, is shown in the following table.

STR Loci	Allele
D3S1358	15
THO1	6, 9.3
D21S11	27
D18S51	15, 16
PENTA E	10
D5S818	11
D13S807	10, 13
D7S820	9, 10
D16S539	11, 12
CSF1PO	13
PENTA D	12, 13
AMELOGENIN	XY
VWA	17, 19
D8S1170	10, 13
TPOX	8, 12
FGA	21

Decomposing remains were found deep in the woods near Dittman's house. DNA from these remains was extracted, amplified, and analyzed at 15 STR loci. Compare the STR readout for Dittman in the table with the chart on page 405 to determine whether the remains could belong to James Dittman. If not, at which STR loci do the profiles differ?

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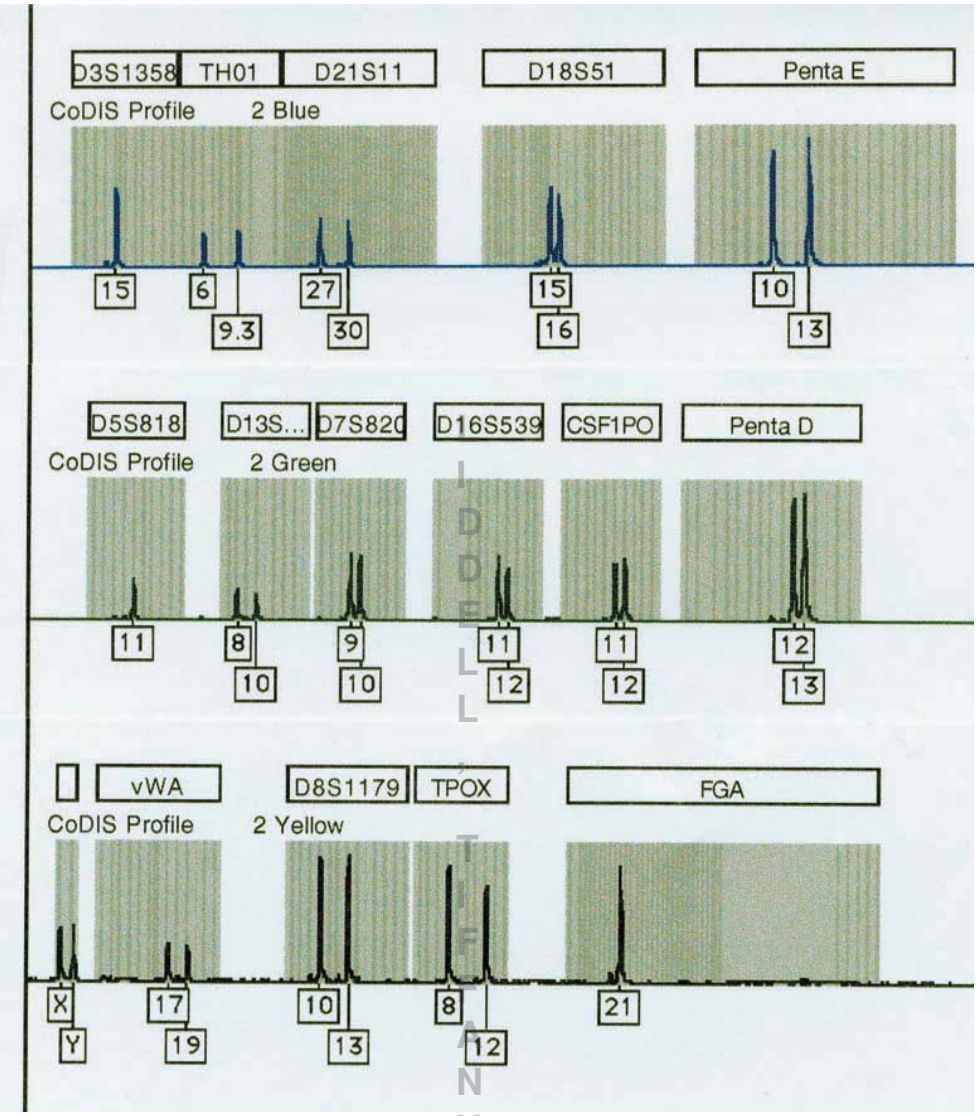

Richard Saferstein

further references

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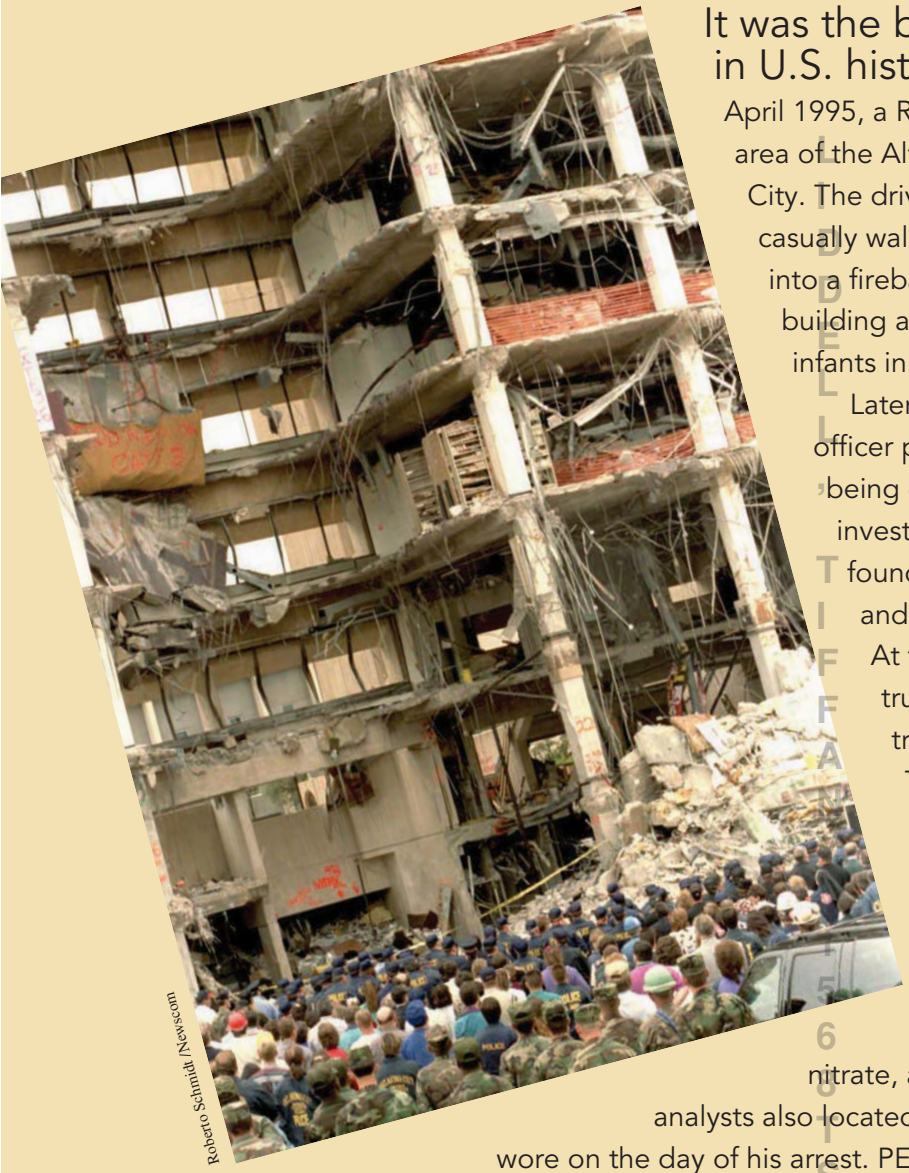
headline news

The Oklahoma City Bombing



Robert Schmidt/Newscom

It was the biggest act of mass murder in U.S. history. On a sunny spring morning in April 1995, a Ryder rental truck pulled into the parking area of the Alfred P. Murrah federal building in Oklahoma City. The driver stepped down from the truck's cab and casually walked away. Minutes later, the truck exploded into a fireball, unleashing enough energy to destroy the building and kill 168 people, including 19 children and infants in the building's day care center.

Later that morning, an Oklahoma Highway Patrol officer pulled over a beat-up 1977 Mercury Marquis being driven without a license plate. On further investigation, the driver, Timothy McVeigh, was found to be in possession of a loaded firearm and charged with transporting a firearm.

At the explosion site, remnants of the Ryder truck were located and the truck was quickly traced to a renter—Robert Kling, an alias for Timothy McVeigh. Coincidentally, the rental agreement and McVeigh's driver's license both used the address of McVeigh's friend Terry Nichols.

Investigators later recovered McVeigh's fingerprint on a receipt for two thousand pounds of ammonium nitrate, a basic explosive ingredient. Forensic analysts also located PETN residues on the clothing McVeigh wore on the day of his arrest. PETN is a component of detonating cord. After three days of deliberation, a jury declared McVeigh guilty of the bombing and sentenced him to die by lethal injection.

chapter 16

forensic aspects of fire and explosion investigation

DIDDELL, TIFFANY

Learning Objectives

After studying this chapter you should be able to:

- List the conditions necessary to initiate and sustain combustion
- Understand the three mechanisms of heat transfer
- Recognize the telltale signs of an accelerant-initiated fire
- Describe how to collect physical evidence at the scene of a suspected arson
- Describe laboratory procedures used to detect and identify hydrocarbon residues
- Understand how explosives are classified
- List some common commercial, homemade, and military explosives
- Describe how to collect physical evidence at the scene of an explosion
- Describe laboratory procedures used to detect and identify explosive residues

KEY TERMS

accelerant
black powder
combustion
deflagration
detonating cord
detonation
endothermic reaction
energy
exothermic reaction
explosion
flammable range
flash point
glowing combustion
heat of combustion
high explosive
hydrocarbon
ignition temperature
low explosive
modus operandi
oxidation
oxidizing agent
primary explosive
pyrolysis
safety fuse
secondary explosive
smokeless powder (double-base)
smokeless powder (single-base)
spontaneous combustion

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Forensic Investigation of Arson

Arson often presents complex and difficult circumstances to investigate. Normally these incidents are committed at the convenience of a perpetrator who has thoroughly planned the criminal act and has left the crime scene long before any official investigation is launched. Furthermore, proving commission of the offense is more difficult because of the extensive destruction that frequently dominates the crime scene. The contribution of the criminalist is only one aspect of a comprehensive and difficult investigative process that must establish a motive, the **modus operandi**, and a suspect.

modus operandi
An offender's pattern of operation.

The criminalist's function is limited; usually he or she is expected only to detect and identify relevant chemical materials collected at the scene and to reconstruct and identify igniters. Although a chemist can identify trace amounts of gasoline or kerosene in debris, no scientific test can determine whether an arsonist has used a pile of rubbish or paper to start a fire. Furthermore, a fire can have many accidental causes, including faulty wiring, overheated electric motors, improperly cleaned and regulated heating systems, and cigarette smoking—which usually leave no chemical traces. Thus, the final determination of the cause of a fire must consider numerous factors and requires an extensive on-site investigation. The ultimate determination must be made by an investigator whose training and knowledge have been augmented by the practical experiences of fire investigation.

The Chemistry of Fire

Humankind's early search to explain the physical concepts underlying the behavior of matter always bestowed a central and fundamental role on fire. To ancient Greek philosophers, fire was one of the four basic elements from which all matter was derived. The medieval alchemist thought of fire as an instrument of transformation, capable of changing one element into another. One ancient recipe expresses its mystical power as follows: "Now the substance of cinnabar is such that the more it is heated, the more exquisite are its sublimations. Cinnabar will become mercury, and passing through a series of other sublimations, it is again turned into cinnabar, and thus it enables man to enjoy eternal life."

oxidation
The combination of oxygen with other substances to produce new substances.

Today, we know of fire not as an element of matter but as a transformation process during which oxygen is united with some other substance to produce noticeable quantities of heat and light (a flame). Therefore, any insight into why and how a fire is initiated and sustained must begin with the knowledge of the fundamental chemical reaction of fire—**oxidation**.

Wallenrock/Shutterstock

FIGURE 16–1
Rust forming on iron is an example of oxidation.

Oxidation

In a simple description of oxidation, oxygen combines with other substances to produce new products. Thus, we may write the chemical equation for the burning of methane gas, a major component of natural gas, as follows:

$$\underset{\text{methane}}{CH_4} + \underset{\text{oxygen}}{2O_2} \longrightarrow \underset{\text{carbon dioxide}}{CO_2} + \underset{\text{water}}{2H_2O}$$

(yields)

However, not all oxidation proceeds in the manner that one associates with fire. For example, oxygen combines with many metals to form oxides. Thus, iron forms a red-brown iron oxide, or rust, as follows (see Figure 16–1):

$$\underset{\text{iron}}{4Fe} + \underset{\text{oxygen}}{3O_2} \longrightarrow \underset{\text{iron oxide}}{2Fe_2O_3}$$

(yields)

Yet chemical equations do not give us a complete insight into the oxidation process. We must consider other factors to understand all of the implications of oxidation or, for that matter, any other chemical reaction. Methane burns when it unites with oxygen, but merely mixing methane and oxygen does not produce a fire. Nor, for example, does gasoline burn when it is simply exposed to air. However, lighting a match in the presence of any one of these fuel–air mixtures (assuming proper proportions) produces an instant fire.

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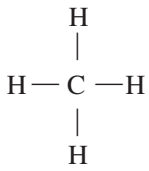
What are the reasons behind these differences? Why do some oxidations proceed with the outward appearances that we associate with a fire, but others do not? Why do we need a match to initiate some oxidations, but others proceed at room temperature? The explanation lies in a fundamental but abstract concept—energy.

Energy

Energy can be defined as the ability or potential of a system or material to do work. Energy takes many forms, such as heat energy, electrical energy, mechanical energy, nuclear energy, light energy, and chemical energy. For example, when methane is burned, the stored chemical energy in methane is converted to energy in the form of heat and light. This heat may be used to boil water or to provide high-pressure steam to turn a turbine. This is an example of converting chemical energy to heat energy to mechanical energy. The turbine can then be used to generate electricity, transforming mechanical energy to electrical energy. Electrical energy may then be used to turn a motor. In other words, energy can enable work to be done; heat is energy.

energy
The ability or potential of a system or material to do work.

The quantity of heat from a chemical reaction comes from the breaking and formation of chemical bonds. Methane is a molecule composed of one carbon atom bonded with four hydrogen atoms:



An oxygen molecule forms when two atoms of the element oxygen bond:

O═O

In chemical changes, atoms are not lost but merely redistributed during the chemical reaction; thus, the products of methane's oxidation will be carbon dioxide:

O═C═O

and water:

H—O—H

This rearrangement, however, means that the chemical bonds holding the atoms together must be broken and new bonds formed. We now have arrived at a fundamental observation in our dissection of a chemical reaction—that molecules must absorb energy to break apart their chemical bonds, and that they liberate energy when their bonds are re-formed.

The amount of energy needed to break a bond and the amount of energy liberated when a bond is formed are characteristic of the type of chemical bond involved. Hence, a chemical reaction involves a change in energy content; energy is going in and energy is given off. The quantities of energies involved are different for each reaction and are determined by the participants in the chemical reaction.

combustion
Rapid combination of oxygen with another substance, accompanied by production of noticeable heat and light.

exothermic reaction
A chemical transformation in which heat energy is liberated.

heat of combustion
The heat liberated during combustion.

endothermic reaction
A chemical transformation in which heat energy is absorbed from the surroundings.

Combustion

All oxidation reactions, including the **combustion** of methane, are examples of reactions in which more energy is liberated than is required to break the chemical bonds between atoms. Such reactions are said to be **exothermic**. The excess energy is liberated as heat, and often as light, and is known as the **heat of combustion**. Table 16–1 summarizes the heats of combustion of some important fuels in fire investigation.

Although we will not be concerned with them, some reactions require more energy than they eventually liberate. These reactions are known as **endothermic reactions**.

Thus, all reactions require an energy input to start them. We can think of this requirement as an invisible energy barrier between the reactants and the products of a reaction (see Figure 16–2). The higher this barrier, the more energy required to initiate the reaction. Where does this initial energy come from? There are many sources of energy; however, for the purpose of this discussion we need to look at only one—heat.

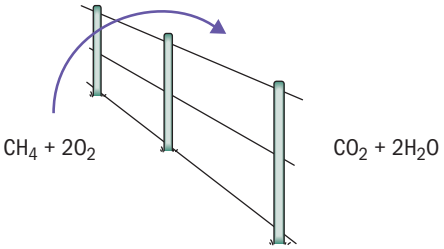


FIGURE 16–2
An energy barrier must be hurdled before reactants such as methane and oxygen can combine with one another to form the products of carbon dioxide and water.

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TABLE 16–1

Heats of Combustion of Fuels

Fuel	Heat of Combustion[a]
Crude oil	19,650 Btu/gal
Diesel fuel	19,550 Btu/lb
Gasoline	19,250 Btu/lb
Methane	995 Btu/cu ft
Natural gas	128–1,868 Btu/cu ft
Octane	121,300 Btu/gal
Wood	7,500 Btu/lb
Coal, bituminous	11,000–14,000 Btu/lb
Anthracite	13,351 Btu/lb

[a]A BTU (British thermal unit) is defined as the quantity of heat required to raise the temperature of 1 pound of water 1°F at or near its point of maximum density.

Source: John D. DeHaan, *Kirk's Fire Investigation*, 2nd ed. Upper Saddle River, N.J.: Prentice Hall, 1983.

HEAT The energy barrier in the conversion of iron to rust is relatively small, and it can be surmounted with the help of heat energy in the surrounding environment at normal outdoor temperatures. Not so for methane or gasoline; these energy barriers are quite high, and a high temperature must be applied to start the oxidation of these fuels. Hence, before any fire can result, the temperature of these fuels must be raised enough to exceed the energy barrier. Table 16–2 shows that this temperature, known as the **ignition temperature**, is quite high for common fuels.

ignition temperature
The minimum temperature at which a fuel spontaneously ignites.

Once combustion starts, enough heat is liberated to keep the reaction going by itself. In essence, the fire becomes a chain reaction, absorbing a portion of its own liberated heat to generate even more heat. The fire burns until either the oxygen or the fuel is exhausted.

Normally, a lighted match provides a convenient igniter of fuels. However, the fire investigator must also consider other potential sources of ignition—for example, electrical discharges, sparks, and chemicals—while reconstructing the initiation of a fire. All of these sources have temperatures higher than the ignition temperature of most fuels.

SPEED OF REACTION Although the liberation of energy explains many important features of oxidation, it does not explain all characteristics of the reaction. Obviously, although all oxidations liberate energy, not all are accompanied by a flame; witness the oxidation of iron to rust. Therefore, one other important consideration will make our understanding of oxidation and fire complete: the rate or speed at which the reaction takes place.

A chemical reaction, such as oxidation, takes place when molecules combine or collide with one another. The faster the molecules move, the greater the number of collisions between

TABLE 16–2

Ignition Temperatures of Some Common Fuels

Fuel	Ignition Temperature, °F
Acetone	869
Benzene	928
Fuel oil #2	495
Gasoline (low octane)	536
Kerosene (fuel oil #1)	410
n-Octane	428
Petroleum ether	550
Turpentine	488

Source: John D. DeHaan, *Kirk's Fire Investigation*, 4th ed. Upper Saddle River, N.J.: Prentice Hall, 1997.

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them and the faster the rate of reaction. Many factors influence the rate of these collisions. In our description of fire and oxidation, we consider only two: the physical state of the fuel and the temperature.

Physical State of Fuel A fuel achieves a reaction rate with oxygen sufficient to produce a flame only when it is in the gaseous state because only in this state can molecules collide frequently enough to support a flaming fire. This remains true whether the fuel is a solid such as wood, paper, cloth, or plastic, or a liquid such as gasoline or kerosene.

For example, the conversion of iron to rust proceeds slowly because the iron atoms cannot achieve a gaseous state. The combination of oxygen with iron is thus restricted to the surface area of the metal exposed to air, a limitation that severely reduces the rate of reaction. On the other hand, the reaction of methane and oxygen proceeds rapidly because all the reactants are in the gaseous state. The speed of the reaction is reflected by the production of noticeable quantities of heat and light (a flame).

Fuel Temperature How, then, does a liquid or solid maintain a gaseous reaction? In the case of a liquid fuel, the temperature must be high enough to vaporize the fuel. The vapor that forms burns when it mixes with oxygen and combusts as a flame. The **flash point** is the *lowest* temperature at which a liquid gives off sufficient vapor to form a mixture with air that will support combustion. Once the flash point is reached, the fuel can be ignited by some outside source of temperature to start a fire. The ignition temperature of a fuel is always considerably higher than the flash point. For example, gasoline has a flash point of –50°F; however, an ignition temperature of 495°F is needed to start a gasoline fire.

flash point
The minimum temperature at which a liquid fuel produces enough vapor to burn.

With a solid fuel such as wood, the process of generating vapor is more complex. A solid fuel burns only when exposed to heat intense enough to decompose the solid into gaseous products. This chemical breakdown of solid material is known as **pyrolysis**. The gaseous products of pyrolysis combine with oxygen to produce a fire (see Figure 16–3). Here again, fire can be described as a chain reaction. A match or other source of heat initiates the pyrolysis of the solid fuel, the gaseous products react with oxygen in the air to produce heat and light, and this heat in turn pyrolyzes more solid fuel into volatile gases.

pyrolysis
The decomposition of solid organic matter by heat.

LiveMan/Shutterstock

FIGURE 16–3
Intense heat causes solid fuels such as wood to decompose into gaseous products, a process called pyrolysis.

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Typically, the rate of a chemical reaction increases when the temperature is raised. The magnitude of the increase varies from one reaction to another and also from one temperature range to another. For most reactions, a 10°C (18°F) rise in temperature doubles or triples the reaction rate. This observation explains in part why burning is so rapid. As the fire spreads, it raises the temperature of the fuel–air mixture, thus increasing the rate of reaction; this in turn generates more heat, again increasing the rate of reaction. Only when the fuel or oxygen is depleted does this vicious cycle come to a halt.

THE FUEL–AIR MIX As we have seen from our discussion about gaseous fuel, air (oxygen) and sufficient heat are the basic ingredients of a flaming fire. There is also one other consideration—the gas fuel–air mix. A mixture of gaseous fuel and air burns only if its composition lies within certain limits. If the fuel concentration is too low (lean) or too great (rich), combustion does not occur. The concentration range between the upper and lower limits is called the **flammable range**. For example, the flammable range for gasoline is 1.3–6.0 percent. Thus, in order for a gasoline–air mix to burn, gasoline must make up at least 1.3 percent, and no more than 6 percent, of the mixture.

flammable range
The entire range of possible gas or vapor fuel concentrations in air that are capable of burning.

GLOWING COMBUSTION Although a flaming fire can be supported only by a gaseous fuel, in some instances a fuel can burn without a flame. Witness a burning cigarette or the red glow of hot charcoal (see Figure 16–4). These are examples of **glowing combustion** or *smoldering*. Here combustion occurs on the surface of a solid fuel in the absence of heat high enough to pyrolyze the fuel. Interestingly, this phenomenon generally ensues long after the flames have gone out. Wood, for example, tends to burn with a flame until all of its pyrolyzable components have been expended; however, wood's carbonaceous residue continues to smolder long after the flame has extinguished itself.

glowing combustion
Combustion on the surface of a solid fuel in the absence of heat high enough to pyrolyze the fuel.

SPONTANEOUS COMBUSTION One interesting phenomenon often invoked by arson suspects as the cause of a fire is **spontaneous combustion**. Actually, the conditions under which spontaneous combustion can develop are rather limited and rarely account for the cause of a fire. Spontaneous combustion is the result of a natural heat-producing process in poorly ventilated containers or areas. For example, hay stored in barns provides an excellent growing medium for bacteria whose activities generate heat. If the hay is not properly ventilated, the heat builds to a level that supports other types of heat-producing chemical reactions in the hay. Eventually, as the heat rises, the ignition temperature of hay is reached, spontaneously setting off a fire.

spontaneous combustion
A fire caused by a natural heat-producing process in the presence of sufficient air and fuel.

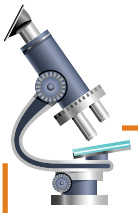
Lev Kropotov/Shutterstock

FIGURE 16–4
Glowing red charcoals.

Another example of spontaneous combustion involves the ignition of improperly ventilated containers containing rags soaked with certain types of highly unsaturated oils, such as linseed oil. Heat can build up to the point of ignition as a result of a slow heat-producing chemical oxidation between the air and the oil. Of course, storage conditions must encourage the accumulation of the heat over a prolonged period of time. However, spontaneous combustion does not occur with hydrocarbon lubricating oils, and it is not expected to occur with most household fats and oils.

In summary, three requirements must be satisfied to initiate and sustain combustion:

1. A fuel must be present.
2. Oxygen must be available in sufficient quantity to combine with the fuel.
3. Heat must be applied to initiate the combustion, and sufficient heat must be generated to sustain the reaction.



inside the science

Heat Transfer

Consider how a structural fire begins. The typical scenario starts with heat ignition at a single location. It may be an arsonist lighting a gasoline-soaked rag, a malfunctioning electric appliance sparking, or an individual falling asleep while smoking a cigarette in bed. How does a flame initially confined to a single location spread to engulf an entire structure? Understanding the anatomy of a fire begins with comprehending how heat travels through a burning structure.

The previous section stressed the importance of the role of heat in generating sufficient fuel vapors to support combustion, as well as the requirement that the heat source be hot enough to ignite the fuel's vapor. Once the fire begins, the heat generated by the fuel's reaction with air is fed back into the fuel–air mix to keep the chemical reaction going.

As a fire progresses, the heat created by the combustion process tends to move from a high-temperature region to one at a lower temperature. Understanding heat transfer from one location to another is important for reconstructing the origin of a fire, as well as for understanding why and how fire spreads through a structure. The three mechanisms of heat transfer are conduction, radiation, and convection.

Conduction

Movement of heat through a solid object is caused by a process called *conduction*, in which electrons and atoms within the heated object collide with one another.

Topham/The Image Works

The wooden handle on this saucepan is a poor conductor of heat.

(*continued*)

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Heat always travels from hot areas of a solid to cold ones by conduction. Solids whose atoms or molecules have loosely held electrons are good conductors of heat. Metals have the most loosely held electrons and are therefore excellent conductors of heat. Thus, when you insert one end of a metal object into an open flame, the entire object quickly becomes hot to the touch.

Materials that have electrons firmly attached to their molecules are poor conductors of heat. Poor conductors are called *insulators*. Wood is a good insulator; for that reason, metal objects that are subject to intense heat (such as skillets and saucepans) often have wooden handles (see the figure).

In reconstructing a fire scene, it's important to keep in mind that heat may be transported through metals such as beams, nails, fasteners, bolts, and other good conductors to a location far from the initial heat source. Any fuel in contact with the conductor may be ignited, creating a new fire location. On the other hand, the conductivity of wood, plastic, and paper is very low, meaning that heat emanating from these surfaces does not spread well and does not cause ignitions far from the initial heat source.

Radiation

Radiation is the transfer of heat energy from a heated surface to a cooler surface by electromagnetic radiation. A hot surface emits electromagnetic radiation of various wavelengths, and in a fire scene the electromagnetic radiation moves in a straight line from one surface to another. Radiant heat plays a key role in understanding how fire spreads throughout a structure. For example, all surfaces that face the fire are exposed to radiant heat and burst into flames when the surface reaches their ignition temperature. In very large fires, nearby structures and vehicles are often ignited at a distance by radiant heat.

Convection

Convection is the transfer of heat energy by movement of molecules within a liquid or gas. Water being heated on a stove illustrates the concept of convection. As the water molecules on the bottom of the pot move faster, they spread apart and become less dense, causing them to move upward. Denser, cooler water molecules then migrate to the bottom of the pot. In this way convection currents keep the fluid stirred up as warmer fluid moves away from the heat source and cooler fluid moves toward the heat source. Likewise, warm air expands, becoming less dense and causing it to rise and move toward the cooler surrounding air.

In a structural fire, the gaseous hot products of combustion expand, and convection moves the hot gases to the upper portions of the structure (see the figure). The convected hot gases become a source of heat, radiating heat energy downward onto all the surfaces below them. The hot surfaces of the exposed objects often pyrolyze or break down, releasing gaseous molecules. The phenomenon known as *flashover* occurs when all the combustible fuels simultaneously ignite, engulfing the entire structure in flame.

Courtesy Dave Frazier, Danita Delimont Photography

Convection causes flames to rise to the upper floor of a burning structure.

Searching the Fire Scene

accelerant
Any material used to start or sustain a fire.

The arson investigator should begin examining a fire scene for signs of arson as soon as the fire has been extinguished. Most arsons are started with petroleum-based **accelerants** such as gasoline or kerosene. Thus, the presence of containers capable of holding an accelerant arouse suspicions of arson. Discovery of an ignition device ranging in sophistication from a candle to a time-delay device is another indication of possible arson. A common telltale sign of arson is an

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irregularly shaped pattern on a floor or on the ground (see Figure 16–5) resulting from pouring an accelerant onto the surface. In addition to these visual indicators, investigators should look for signs of breaking and entering and theft, and they should begin interviewing any eyewitnesses to the fire.

Franklin County Sheriff's Office of North Carolina

FIGURE 16–5
Irregularly shaped pattern on the ground resulting from a poured ignitable liquid.

Timeliness of Investigation

Time constantly works against the arson investigator. Any accelerant residues that remain after a fire is extinguished may evaporate within a few days or even hours. Furthermore, safety and health conditions may necessitate that cleanup and salvage operations begin as quickly as possible. Once this occurs, a meaningful investigation of the fire scene is impossible. Accelerants in soil and vegetation can be rapidly degraded by bacterial action. Freezing samples containing soil or vegetation is an effective way to prevent this degradation.

The need to begin an *immediate* investigation of the circumstances surrounding a fire even takes precedence over the requirement to obtain a search warrant to enter and search the premises. The Supreme Court, explaining its position on this issue, stated in part:

> . . . Fire officials are charged not only with extinguishing fires, but with finding their causes. Prompt determination of the fire's origin may be necessary to prevent its recurrence, as through the detection of continuing dangers such as faulty wiring or a defective furnace. Immediate investigation may also be necessary to preserve evidence from intentional or accidental destruction. And, of course, the sooner the officials complete their duties, the less will be their subsequent interference with the privacy and the recovery efforts of the victims. For these reasons, officials need no warrant to remain in a building for a reasonable time to investigate the cause of a blaze after it has been extinguished. And if the warrantless entry to put out the fire and determine its cause is constitutional, the warrantless seizure of evidence while inspecting the premises for these purposes also is constitutional. . . .

In determining what constitutes a reasonable time to investigate, appropriate recognition must be given to the exigencies that confront officials serving under these conditions, as well as to individuals' reasonable expectations of privacy.[1]

Locating the Fire's Origin

A search of the fire scene must focus on finding the fire's origin, which will prove most productive in any search for an accelerant or ignition device. In searching for a fire's specific point of origin, the investigator may uncover telltale signs of arson such as evidence of separate and unconnected fires or the use of "streamers" to spread the fire from one area to another. For example, the arsonist may have spread a trail of gasoline or paper to cause the fire to move rapidly from one room to another.

There are no fast and simple rules for identifying a fire's origin. Normally a fire tends to move upward, and thus the probable origin is most likely closest to the lowest point that shows the most intense characteristics of burning. Sometimes as the fire burns upward, a V-shaped pattern forms against a vertical wall, as shown in Figure 16–6. Because flammable liquids always flow to the lowest point, more severe burning found on the floor than on the ceiling may indicate the presence of an accelerant. If a flammable liquid was used, charring is expected to be more intense on the bottom of furniture, shelves, and other items rather than the top.

However, many factors can contribute to the deviation of a fire from normal behavior. Using burn patterns, such as depth of char, a V-shaped pattern, or low intense burn area, as indicators of a fire's origin can prove to be misleading, particularly when a structural fire burns beyond flashover to full-room involvement. In these situations, air flow currents through the burning room can become a dominant factor in creating burn patterns.

Prevailing drafts and winds; secondary fires due to collapsing floors and roofs; the physical arrangement of the burning structure; stairways and elevator shafts; holes in the floor, wall, or roof; and the effects of the firefighter in suppressing the fire are other factors that the fire investigator must consider before determining conclusive findings regarding a fire's origin.

[1] *Michigan* v. *Tyler*, 436 U.S. 499 (1978).

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David Schalliol/Getty Images

FIGURE 16–6
Typical V pattern illustrating upward movement of the fire.

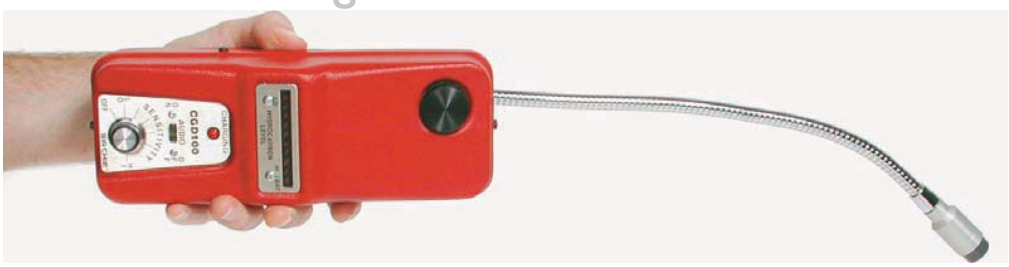
Once located, the point of origin should be protected to permit careful investigation. As at any crime scene, nothing should be touched or moved before notes, sketches, and photographs are taken. An examination must also be made for possible accidental causes, as well as for evidence of arson. The most common materials used by an arsonist to ensure the rapid spread and intensity of a fire are gasoline and kerosene or, for that matter, any volatile flammable liquid.

Searching for Accelerants

Fortunately, only under the most ideal conditions will combustible liquids be entirely consumed during a fire. When the liquid is poured over a large area, a portion of it will likely seep into a porous surface, such as cracks in the floor, upholstery, rags, plaster, wallboards, or carpet. Enough of the liquid may remain unchanged to permit its detection in the crime laboratory. In addition, when a fire is extinguished with water, the evaporation rate of volatile fluids may be slowed because water cools and covers materials through which the combustible liquid may have soaked. Fortunately, water does not interfere with laboratory methods used to detect and characterize flammable liquid residues.

The search for traces of flammable liquid residues may be aided by the use of a sensitive portable vapor detector or "sniffer" (see Figure 16–7). This device can rapidly screen suspect materials for volatile residues by sucking in the air surrounding the questioned sample. The air is passed over a heated filament; if a combustible vapor is present, it oxidizes and immediately increases the temperature of the filament. The rise in filament temperature is then registered as a deflection on the detector's meter.

Of course, such a device is not a conclusive test for a flammable vapor, but it is an excellent screening device for checking suspect samples at the fire scene. Another approach is to use dogs that have been trained to recognize the odor of hydrocarbon accelerants.



Courtesy Sirchie Fingerprint Laboratories, Youngsville, NC, www.sirchie.com

FIGURE 16–7
Portable hydrocarbon detector.

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Collection and Preservation of Arson Evidence

Two to three quarts of ash and soot debris must be collected at the point of origin of a fire when arson is suspected. The collection should include all porous materials and all other substances thought likely to contain flammable residues. These include such things as wood flooring, rugs, upholstery, and rags.

Courtesy Sirchie Fingerprint Laboratories, Youngsville, NC, www.sirchie.com

FIGURE 16–8
Various sizes of paint cans suitable for collecting debris at fire scenes.

Packaging and Preservation of Evidence

Specimens should be packaged immediately in airtight containers so possible residues are not lost through evaporation. New, clean paint cans with friction lids are good containers because they are low cost, airtight, unbreakable, and available in a variety of sizes (see Figure 16–8). Wide-mouthed glass jars are also useful for packaging suspect specimens, provided that they have airtight lids. Cans and jars should be filled one-half to two-thirds full, leaving an air space in the container above the debris.

Large bulky samples should be cut to size at the scene as needed so that they will fit into available containers. Plastic polyethylene bags are not suitable for packaging specimens because they react with hydrocarbons and permit volatile hydrocarbon vapors to be depleted. Fluids found in open bottles or cans must be collected and sealed. Even when such containers appear empty, the investigator is wise to seal and preserve them in case they contain trace amounts of liquids or vapors.

Substrate Control

The collection of all materials suspected of containing volatile liquids must be accompanied by a thorough sampling of similar but uncontaminated control specimens from another area of the fire scene. This is known as *substrate control*. For example, if an investigator collects carpeting at the point of origin, he or she must sample the same carpet from another part of the room, where it can be reasonably assumed that no flammable substance was placed.

In the laboratory, the criminalist checks the substrate control to be sure that it is free of any flammables. This procedure reduces the possibility (and subsequent argument) that the carpet was exposed to a flammable liquid such as a cleaning solution during normal maintenance. In addition, laboratory tests on the unburned control material may help analyze the breakdown products from the material's exposure to intense heat during the fire. Common materials such as plastic floor tiles, carpet, linoleum, and adhesives can produce volatile hydrocarbons when they are burned. These breakdown products can sometimes be mistaken for an accelerant.

Igniters and Other Evidence

The scene should also be thoroughly searched for igniters. The most common igniter is a match. Normally the match is completely consumed during a fire and is impossible to locate. However, there have been cases in which, by force of habit, matches have been extinguished and tossed aside only to be recovered later by the investigator. This evidence may prove valuable if the criminalist can fit the match to a book found in the possession of a suspect.

Arsonists can construct many other types of devices to start a fire. These include burning cigarettes, firearms, ammunition, a mechanical match striker, electrical sparking devices, and a "Molotov cocktail"—a glass bottle containing flammable liquid with a cloth rag stuffed into it and lit as a fuse. Relatively complex mechanical devices are much more likely to survive the fire for later discovery. The broken glass and wick of the Molotov cocktail, if recovered, must be preserved as well.

One important piece of evidence is the clothing of the suspect perpetrator. If this individual is arrested within a few hours of initiating the fire, residual quantities of the accelerant may still be present in the clothing. As we will see in the next section, the forensic laboratory can detect extremely small quantities of accelerants, making the examination of a suspect's clothing a feasible investigative approach. Each item of clothing should be placed in a separate airtight container, preferably a new, clean paint can.

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Richard Saferstein, Ph.D.

FIGURE 16–9
Removal of vapor from an enclosed container for gas chromatographic analysis.

Analysis of Flammable Residues

Criminalists are nearly unanimous in judging the gas chromatograph to be the most sensitive and reliable instrument for detecting and characterizing flammable residues. Most arsons are initiated by petroleum distillates, such as gasoline and kerosene, that are composed of a complex mixture of **hydrocarbons**. The gas chromatograph separates the hydrocarbon components of these liquids and produces a chromatographic pattern characteristic of a particular petroleum product.

The Headspace Technique

Before accelerant residues can be analyzed, they first must be recovered from the debris collected at the scene. The easiest way to recover accelerant residues from fire-scene debris is to heat the airtight container in which the sample is sent to the laboratory. When the container is heated, any volatile residue in the debris is driven off and trapped in the container's enclosed airspace. The vapor or *headspace* is then removed with a syringe, as shown in Figure 16–9.

When the vapor is injected into the gas chromatograph, it is separated into its components, and each peak is recorded on the chromatogram. One way of classifying ignitable liquids is by their boiling point range, which is related to the number of carbon molecules that are present in the mixture of hydrocarbons that make up an ignitable fuel. A common classification system characterizes ignitable liquids based on their boiling point ranges and number of carbon molecules as light, medium, and heavy petroleum distillates. Figure 16–10 illustrates examples of chromatograms for light, medium, and heavy petroleum distillates. The identity of the volatile residue is determined when the pattern of the resultant chromatogram is compared to patterns produced by known petroleum products. For example, in Figure 16–11, a gas chromatographic analysis of debris recovered from a fire site shows a chromatogram similar to a known gasoline standard, thus proving the presence of gasoline.

hydrocarbon
Any compound consisting of only carbon and hydrogen.

In the absence of any recognizable pattern, the individual peaks can be identified when the investigator compares their retention times to known hydrocarbon standards (such as hexane, benzene, toluene, and xylenes). The brand name of a gasoline sample cannot currently be determined by gas chromatography or any other technique. Fluctuating gasoline markets and exchange agreements among the various oil companies preclude this possibility.

Vapor Concentration

One major disadvantage of the headspace technique is that the size of the syringe limits the volume of vapor that can be removed from the container and injected into the gas chromatograph. To overcome this deficiency, many crime laboratories augment the headspace technique with a method called *vapor concentration*. One setup for this analysis is shown in Figure 16–12.

A charcoal-coated strip, similar to that used in environmental monitoring badges, is placed within the container holding the debris that has been collected from the fire scene.[2] The container is then heated to about 60°C for about one hour. At this temperature, a significant quantity of accelerant vaporizes into the container airspace. The charcoal absorbs the accelerant vapor with which it comes into contact. In this manner, over a short period of time a significant quantity of the accelerant will be trapped and concentrated onto the charcoal strip.

Once the heating procedure is complete, the analyst removes the charcoal strip from the container and recovers the accelerant from the strip by washing it with a small volume of solvent (carbon disulfide). The solvent is then injected into the gas chromatograph for analysis. The major advantage of using vapor concentration with gas chromatography is its sensitivity. By absorbing the accelerant into a charcoal strip, the forensic analyst can increase the sensitivity of accelerant detection at least a hundredfold over that of the conventional headspace technique.

An examination of Figure 16–11 shows that identifying an accelerant such as gasoline by gas chromatography is an exercise in pattern recognition. Typically a forensic analyst compares the pattern generated by the sample to chromatograms from accelerant standards obtained under the same conditions. The pattern of gasoline, as with many other accelerants, can easily be placed in a searchable library. An invaluable reference known as the Ignitable Liquids Reference

[2] R. T. Newman et al., "The Use of Activated Charcoal Strips for Fire Debris Extractions by Passive Diffusion. Part 1: The Effects of Time, Temperature, Strip Size, and Sample Concentration," *Journal of Forensic Sciences* 41 (1996): 361.

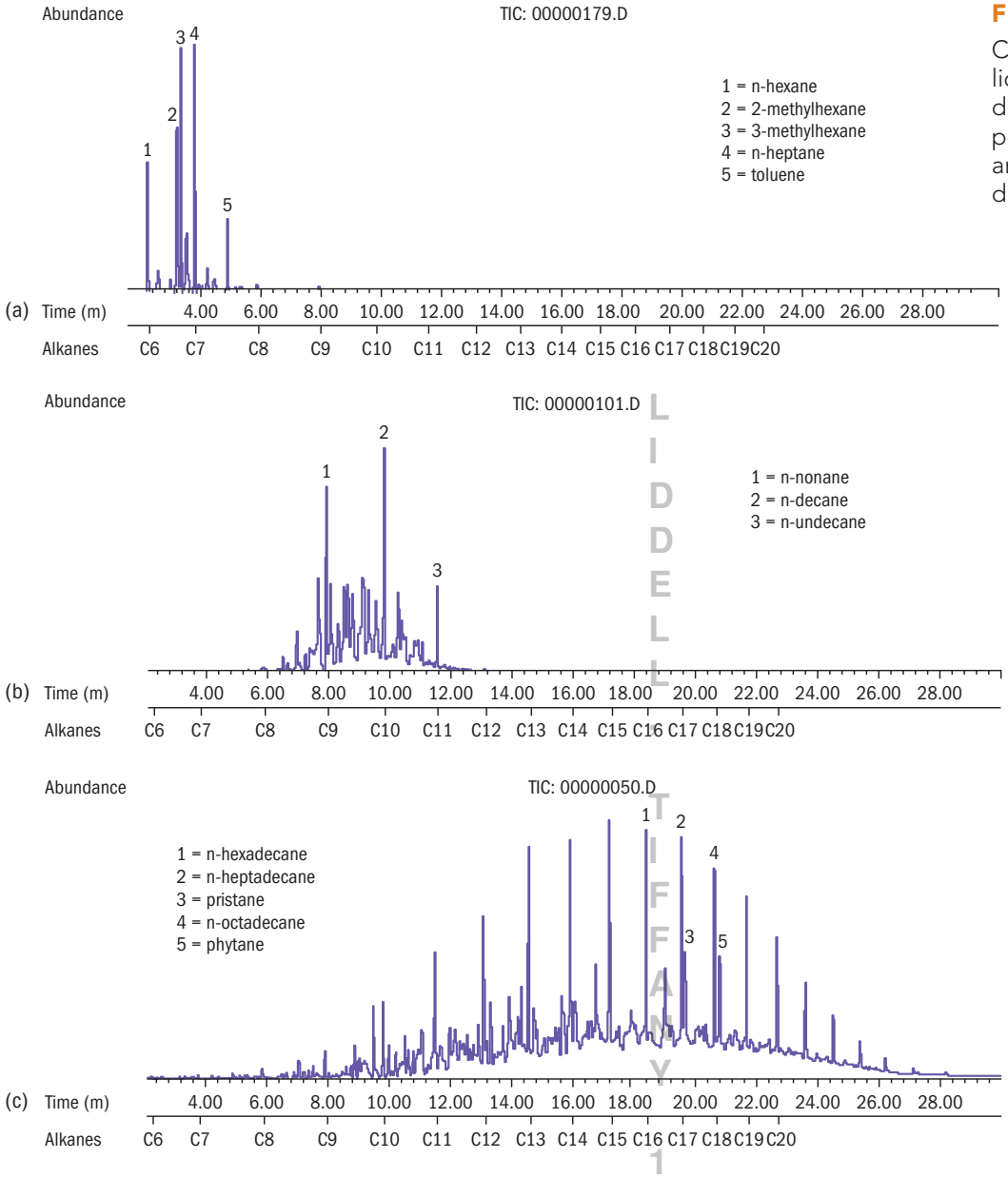

FIGURE 16–10
Chromatograms of ignitable liquids. (a) light petroleum distillate, (b) medium petroleum distillate, and (c) heavy petroleum distillate.

Collection (ILRC) is found on the Internet at http://ilrc.ucf.edu. The ILRC is a useful collection showing chromatographic patterns for approximately five hundred ignitable liquids.

Explosions and Explosives

The ready accessibility of potentially explosive laboratory chemicals, dynamite, and, in some countries, an assortment of military explosives has provided the criminal element of society with a lethal weapon. Unfortunately for society, explosives have become an attractive weapon to criminals bent on revenge, destruction of commercial operations, or just plain mischief.

Although politically motivated bombings have received considerable publicity worldwide, in the United States most bombing incidents are perpetrated by isolated individuals rather than by organized terrorists. These incidents typically involve homemade explosives and incendiary devices. The design of such weapons is limited only by the imagination and ingenuity of the bomber.

Like arson investigation, bomb investigation requires close cooperation of a group of highly specialized individuals trained and experienced in bomb disposal, bomb-site investigation, forensic analysis, and criminal investigation. The criminalist must detect and identify explosive chemicals recovered from the crime scene as well as identify the detonating mechanisms. This special responsibility concerns us for the remainder of this chapter.

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FIGURE 16–11

(Top) Gas chromatograph of vapor from a genuine gasoline sample. (Bottom) Gas chromatograph of vapor from debris recovered at a fire site. Note the similarity of the known gasoline to vapor removed from the debris.

Source: Richard Saferstein, Ph.D.

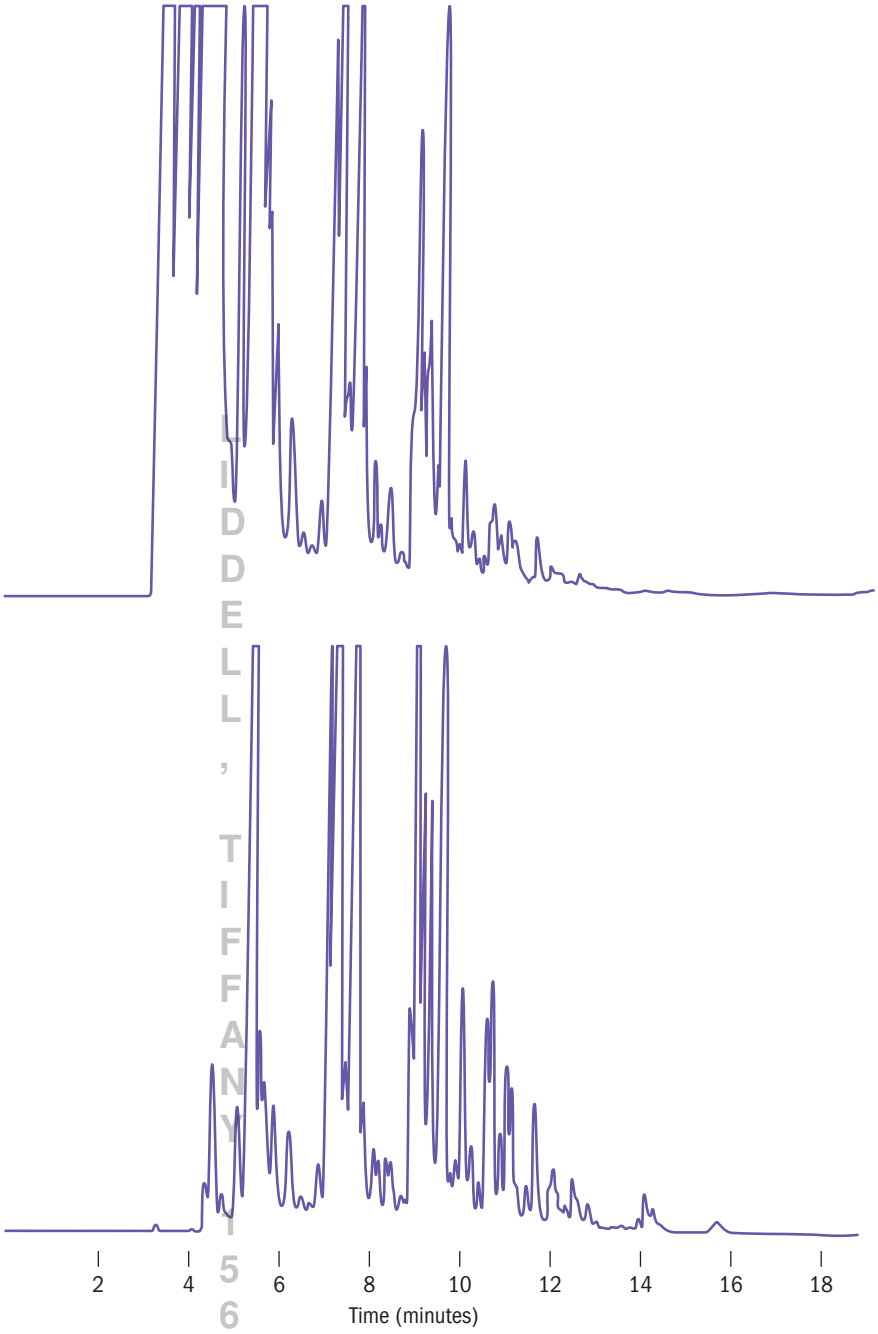
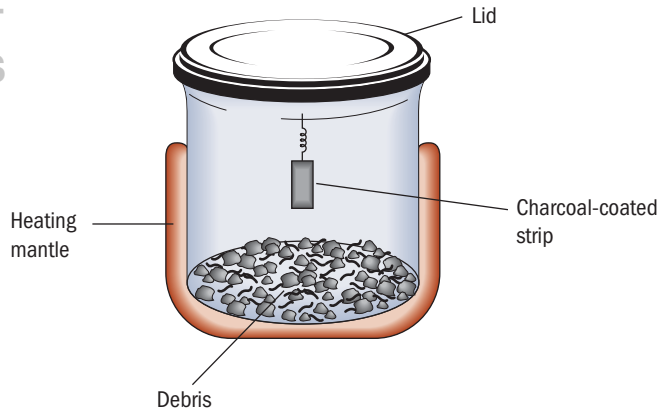


FIGURE 16–12

Apparatus for accelerant recovery by vapor concentration. The vapor in the enclosed container is exposed to charcoal, a chemical absorbent, where it is trapped for later analysis.



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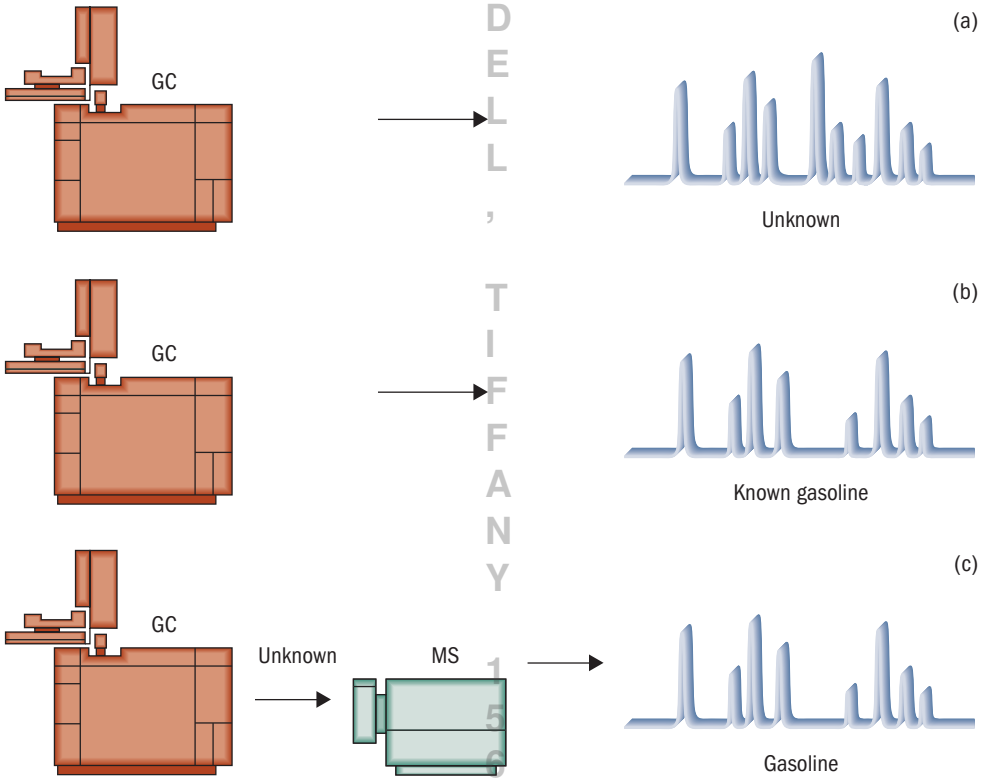
inside the science

Gas Chromatography/ Mass Spectrometry

On occasion, discernible patterns are not attainable by gas chromatography. This may be due to a combination of accelerants, or to the mixing of accelerant residue with heat-generated breakdown products of materials burning at the fire scene. Under such conditions, a gas chromatographic pattern can be difficult if not impossible to interpret. In these cases, gas chromatography combined with mass spectrometry (discussed in Chapter 11) has proven valuable for solving difficult problems in the detection of accelerant residues.

Complex chromatographic patterns can be simplified by passing the separated components emerging from the gas chromatographic column through a mass spectrometer. As each component enters the mass spectrometer, it is fragmented into a collection of ions. The analyst can then control which ions will be detected and which will go unnoticed. In essence, the mass spectrometer acts as a filter allowing the analyst to see only the peaks associated with the ions selected for a particular accelerant. In this manner, the chromatographic pattern can be simplified by eliminating extraneous peaks that may obliterate the pattern.[3] The process is illustrated in the figure.



Chromatogram of a residue sample collected at a fire scene (a) shows a pattern somewhat like that of gasoline (b). However, a definitive conclusion that the unknown contained gasoline could be obtained only after extraneous peaks were eliminated from the unknown by the use of GC/MS (c).

The Chemistry of Explosions

Like fire, an **explosion** is the product of combustion accompanied by the creation of gases and heat. However, the distinguishing characteristic of an explosion is the rapid rate of the reaction. The sudden buildup of expanding gas pressure at the origin of the explosion produces the violent physical disruption of the surrounding environment.

explosion
A chemical or mechanical action caused by combustion, accompanied by creation of heat and rapid expansion of gases.

[3] M. W. Gilbert, "The Use of Individual Extracted Ion Profiles versus Summed Extracted Ion Profiles in Fire Debris Analysis," *Journal of Forensic Sciences* 43 (1998): 871.

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Our previous discussion of the chemistry of fire referred only to oxidation reactions that rely on air as the sole source of oxygen. However, we need not restrict ourselves to this type of situation. For example, explosives are substances that undergo a rapid exothermic oxidation reaction, producing large quantities of gases. This sudden buildup of gas pressure constitutes an explosion. Detonation occurs so rapidly that oxygen in the air cannot participate in the reaction; thus, many explosives must have their own source of oxygen.

oxidizing agent
A substance that supplies oxygen to a chemical reaction.

Chemicals that supply oxygen are known as **oxidizing agents**. One such agent is found in black powder, a *low explosive*, which is composed of a mixture of the following chemical ingredients:

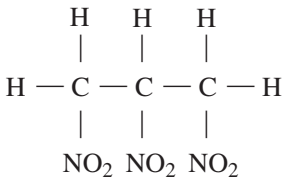
75 percent potassium nitrate (KNO_3)
15 percent charcoal (C)
10 percent sulfur (S)

In this combination, oxygen containing potassium nitrate acts as an oxidizing agent for the charcoal and sulfur fuels. As heat is applied to black powder, oxygen is liberated from potassium nitrate and simultaneously combines with charcoal and sulfur to produce heat and gases (symbolized by ↑), as represented in the following chemical equation:

$$\underset{\text{carbon}}{3C} + \underset{\text{sulfur}}{S} + \underset{\text{potassium nitrate}}{2KNO_3} \rightarrow \text{yields}$$

$$\underset{\text{carbon dioxide}}{3CO_2\uparrow} + \underset{\text{nitrogen}}{N_2\uparrow} + \underset{\text{potassium sulfide}}{K_2S}$$

Some explosives have their oxygen and fuel components combined within one molecule. For example, the chemical structure of nitroglycerin, the major constituent of dynamite, combines carbon, hydrogen, nitrogen, and oxygen:



When nitroglycerin detonates, large quantities of energy are released as the molecule decomposes, and the oxygen recombines to produce large volumes of carbon dioxide, nitrogen, and water.

Consider, for example, the effect of confining an explosive charge to a relatively small, closed container. On detonation, the explosive almost instantaneously produces large volumes of gases that exert enormously high pressures on the interior walls of the container. In addition, the heat energy released by the explosion expands the gases, causing them to push on the walls with an even greater force. If we could observe the effects of an exploding lead pipe in slow motion, we would first see the pipe's walls stretch and balloon under pressures as high as several hundred tons per square inch. Finally, the walls would fragment and fly outward in all directions. This flying debris or shrapnel constitutes a great danger to life and limb in the immediate vicinity.

On release from confinement, the gaseous products of the explosion suddenly expand and compress layers of surrounding air as they move outward from the origin of the explosion. This blast effect, or outward rush of gases, at a rate that may be as high as 7,000 miles per hour creates an artificial gale that can overthrow walls, collapse roofs, and disturb any object in its path. If a bomb is sufficiently powerful, more serious damage will be inflicted by the blast effect than by fragmentation debris (see Figure 16–13).

Stefan Zaklin/Corbis Images

FIGURE 16–13
A violent explosion.

Types of Explosives

The speed at which explosives decompose varies greatly from one to another and permits their classification as *high* and *low explosives*. In a low explosive, this speed is called the speed of

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deflagration (burning). It is characterized by very rapid oxidation that produces heat, light, and a subsonic pressure wave. In a high explosive, it is called the speed of detonation. **Detonation** refers to the creation of a supersonic shock wave within the explosive charge. This shock wave breaks the chemical bonds of the explosive charge, leading to the new instantaneous buildup of heat and gases.

deflagration
A very rapid oxidation reaction accompanied by the generation of a low-intensity pressure wave that can disrupt the surroundings.

detonation
An extremely rapid oxidation reaction accompanied by a violent disruptive effect and an intense, high-speed shock wave.

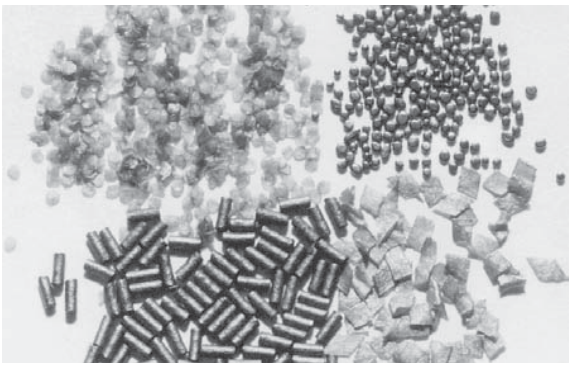
low explosive
An explosive with a velocity of detonation less than 1,000 meters per second.

LOW EXPLOSIVES **Low explosives**, such as black and smokeless powders, decompose relatively slowly at rates up to 1,000 meters per second. Because of their slow burning rates, they produce a propelling or throwing action that makes them suitable as propellants for ammunition or skyrockets. However, the danger of this group of explosives must not be underestimated because, when any one of them is confined to a relatively small container, it can explode with a force as lethal as that of any known explosive.

Black Powder and Smokeless Powder The most widely used explosives in the low-explosive group are black powder and smokeless powder. The popularity of these two explosives is enhanced by their accessibility to the public. Both are available in any gun store, and black powder can easily be made from ingredients purchased at any chemical supply house as well.

Black powder is a relatively stable mixture of potassium nitrate or sodium nitrate, charcoal, and sulfur. Unconfined, it merely burns; thus it commonly is used in safety fuses that carry a flame to an explosive charge. A **safety fuse** usually consists of black powder wrapped in a fabric or plastic casing. When ignited, a sufficient length of fuse will burn at a rate slow enough to allow a person adequate time to leave the site of the pending explosion. Black powder, like any other low explosive, becomes explosive and lethal only when it is confined.

The safest and most powerful low explosive is **smokeless powder**. This explosive usually consists of nitrated cotton or nitrocellulose (**single-base powder**) or nitroglycerin mixed with nitrocellulose (**double-base powder**). The powder is manufactured in a variety of grain sizes and shapes, depending on the desired application (see Figure 16–14).



Courtesy Bureau of Alcohol, Tobacco, Firearms & Explosives

FIGURE 16–14
Samples of smokeless powders.

black powder
Normally, a mixture of potassium nitrate, carbon, and sulfur in the ratio 75/15/10.

safety fuse
A cord containing a core of black powder, used to carry a flame at a uniform rate to an explosive charge.

smokeless powder (single-base)
An explosive consisting of nitrated cotton or nitrocellulose.

smokeless powder (double-base)
An explosive consisting of a mixture of nitroglycerin and nitrocellulose.

Chlorate Mixtures The only ingredients required for a low explosive are fuel and a good oxidizing agent. The oxidizing agent potassium chlorate, for example, when mixed with sugar, produces a popular and accessible explosive mix. When confined to a small container—for example, a pipe—and ignited, this mixture can explode with a force equivalent to a stick of 40 percent dynamite.

Some other commonly encountered ingredients that may be combined with chlorate to produce an explosive are carbon, sulfur, starch, phosphorus, and magnesium filings. Chlorate mixtures may also be ignited by the heat generated from a chemical reaction. For instance, sufficient heat can be generated to initiate combustion when concentrated sulfuric acid comes in contact with a sugar–chlorate mix.

Gas–Air Mixtures Another form of low explosive is created when a considerable quantity of natural gas escapes into a confined area and mixes with a sufficient amount of air. If ignited, this mixture results in simultaneous combustion and sudden production of large volumes of gases and heat. In a building, walls are forced outward by the expanding gases, causing the roof to fall into the interiors, and objects are thrown outward and scattered in erratic directions with no semblance of pattern.

Mixtures of air and a gaseous fuel explode or burn only within a limited concentration range. For example, the concentration limits for methane in air range from 5.3 to 13.9 percent. In the presence of too much air, the fuel becomes too diluted and does not ignite. On the other hand, if the fuel becomes too concentrated, ignition is prevented because there is not enough oxygen to support the combustion.

Mixtures at or near the upper concentration limit ("rich" mixtures) explode; however, some gas remains unconsumed because there is not enough oxygen to complete the combustion. As air rushes back into the origin of the explosion, it combines with the residual hot gas, producing a fire that is characterized by a *whoosh* sound. This fire is often more destructive than the explosion that preceded it. Mixtures near the lower end of the limit ("lean" mixtures) generally cause an explosion without accompanying damage due to fire.

high explosive
An explosive with a velocity of detonation greater than 1,000 meters per second.

primary explosive
A high explosive that is easily detonated by heat, shock, or friction.

secondary explosive
A high explosive that is relatively insensitive to heat, shock, or friction.

HIGH EXPLOSIVES **High explosives** include dynamite, TNT, PETN, and RDX. They detonate almost instantaneously at rates of 1,000–8,500 meters per second, producing a smashing or shattering effect on their target. High explosives are classified into two groups—primary and secondary explosives—based on their sensitivity to heat, shock, or friction.

Primary explosives are ultrasensitive to heat, shock, or friction, and under normal conditions they detonate violently instead of burning. For this reason, they are used to detonate other explosives through a chain reaction and are often referred to as *primers*. Primary explosives provide the major ingredients of blasting caps and include lead azide, lead styphnate, and diazodinitrophenol (see Figure 16–15). Because of their extreme sensitivity, these explosives are rarely used as the main charge of a homemade bomb.

Secondary explosives are relatively insensitive to heat, shock, or friction, and they normally burn rather than detonate when ignited in small quantities in open air. This group comprises most high explosives used for commercial and military blasting. Some common examples of secondary explosives are dynamite, TNT (trinitrotoluene), PETN (pentaerythritol tetranitrate), RDX (cyclotrimethylenetrinitramine), and tetryl (2,4,6-trinitrophenylmethylnitramine).

Dynamite It is an irony of history that the prize most symbolic of humanity's search for peace—the Nobel Peace Prize—should bear the name of the developer of one of our most lethal discoveries—dynamite. In 1867, the Swedish chemist Alfred Nobel, searching for a method to desensitize nitroglycerin, found that when kieselguhr, a variety of diatomaceous earth, absorbed a large portion of nitroglycerin, it became far less sensitive but still retained its explosive force. Nobel later decided to use pulp as an absorbent because kieselguhr was a heat-absorbing material.

This so-called pulp dynamite was the beginning of what is now known as the straight dynamite series. These dynamites are used when a quick shattering action is desired. In addition to nitroglycerine and pulp, present-day straight dynamites also include sodium nitrate (which furnishes oxygen for complete combustion) and a small percentage of a stabilizer, such as calcium carbonate.

All straight dynamite is rated by strength; the strength rating is determined by the weight percentage of nitroglycerin in the formula. Thus, a 40 percent straight dynamite contains 40 percent nitroglycerin, a 60 percent grade contains 60 percent nitroglycerin, and so forth. However, the relative blasting power of different strengths of dynamite is not directly proportional to their strength ratings. A 60 percent straight dynamite, rather than being three times as strong as a 20 percent, is only one and one-half times as strong (see Figure 16–16).

Ammonium Nitrate Explosives In recent years, nitroglycerin-based dynamite has all but disappeared from the industrial explosives market. Commercially, these explosives have been replaced mainly by ammonium nitrate–based explosives, that is, water gels, emulsions, and ANFO explosives. These explosives mix oxygen-rich ammonium nitrate with a fuel to form a low-cost, stable explosive.

Typically, water gels have a consistency resembling that of set gelatin or gel-type toothpaste. They are characterized by their water-resistant nature and are employed for all types of blasting under wet conditions. These explosives are based on formulations of ammonium nitrate and sodium nitrate gelled with a natural polysaccharide such as guar gum. Commonly, a combustible material such as aluminum is mixed into the gel to serve as the explosive's fuel.

Emulsion explosives differ from gels in that they consist of two distinct phases, an oil phase and a water phase. In these emulsions, a droplet of a supersaturated solution of ammonium nitrate is surrounded by a hydrocarbon serving as a fuel. A typical emulsion

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FIGURE 16–15
Blasting caps. The left and center caps are initiated by an electrical current; the right cap is initiated by a safety fuse.

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U.S. Department of Justice

FIGURE 16–16
Sticks of dynamite.

consists of water, one or more inorganic nitrate oxidizers, oil, and emulsifying agents. Commonly, emulsions contain micron-sized glass, resin, or ceramic spheres known as *microspheres* or *microballoons*. The size of these spheres controls the explosive's sensitivity and detonation velocity.

Ammonium nitrate soaked in fuel oil is an explosive known as *ANFO*. Such commercial explosives are inexpensive and safe to handle and have found wide applications in blasting operations in the mining industry. Ammonium nitrate in the form of fertilizer makes a readily obtainable ingredient for homemade explosives. Indeed, in an incident related to the 1993 bombing of New York City's World Trade Center, the FBI arrested five men during a raid on their hideout in New York City, where they were mixing a "witches' brew" of fuel oil and an ammonium nitrate–based fertilizer.

TATP *Triacetone triperoxide* (TATP) is a homemade explosive that has been used as an improvised explosive by terrorist organizations in Israel and other Middle Eastern countries. It is prepared by reacting the common ingredients of acetone and hydrogen peroxide in the presence of an acid catalyst such as hydrochloric acid.

TATP is a friction- and impact-sensitive explosive that is extremely potent when confined in a container such as a pipe. The 2005 London transit bombings were caused by TATP-based explosives and provide ample evidence that terrorist cells have moved TATP outside the Middle East. A London bus destroyed by one of the TATP bombs is shown in Figure 16–17.

A plot to blow up ten international plane flights leaving Britain for the United States with a "liquid explosive" apparently involved plans to smuggle the peroxide-based TATP explosive onto the planes. This plot has prompted authorities to prohibit airline passengers from carrying liquids and gels onto planes.

Military High Explosives No discussion of high explosives would be complete without a mention of military high explosives. In many countries outside the United States, the accessibility of high explosives to terrorist organizations makes them common constituents of homemade bombs.

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Dylan Martinez/AP Images

FIGURE 16–17
A London bus destroyed by a TATP-based bomb.

RDX, the most popular and powerful military explosive, is often encountered in the form of a pliable plastic of doughlike consistency known as *composition C–4* (a U.S. military designation).

TNT was produced and used on an enormous scale during World War II and may be considered the most important military bursting charge explosive. Alone or in combination with other explosives, it has found wide application in shells, bombs, grenades, demolition explosives, and propellant compositions. Interestingly, military "dynamite" contains no nitroglycerin but is actually composed of a mixture of RDX and TNT. Like other military explosives, TNT is rarely encountered in bombings in the United States.

PETN is used by the military in TNT mixtures for small-caliber projectiles and grenades. Commercially, the chemical is used as the explosive core in a **detonating cord** or *primacord*. Instead of the slower-burning safety fuse, a detonating cord is often used to connect a series of explosive charges so that they will detonate simultaneously.

detonating cord
A cordlike explosive containing a core of high-explosive material, usually PETN; also called primacord.

Detonators Unlike low explosives, bombs made of high explosives must be detonated by an initiating explosion. In most cases, detonators are blasting caps composed of copper or aluminum cases filled with lead azide as an initiating charge and PETN or RDX as a detonating charge. Blasting caps can be initiated by means of a burning safety fuse or by an electrical current.

Homemade bombs camouflaged in packages, suitcases, and the like are usually initiated with an electrical blasting cap wired to a battery. An unlimited number of switching-mechanism designs have been devised for setting off these devices; clocks and mercury switches are favored. Bombers sometimes prefer to employ outside electrical sources. For instance, most automobile bombs are detonated when the ignition switch of a car is turned on.

Collection and Analysis of Evidence of Explosives

The most important step in the detection and analysis of explosive residues is the collection of appropriate samples from the explosion scene. Invariably, undetonated residues of the explosive remain at the site of the explosion. The detection and identification of these explosives in the laboratory depends on the bomb-scene investigator's skill and ability to recognize and sample the areas most likely to contain such materials.

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case files

Liquid Explosives

In 2006, security agencies in the United States and Great Britain uncovered a terrorist plot to use liquid explosives to destroy commercial airlines operating between the two countries. Of the hundreds of types of explosives, most are solid. Only about a dozen are liquid. But some of those liquid explosives can be readily purchased and others can be made from hundreds of different kinds of chemicals that are not difficult to obtain. After the September 11 attacks, worries about solid explosives became the primary concern. In 2001, Richard Reid was arrested for attempting to destroy an American Airlines flight out of Paris. Authorities later found a high explosive with a TATP detonator hidden in the lining of his shoe. It is therefore not surprising that terrorists turned to liquids in this latest plot. A memo issued by federal security officials about the plot to blow up ten international planes highlighted a type of liquid explosive based on peroxide.

The most common peroxide-based explosive is TATP (triacetone triperoxide), which is made up of acetone and hydrogen peroxide, two widely available substances. TATP can be used as a detonator or a primary explosive and has been used in al Qaeda–related bomb plots and by Palestinian suicide bombers. TATP itself is a white powder made up of crystals that form when acetone and hydrogen peroxide are mixed together, usually with a catalyst added to speed the chemical reactions. Acetone is the main ingredient in nail polish remover, while hydrogen peroxide is a popular antiseptic. When the two main ingredients are mixed, they form a white powder that can be easily detonated using an electrical spark.

Commercially available hydrogen peroxide, however, is not concentrated enough to create TATP. The solution sold in stores contains about 3 percent hydrogen peroxide, compared to the approximately 70 percent concentration need for TATP. However, hydrogen peroxide solutions of up to 30 percent can be obtained from chemical supply houses. According to explosives experts, a mixture of 30 percent hydrogen peroxide and acetone can create a fire hot enough to burn though the fuselage of an aircraft.

In theory, scientists know how to detect peroxide-based explosives. The challenge is to design machines that can perform scans quickly and efficiently on thousands of passengers passing through airport security checks. Current scanning machines at airports are designed to detect nitrogen-containing chemicals and are not designed to detect peroxide-containing explosive ingredients. Since 9/11, security experts have worried about the possibility of liquid explosives in the form of liquids and gels getting onto airliners.

Without the luxury of waiting for newly designed scanning devices capable of ferreting out dangerous liquids to be in place at airports, experts decided to use a commonsense approach—that is, to restrict the types and quantities of liquids that a passenger can carry onto a plane.

Stefano Paltera/AP Images

Gels and liquids discarded by airline passengers before boarding.

Detecting and Recovering Evidence of Explosives

The most obvious characteristic of a high or contained low explosive is the presence of a crater at the origin of the blast. Once the crater has been located, all loose soil and other debris must immediately be removed from the interior of the hole and preserved for laboratory analysis. Other good sources of explosive residues are objects located near the origin of detonation. Wood, insulation, rubber, and other soft materials that are readily penetrated often collect traces of the explosive. However, nonporous objects near the blast must not be overlooked. For instance, residues can be found on the surfaces of metal objects near the site of an explosion. Material blown away from the blast's origin should also be recovered because it, too, may retain explosive residues.

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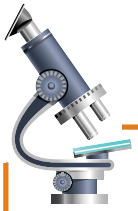
The entire area must be systematically searched, with great care given to recovering any trace of a detonating mechanism or any other item foreign to the explosion site. Wire-mesh screens are best used for sifting through debris. All personnel involved in searching the bomb scene must take appropriate measures to avoid contaminating the scene, including dressing in disposable gloves, shoe covers, and overalls.

COLLECTION AND PACKAGING All materials collected for examination by the laboratory must be placed in airtight sealed containers and labeled with all pertinent information. In pipe-bomb explosions, particles of the explosive are frequently found adhering to the pipe cap or to the pipe threads, as a result of either being impacted into the metal by the force of the explosion or being deposited in the threads during the construction of the bomb. Soil and other soft loose materials are best stored in metal airtight containers such as clean paint cans. Debris and articles collected from different areas are to be packaged in separate airtight containers. Plastic bags should not be used to store evidence suspected of containing explosive residues. Some explosives can actually escape through the plastic. Sharp-edged objects should not be allowed to pierce the sides of a plastic bag. It is best to place these types of items in metal containers.



inside the science

Analysis of Evidence of Explosives

When the bomb-scene debris and other materials arrive at the laboratory, everything is first examined microscopically to detect particles of unconsumed explosive. Portions of the recovered debris and detonating mechanism, if found, are carefully viewed under a low-power stereoscopic microscope in a painstaking effort to locate particles of the explosive. Black powder and smokeless powder are relatively easy to locate in debris because of their characteristic shapes and colors (see Figure 16–14). However, dynamite and other high explosives present the microscopist with a much more difficult task and often must be detected by other means.

One approach for screening objects for the presence of explosive residues in the field or the laboratory is the ion mobility spectrometer (IMS).[4] A portable IMS is shown in the figure.

This handheld detector uses a vacuum to collect explosive residues from suspect surfaces. Alternatively, the surface suspected of containing explosive residues is wiped down with a Teflon-coated fiberglass disc and the collected residues are then drawn into the spectrometer off the disc. Once in the IMS, the explosive residues are vaporized by the application of heat. These vaporized substances are exposed to a beam of electrons or beta rays emitted by radioactive nickel and converted into electrically charged molecules or ions. The ions are then allowed to move through a tube (drift region) under the influence of an electric field. A schematic diagram of an IMS is shown in the figure.

The preliminary identification of an explosive residue can be made by noting the time it takes the explosive to move through the tube. Because ions

Hardened MobileTrace®, a portable ion mobility spectrometer used to rapidly detect and tentatively identify trace quantities of explosives.

[4] T. Keller et al., "Application of Ion Mobility Spectrometry in Cases of Forensic Interest," *Forensic Science International* 161 (2006): 130.

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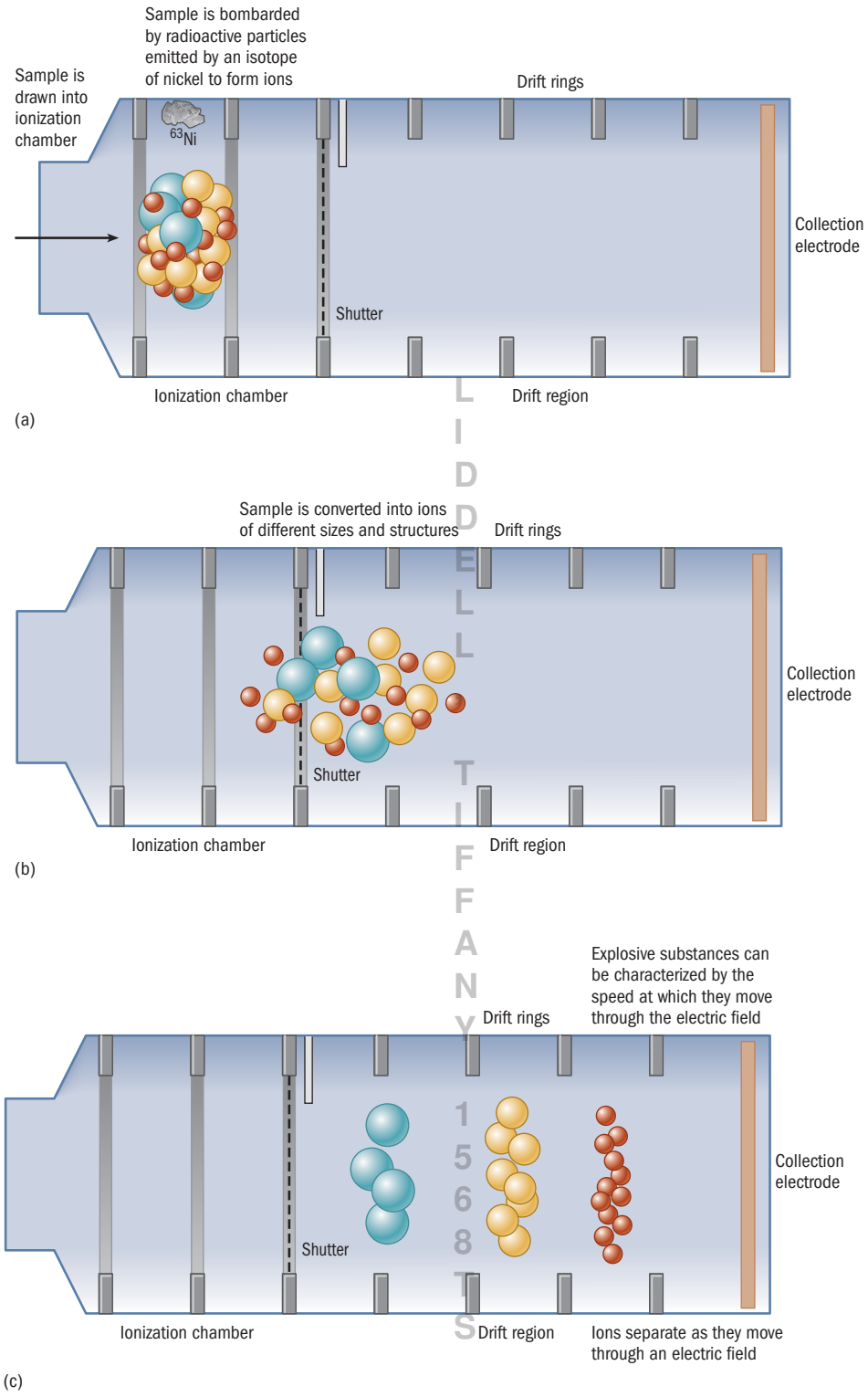

Schematic diagram of an ion mobility spectrometer. A sample is introduced into an ionization chamber, where bombardment with radioactive particles emitted by an isotope of nickel converts the sample to ions. The ions move into a drift region where ion separation occurs based on the speed of the ions as they move through an electric field.

(*continued*)

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move at different speeds depending on their size and structure, they can be characterized by the speed at which they pass through the tube. Used as a screening tool, this method rapidly detects a full range of explosives, even at low detection levels. However, all results need to be verified through confirmatory tests.

Following microscopic examination, the recovered debris is thoroughly rinsed with acetone. The high solubility of most explosives in acetone ensures their quick removal from the debris. When a water-gel explosive containing ammonium nitrate or a low explosive is suspected, the debris should be rinsed with water so that water-soluble substances (such as nitrates and chlorates) will be extracted. Table 16–3 lists a number of simple color tests the examiner can perform on the acetone and water extracts to screen for the presence of organic and inorganic explosives, respectively.

Once collected, the acetone extract is concentrated and analyzed using color spot tests, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography/mass spectrometry. The presence of an explosive is indicated by a well-defined spot on a TLC plate corresponding to a known explosive—for example, nitroglycerin, RDX, or PETN.

The high sensitivity of HPLC also makes it useful for analyzing trace evidence of explosives. HPLC operates at room temperature and hence does not cause explosives, many of which are temperature sensitive, to decompose during their analysis. When a water-gel explosive containing ammonium nitrate or a low explosive is suspected, the debris should be rinsed with water so that water-soluble substances (such as nitrates and chlorates) will be extracted.

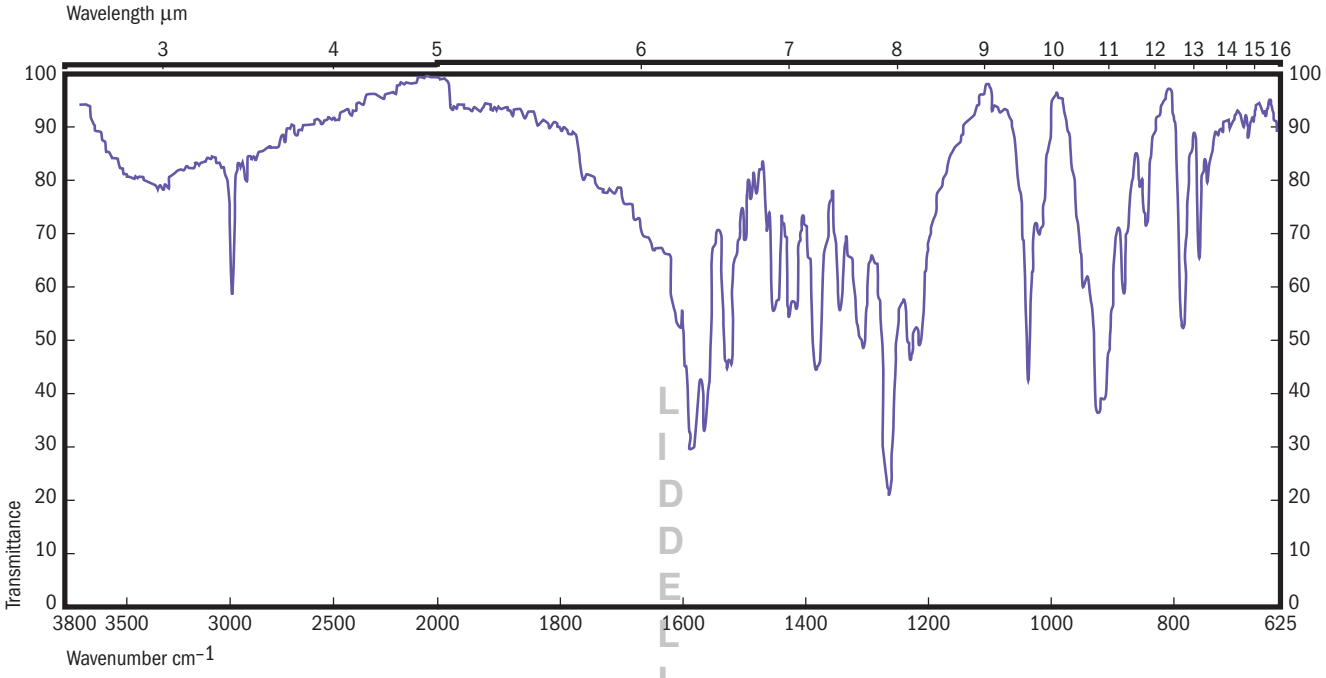
When sufficient quantities of explosives are recoverable, confirmatory tests may be performed by infrared spectrophotometry. The former produces a unique "fingerprint" pattern for an organic explosive, as shown by the IR spectrum of RDX in the figure.

TABLE 16–3

Color Spot Tests for Common Explosives

	Reagent		
Substance	Griess[a]	Diphenylamine[b]	Alcoholic KOH[c]
Chlorate	No color	Blue	No color
Nitrate	Pink to red	Blue	No color
Nitrocellulose	Pink	Blue-black	No color
Nitroglycerin	Pink to red	Blue	No color
PETN	Pink to red	Blue	No color
RDX	Pink to red	Blue	No color
TNT	No color	No color	Red
Tetryl	Pink to red	Blue	Red-violet

[a] Griess reagent: Solution 1—Dissolve 1 g sulfanilic acid in 100 mL 30 percent acetic acid. Solution 2—Dissolve 0.5 g *N*-(1-napthyl) ethylenediamine in 100 mL methyl alcohol. Add solutions 1 and 2 and a few milligrams of zinc dust to the suspect extract.
[b] Diphenylamine reagent: Dissolve 1 g diphenylamine in 100 mL concentrated sulfuric acid.
[c] Alcoholic KOH reagent: Dissolve 10 g of potassium hydroxide in 100 mL absolute alcohol.



Infrared spectrum of RDX.

chapter summary

When a fire occurs, oxygen combines with a fuel to produce noticeable quantities of heat and light (flames). Three requirements must be satisfied to initiate and sustain combustion: (1) a fuel must be present, (2) oxygen must be available in sufficient quantity to combine with the fuel, and (3) sufficient heat must be applied to initiate the combustion and generated to sustain the reaction. A fuel achieves a reaction rate with oxygen sufficient to sustain a fire only when it is in the gaseous state.

The arson investigator must begin examining a fire scene for signs of arson as soon as the fire has been extinguished. Some telltale signs of arson include evidence of separate and unconnected fires, the use of "streamers" to spread the fire from one area to another, and evidence of severe burning found on the floor as opposed to the ceiling of a structure.

The search of the fire scene must focus on finding the fire's origin. There are no fast and simple rules for identifying a fire's origin. Normally a fire tends to move upward, and thus the probable origin is most likely closest to the lowest point that shows the most intense characteristics of burning. Sometimes as the fire burns upward, a V-shaped pattern forms against a vertical wall. At the suspect point of origin of a fire, porous materials should be collected and stored in airtight containers.

In the laboratory, the gas chromatograph is the most sensitive and reliable instrument for detecting and characterizing flammable residues. Most arsons are initiated by petroleum distillates such as gasoline and kerosene. The gas chromatograph separates the hydrocarbon components and produces a chromatographic pattern characteristic of a particular petroleum product. By comparing select gas chromatographic peaks recovered from fire-scene debris to known flammable liquids, a forensic analyst may be able to identify the accelerant used to initiate the fire.

Explosives are substances that undergo a rapid oxidation reaction with the production of large quantities of gases. This sudden buildup of gas pressure constitutes an explosion. The speed at which explosives decompose permits their classification as high or low explosives.

The most widely used low explosives are black powder and smokeless powder. Among the high explosives, primary explosives are ultrasensitive to heat, shock, or friction and provide the major ingredients found in blasting caps. Secondary explosives normally constitute the main charge of a high explosive.

Among the high explosives, nitroglycerin-based dynamite has all but disappeared from the industrial explosives

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market and has been replaced by ammonium nitrate–based explosives (such as water gels, emulsions, and ANFO explosives). In many countries outside the United States, the accessibility of military high explosives to terrorist organizations makes them common constituents of homemade bombs. RDX is the most popular and powerful of the military explosives.

The entire bomb site must be systematically searched, with great care given to recovering any trace of a detonating mechanism or any other item foreign to the explosion site. Objects located at or near the origin of the explosion must be collected for laboratory examination.

Typically, in the laboratory, debris collected at explosion scenes is examined microscopically for unconsumed explosive particles. Recovered debris may also be thoroughly rinsed with organic solvents and analyzed by testing procedures that include color spot tests, thin-layer chromatography, high-performance liquid chromatography, and gas chromatography/mass spectrometry.

review questions

1. True or False: The absence of chemical residues always rules out the possibility of arson. _____
2. The combination of oxygen with other substances to produce new chemical products is called _____.
3. True or False: All oxidation reactions produce noticeable quantities of heat and light. _____
4. _____ is the capacity for doing work.
5. Burning methane for the purpose of heating water to produce steam in order to drive a turbine is an example of converting _____ energy to _____ energy.
6. The quantity of heat evolved from a chemical reaction arises out of the _____ and _____ of chemical bonds.
7. Molecules must (absorb, liberate) energy to break their bonds and (absorb, liberate) energy when their bonds are re-formed.
8. All oxidation reactions (absorb, liberate) heat.
9. Reactions that liberate heat are said to be _____.
10. Excess heat energy liberated by an oxidation reaction is called the _____.
11. A chemical reaction in which heat is absorbed from the surroundings is said to be _____.
12. True or False: All reactions require an energy input to start them. _____
13. The minimum temperature at which a fuel burns is known as the _____ temperature.
14. A fuel achieves a sufficient reaction rate with oxygen to produce a flame only in the (gaseous, liquid) state.
15. The lowest temperature at which a liquid fuel produces enough vapor to burn is the _____.
16. _____ is the chemical breakdown of a solid material to gaseous products.
17. _____ is a phenomenon in which a fuel burns without the presence of a flame.
18. The rate of a chemical reaction (increases, decreases) as the temperature rises.
19. _____ describes a fire caused by a natural heat-producing process.
20. True or False: An immediate search of a fire scene can commence without obtaining a search warrant. _____
21. A search of the fire scene must focus on finding the fire's _____.
22. True or False: The probable origin of a fire is most likely closest to the lowest point that shows the most intense characteristics of burning. _____
23. The collection of debris at the origin of a fire should include all (porous, nonporous) materials.
24. _____ containers must be used to package all materials suspected of containing hydrocarbon residues.
25. The most sensitive and reliable instrument for detecting and characterizing flammable residues is the (gas chromatograph, infrared spectrophotometer).
26. The identity of a volatile petroleum residue is determined by the (size, pattern) of its gas chromatogram.
27. True or False: The major advantage of using the vapor concentration technique in combination with gas chromatography is its extreme sensitivity for detecting volatile residues from fire-scene evidence. _____
28. True or False: A forensic analyst typically compares the gas chromatographic pattern generated from a fire-scene sample to a library of patterns in order to identify the accelerant. _____
29. The criminalist (can, cannot) identify gasoline residues by brand name.
30. Rapid combustion accompanied by the creation of large volumes of gases describes a(n) _____.
31. True or False: Chemicals that supply oxygen are known as oxidizing agents. _____
32. Explosives that decompose at relatively slow rates are classified as _____ explosives.
33. The speed at which low explosives decompose is called the speed of _____.

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34. Three ingredients of black powder are_____, _____, and _____.
35. _____ explosives detonate almost instantaneously to produce a smashing or shattering effect.
36. The most widely used low explosives are _____ and _____.
37. A low explosive becomes explosive and lethal only when it is _____.
38. True or False: Air and a gaseous fuel burn when mixed in any proportions. _____
39. High explosives can be classified as either _____ or _____ explosives.
40. The most widely used explosive in the military is _____.
41. The explosive core in detonating cord is _____.
42. A high explosive is normally detonated by a(n) _____ explosive contained within a blasting cap.
43. An obvious characteristic of a high explosive is the presence of a(n) _____ at the origin of the blast.
44. True or False: Debris and articles at an explosion scene that are collected from different areas are to be packaged in separate airtight containers. _____

review questions for inside the science

1. A fire moves away from the original point of ignition because the _____ created by the combustion process tends to move from a high-temperature region to one at a lower temperature.
2. Electrons and atoms within a solid object exposed to heat collide with one another, causing movement of heat through the object in a process called _____.
3. In a process known as _____, a heated surface emits electromagnetic radiation of various wavelengths that moves in a straight line from one surface to another, helping the fire to spread throughout a structure.
4. Complex chromatographic patterns can be simplified by passing the components emerging from the gas chromatographic column through a(n) _____.
5. To screen objects for the presence of explosive residues in the field or the laboratory, the investigator may use a handheld _____.
6. Unconsumed explosive residues may be detected in the laboratory through a careful _____ examination of the debris.
7. Debris recovered from the site of an explosion is routinely rinsed with _____ in an attempt to recover high-explosive residues.
8. Once collected, the acetone extract is initially analyzed by _____, _____, and _____.
9. The technique of _____ produces a unique absorption spectrum for an organic explosive.

application and critical thinking

1. Indicate which method of heat transfer is most likely to be responsible for each of the following:
   a. Ignition of papers in the room where a fire starts
   b. Ignition of electrical wiring in a room adjoining the fire's point or origin
   c. Ignition of roof timbers
   d. Ignition of a neighboring house
2. It is late August in Houston, Texas, and you are investigating a fire that occurred at a facility that stores motor oils and other lubricating oils. A witness points out a man who allegedly ran from the structure about the same time that the fire started. You question the man, who turns out to be the owner of the facility. He tells you that he was checking his inventory when barrels of waste motor oil stored in an unventilated back room spontaneously burst into flames. The owner claims that the fire spread so rapidly that he had to flee the building before he could call 911. After speaking with several employees, you learn that the building has no air conditioning and that the oil had been stored for almost a year in the cramped back room. You also learn from a detective assisting on the case that the owner increased his insurance coverage on the facility within the past three months. Should you believe the owner's story, or should you suspect arson? On what do you base your conclusion?
3. Criminalist Mick Mickelson is collecting evidence from a fire scene. He gathers about a quart of ash and soot debris from several rooms surrounding the point of origin. He stores the debris in a new, clean paint can,

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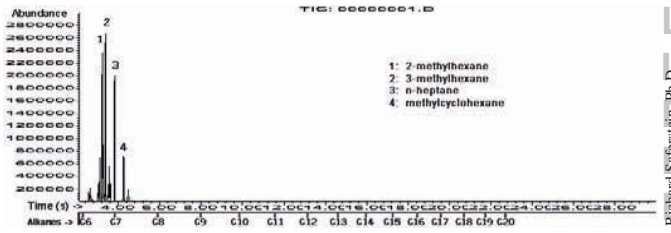
filled about three-quarters full. Seeing several pieces of timber that he believes may contain accelerant residues, he cuts them and places them in airtight plastic bags. A short time later, a suspect is arrested and Mick searches him for any signs of an igniter or accelerants. He finds a cigarette lighter on the suspect and seizes it for evidence before turning the suspect over to the police. What mistakes, if any, did Mick make in collecting evidence?

4. Classify the following chromatograms of ignitable liquids as low, medium, or high petroleum distillates. Refer to Figure 16–10.



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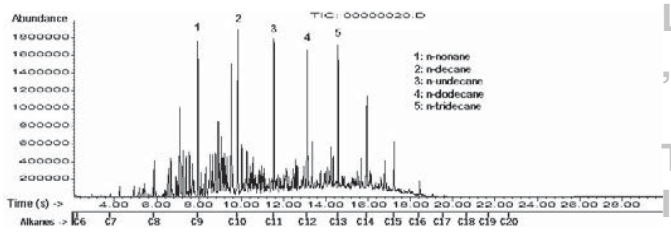
a. _____



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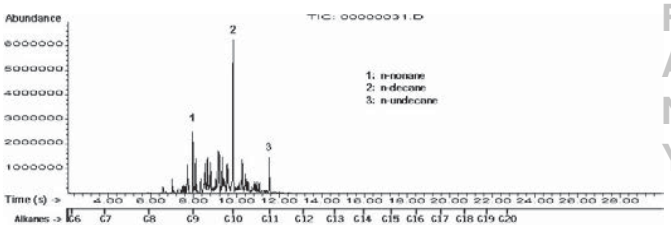
b. _____



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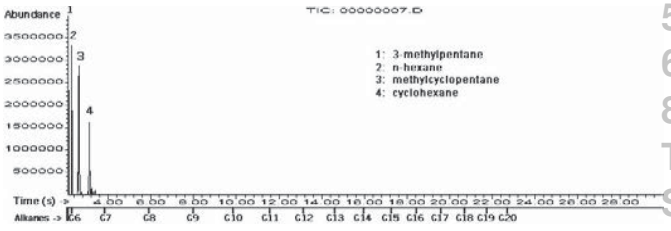
c. _____



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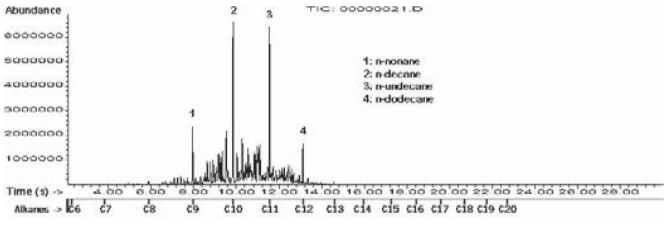
d. _____



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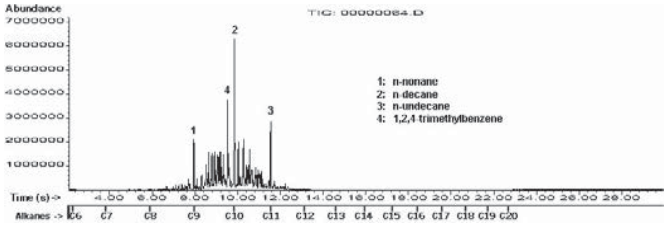
e. _____



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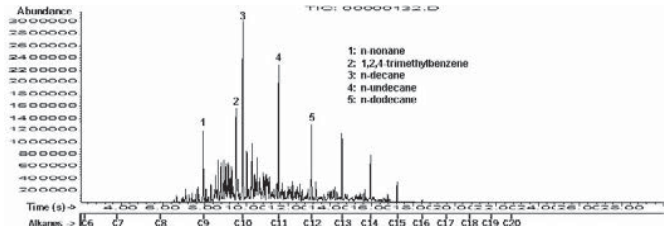
f. _____



Richard Saferstein, Ph.D.

g. _____

5. The following pieces of evidence were found at separate explosion sites. For each item, indicate whether the explosion was more likely caused by low or high explosives, and explain your answer:
   a. Lead azide residues
   b. Nitrocellulose residues
   c. Ammonium nitrate residues
   d. Scraps of primacord
   e. Potassium chlorate residues
6. Which color test or tests would you run first on a suspect sample to test for evidence of each of the following explosives? Explain your answers.
   a. Tetryl
   b. TNT
   c. Chlorate
   d. Nitrocellulose
7. Criminalist Matt Weir is collecting evidence from the site of an explosion. Arriving on the scene, he immediately proceeds to look for the crater caused by the blast. After finding the crater, he picks through the debris at the site by hand, looking for evidence of detonators or foreign materials. Matt collects loose soil and debris from the immediate area, placing the smaller bits in paper folded into a druggist fold. Larger items he stores in plastic bags for transportation to the laboratory. What mistakes, if any, did Matt make in collecting and storing this evidence?

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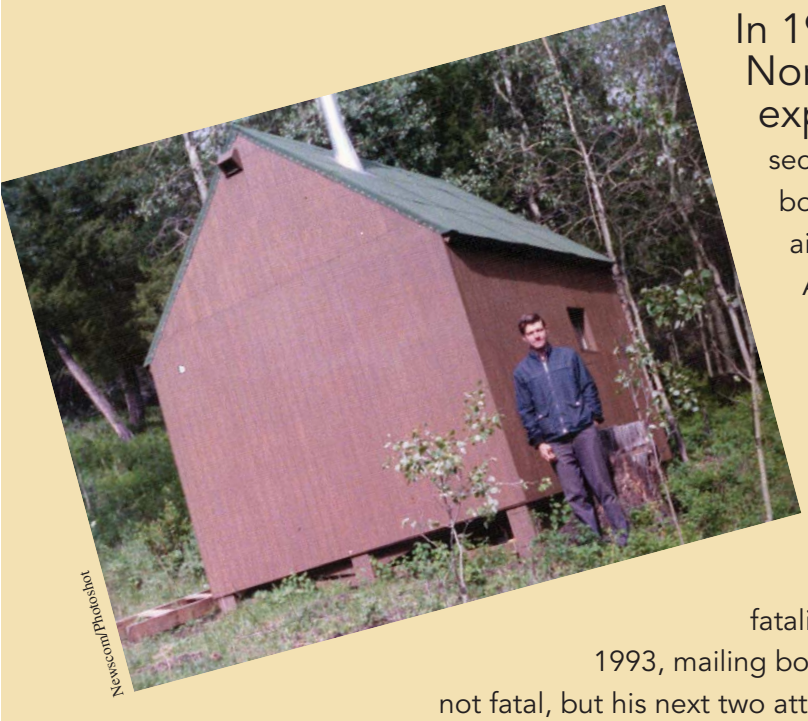
headline news

The Unabomber



Newscom/Photoshot

In 1978, a parcel addressed to a Northwestern University professor exploded as it was being opened by a campus security officer. This was the first of a series of bomb-containing packages sent to universities and airlines. The perpetrator was dubbed UN (university) A (airlines) BOM; hence, the Unabomber. The explosives were usually housed in a pipe within a wooden box. The explosive ingredients generally were black powder, smokeless powder, or an ammonium nitrate mix. The box was filled with metal objects to create a shrapnel effect on explosion. The device typically had the initials "FC" punched into it. The first Unabomber fatality came in 1985. The Unabomber surfaced again in 1993, mailing bombs to two university professors. Their injuries were not fatal, but his next two attacks did result in fatalities.

In 1995, the case took an unexpected turn when the Unabomber promised to end his mad spree if his 35,000-page typewritten Manifesto sent to the *New York Times* and the *Washington Post* was published. The Manifesto proved to be a long, rambling rant against technology, but it offered valuable clues that broke the case. David Kaczynski realized that the Manifesto's writing style and the philosophy it espoused closely resembled that of his brother Ted. Linguistic experts carefully pored over the Manifesto's content. Ted Kaczynski was arrested in Montana in 1996. Inside his ramshackle cabin were writings similar to the Manifesto, three manual typewriters, and bomb-making materials. Forensic document examiners matched the typewritten Manifesto to one of the typewriters recovered from the cabin.

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