chapter **13**

DNA—The Indispensable Forensic Science Tool

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 enzymes

restriction fragment length polymorphisms (RFLPs)

sequencing

short tandem repeat (STR)

substrate control

tandem repeat

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 re-
  peating base sequences

■ Explain the technology of polymerase chain reaction (PCR) and how it applies to forensic
  DNA typing

■ Contrast the newest DNA-typing technique, short tandem repeats (STRs), with previous

DNA-typing technologies

■ 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

Y-STRs O.J. Simpson—A Mountain of Evidence

On June 12, 1994, police arrived at the home of Nicole Simpson only to view a horrific scene. The bodies of O. J. Simpson's estranged wife and her friend Ron Goldman were found on the path leading to the front door of Nicole's home. Both bodies were covered in blood and had suffered deep knife wounds. Nicole's head was nearly severed from her body. This was not a well-planned murder. A trail of blood led away from the murder scene. Blood was found in O. J. Simpson's Bronco. Blood drops were on O. J.'s driveway and in the foyer of his home. A blood-soaked sock was located in O. J. Simpson's bedroom, and a bloodstained glove rested outside his residence.

As DNA was extracted and profiled from each bloodstained article, a picture emerged that seemed to irrefutably link Simpson to the murders. A trail of DNA leaving the crime scene was consistent with O. J.'s profile, as was the DNA found entering Simpson's home. Simpson's DNA profile was found in the Bronco along with that of both victims. The glove contained the DNA profiles of Nicole and Ron, and the sock had Nicole's DNA profile. At trial, the defense team valiantly fought back. Miscues in evidence collection were craftily exploited. The defense strategy was to paint a picture of, not only an incompetent investiga-

tion, but one that was tinged with dishonest police planting evidence. The strategy worked. O. J. Simpson was acquitted of murder.

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, for 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.

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.

Before examining the implications of Watson and Crick's discovery, let's see how DNA is constructed. DNA is a **polymer**. As we learned in Chapter 8, a polymer is a very large molecule made by linking a series of repeating units. In this case, the 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 13–1 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 13–1 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 to detail 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 13–2).

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 opposite number in a double-helix configuration, they pair as follows:

$$
\begin{array}{ccc}
\text{T A T T} & \text{G T A A} & \text{G T C A} \\
|\ |\ |\ | & |\ |\ |\ | & |\ |\ |\ | \\
\text{A T A A} & \text{C A T T} & \text{C A G T}
\end{array}
$$

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 three billion base pairs. From these numbers, we can begin to appreciate the diversity of 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.
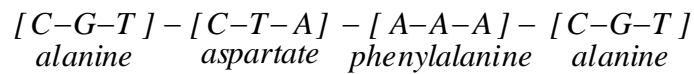
DNA AT WORK

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 twenty 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 13–3(a). Studies of individuals afflicted 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 13–3(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.

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
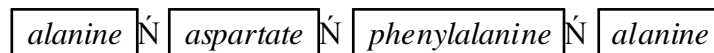
along the DNA strand. The alphabet of DNA is simple—*A, T, G,* and *C*—but the key to deciphering the genetic code is to know that each amino acid is coded by a sequence of three bases. Thus, the amino acid alanine is coded by the combination *C–G–T;* the amino acid aspartate is coded by the combination *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

$$-C–G–T–C–T–A–A–A–A–C–G–T-$$
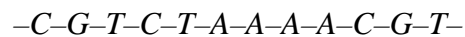
The triplet code contained within this segment translates into

$$[\,C–G–T\,] - [\,C–T–A\,] - [\,A–A–A\,] - [\,C–G–T\,]$$
$$\text{alanine} \qquad \text{aspartate} \quad \text{phenylalanine} \quad \text{alanine}$$

or the protein chain

$$\boxed{alanine}\ \acute{N}\ \boxed{aspartate}\ \acute{N}\ \boxed{phenylalanine}\ \acute{N}\ \boxed{alanine}$$

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 13–3), 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

$$-[\,C–C–T\,]-[\,G–A–G\,]-[\,G–A–G\,]-$$
$$\text{proline} \qquad \text{glutamate} \quad \text{glutamate}$$
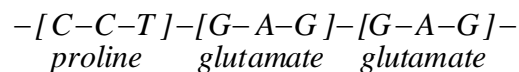
Individuals afflicted with sickle-cell disease carry the sequence

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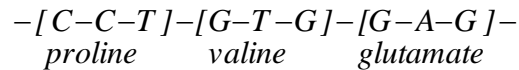
Thus, we see that a single base or letter change (*T* has been substituted for *A* in valine) is the underlying cause of sickle-cell anemia, demonstrating the delicate chemical balance between health and disease in the human body.

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, 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 thirteen-year project to determine the order of bases on all twenty- three 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.

REPLICATION OF DNA

Once the double-helix structure of DNA was discovered, it became apparent how DNA duplicated itself prior to cell division. 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. DNA

replication 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 13–4). A cell can now pass on its genetic identity when it divides.

Many enzymes and proteins are involved in the process of 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 only of academic interest to forensic scientists interested in DNA for identification purposes. However, this changed when researchers perfected the technology of using DNA polymerases to copy a DNA strand located outside a living cell. This relatively new laboratory technique is known as **polymerase chain reaction (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 can be accomplished in an automated fashion using a DNA Thermal Cycler (see Figure 13–5). Each cycle of the PCR technique results in a doubling of the DNA, as shown in Figure 13–4. Within a few hours, thirty 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.

RECOMBINANT DNA: CUTTING AND SPLICING DNA

The relationship between the base letters on a DNA strand and the type of protein specified for manufacture by the sequence of these letters is called the *genetic code*. Once a particular DNA site has been identified as controlling the production of a certain protein, molecular biologists can take advantage of the natural chemical-producing abilities of the DNA site. This undertaking has given rise to the technology known as *recombinant DNA*.

Recombinant DNA relies on the ability of certain chemicals, known as **restriction enzymes**, to cut DNA into fragments that can later be incorporated into another DNA strand. Restriction enzymes can be thought of as highly specialized scissors that cut a DNA molecule when it recognizes a specific sequence of bases. At present, more than 150 restriction enzymes are commercially available. Thus, molecular biologists have a great deal of flexibility in choosing the portion of a DNA strand they wish to cut out. Once a portion of the DNA strand has been cut out with the aid of a restriction enzyme, the next step in the recombinant DNA process is to insert the isolated DNA segment into a foreign DNA strand (normally, bacterium DNA is selected). Many types of bacteria contain DNA shaped in a circle. A restriction enzyme is used to cut open the circular DNA; then the foreign DNA is spliced in to re-form the circle (see Figure 13–6). The newly fashioned DNA is reintroduced into the bacterial cells. As the bacteria multiply rapidly in their usual fashion, copies of altered DNA are passed on to all descendants.

The commercial implications of recombinant DNA technology are enormous. For example, the gene that produces human growth hormone has been introduced into goldfish and carp, and the gene that produces growth hormone in rainbow trout has been introduced into carp. In each case, the gene-altered fish have grown significantly faster and larger than their natural relatives.

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If altered bacteria are infused with the DNA segment that makes human insulin, for example, the bacteria make human insulin. Because bacteria multiply so rapidly, it is not long before significant amounts of insulin can be recovered and used to treat diabetes. In this manner, other naturally occurring substances can be produced in commercial quantities for the treatment of human ailments. Likewise, plant genetic engineering holds promise for increasing global food production.

DNA TYPING

Restriction Fragment Length Polymorphisms

Geneticists concerned with the technology of recombinant DNA are usually interested in finding and reproducing DNA segments that control protein synthesis. However, not all of the letter sequences in DNA code for the production of proteins. Portions of the DNA molecule contain sequences of letters that are repeated numerous times. 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.* More than 30 percent of the human genome is largely 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 to affect our outward appearance, or for that matter control any other basic genetic function, they are nevertheless part of our genetic makeup and are inherited from our parents in the manner illustrated by the Punnett square (p. 366).

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 13–7). 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)**. These repeats are cut out of the DNA double helix by a restriction enzyme that acts like a pair of scissors. A number of different RFLPs were selected by the forensic science community for performing DNA typing. Typically a core sequence is fifteen to thirty-five bases long and repeats itself up to one thousand times.

Let's examine some DNA strands with regions of repeating base sequences to see how this process works. Figure 13–8 illustrates a portion of a pair of chromosomes. Note that each chromosome is composed of two DNA strands wrapped in a double-helix configuration. Each chromosome has a region that contains repeating bases. For the sake of simplicity in illustrating the RFLP method, we assume that the core repeat is only three bases long having a sequence *T–A–G*. Note an important distinction between the two chromosomes: the chromosome on the left has three repeating sequences of *T–A–G,* while the one on the right has two repeating sequences of *T–A–G.* As with any genetic trait, these repeating sequences were inherited from the parents. In this example, one parent contributed the chromosome containing the three repeating sequences, and the other parent passed on the chromosome containing the two repeating sequences.

The key to understanding DNA typing lies in the knowledge that within the world's population, numerous possibilities exist for the number of times a particular sequence of base letters can repeat itself on a DNA strand. The possibilities become even greater when one deals with two chromosomes, each containing different lengths of repeating sequences. During RFLP typing, restriction enzymes cut up chromosomes into hundreds of fragments, some containing repeating sequences from the DNA molecule. In our example, shown in Figure 13–8, the chromo-

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some pair, when cut, will yield two different fragment lengths of *T–A–G.* The length differences associated with DNA strands or RFLPs allow forensic scientists to distinguish one person from another. In actuality, these strands are relatively long, often consisting of thousands of bases. Once the DNA molecules have been cut up by the restriction enzyme, the resulting fragments must be sorted out. This is accomplished by separating the fragments by **electrophoresis** (pp. 142–149). DNA from various sources, cut up by restriction enzymes, is placed in separate lanes on an electrophoretic gel and subjected to an electric field. During the electrophoretic process, the DNA fragments migrate across a gel-coated plate. The smaller DNA fragments move faster along the plate than do the larger fragments (see Figure 5–12). Once the electrophoresis process is completed, the double-stranded fragments of DNA are chemically treated so that the strands separate from each other. The fragments are then transferred to a nylon membrane in much the same way as one would transfer an ink line onto a blotter. This transfer process is called *Southern blotting,* named after its developer, Edward Southern. To visualize the separated RFLPs, the nylon sheet is treated with radioactively labeled probes containing a base sequence complementary to the RFLPs being identified (a process called **hybridization**).

In our example, we aim to identify RFLPs composed of a repeating string of letters spelling *T–A–G.* Hence, the appropriate probes would have the complementary letter sequence *A–T–C,* as shown in the following diagram, so that the probes can specifically bind to the desired RFLP. (Note: The asterisk designates a radioactive label.)

Next, the nylon sheet is placed against X-ray film and exposed for several days. The radioactive decay products strike the film. When the film is processed, bands appear where the radioactive probes stuck to the fragments on the nylon sheet. The length of each fragment is determined by running known DNA fragment lengths alongside the test specimens and comparing the dis-

tances they migrated across the plate. The entire DNA typing process is depicted in Figure 13–9.

A typical DNA fragment pattern shows two bands (one RFLP from each chromosome). When comparing the DNA fragment patterns of two or more specimens, one merely looks for a match between the band sets. For example, in Figure 13–10, DNA extracted from a crime-scene stain matches the DNA recovered from one of three suspects. Although only a limited number of people in a population would have the same DNA fragment pattern as the suspect, this test in itself cannot be used to individualize the stain to the suspect. But by using additional DNA probes, each of which recognizes different repeating DNA segments (other than $T–A–G$), a high degree of discrimination or even near individualization can be achieved. For example, if each probe selected yielded a DNA type having a frequency of occurrence of one in one hundred in a population, then four different probes would have a combined frequency of one in 100 million ($1/100 \times 1/100 \times 1/100 \times 1/100$).

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. A seven-probe 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 13–11.

Polymerase Chain Reaction

For nearly ten years starting in 1985, RFLP was the dominant DNA typing procedure in the United States. However, its utility quickly ended by the mid-1990s. What caused this change? The answer is quite simple: the emergence of a revolutionary and elegant technique known as polymerase chain reaction. Put simply, PCR is a technique designed to copy or multiply DNA strands. For the forensic scientist, who is often presented with minute quantities of materials, the opportunity to multiply the quantity of sample available for analysis was too good to pass up.

PCR is the outgrowth of knowledge gained from an understanding of how DNA strands naturally replicate within a cell. The most important feature of PCR is knowing that an enzyme called *DNA polymerase* can be directed to synthesize a specific region of DNA. In a relatively straight forward 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:

<div align="center">

*–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–*

</div>

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

<div align="center">

–G–T–C–T–C–A–G–C–T–T–C–C–A–G–

</div>

–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 (*G, A, T, C*) to the separated strands. When the test tube is heated to 72°C, the polymerase enzyme directs the re-building 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, and the outcome is a doubling of the number of DNA strands—that is, from one to two. The cycle of heating, cooling, and strand re-building is then repeated, resulting again in a doubling of the DNA strands. On completion of the second cycle, four double-stranded DNA molecules will have been created from the original double-stranded DNA sample. Typically, twenty-eight to thirty-two cycles are carried out to

yield almost a billion copies of the original DNA molecule. Each cycle takes less than two minutes to perform.

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.

From the forensic scientist's viewpoint, PCR offers another distinct advantage in that it can amplify minute quantities of DNA, thus overcoming the limited sample-size problem often associated with crime-scene evidence. With PCR, less than one-billionth of a gram of DNA is required for analysis. Consequently, PCR can characterize DNA extracted from small quantities of blood, semen, and saliva. The extraordinary sensitivity of PCR allows forensic analysts to characterize small quantities of DNA that could never be detected by RFLP. For instance, PCR has been applied to the identification of saliva residues found on envelopes, stamps, soda cans, and cigarette butts.

Short Tandem Repeats (STRs)

Out with the Old, In with the New The latest method of DNA typing, **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 the RFLP procedure. This means that STRs are much less susceptible to degradation and can often be 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 evidence. Only one-billionth of a gram or less of DNA is fifty to one hundred times less than normally required for RFLP analysis.

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 five to eleven repeats of *A–A–T–G*. Figure 13–12 illustrates two such TH01 variants, one containing six repeats and the other containing eight repeats of *A–A–T–G*.

During a forensic examination, TH01 is extracted from biological materials and amplified by PCR in the manner described on pp. 394–396. 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 on an electrophoretic gel. 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.

What makes STRs so attractive to forensic scientists is that hundreds of different types of

STRs are found in human genes. The more STRs one can characterize, the smaller will be the percentage of the population from which these 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. For example, one system on the commercial market is the STR Blue Kit.[2] This kit provides the necessary materials for the coamplification and detection of three STRs *(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 13–13. In the United States, the forensic science community has standardized on thirteen STRs for entry into a national database known as the Combined DNA Index System (CODIS).

One important concept to grasp in understanding STR technology is that 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. Figure 13–14 illustrates how appropriate primers are used to define the region of DNA to be amplified. Also, a mix of different primers aimed at different STRs will be used to simultaneously amplify a multitude of STRs (multiplexing). In fact, one STR kit on the commercial market can simultaneously make copies of fifteen different STRs.

The thirteen CODIS STRs are listed in Table 13–1 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. Hence, the greater the number of STRs characterized, the smaller will be the frequency of occurrence of the analyzed sample in the general population.

The combination of the first three STRs shown in Table 13–1 typically produces a frequency of occurrence of about 1 in 5,000. A combination of the first six STRs typically yields a frequency of occurrence in the range of 1 in two million for the Caucasian population, and if the top nine STRs are determined in combination, this frequency declines to about 1 in one billion. The combination of all thirteen STRs shown in Table 13–1 typically produces frequencies of occurrence that measure in the range of 1 in 575 trillion for Caucasian Americans and 1 in 900 trillion for African-Americans. Importantly, a number of commercially available kits readily allow forensic scientists to profile STRs in the kinds of combinations cited here.

The separation of STRs can typically be carried out on a flat gel-coated electrophoretic plate, as depicted in Figures 5–12 and 13–13. However, with the advent and success of STR analysis, the need to reduce analysis time and to automate the sampling and data collection procedures has led to the emergence of *capillary electrophoresis* as the preferred technology for the 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 Figure 13–15, 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 with a syringe. 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.

Table 13–1   The Thirteen 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,**

Manufacturers of commercial STR kits typically used by crime laboratories provide analysts with 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 (see pp. 364–365). This 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.

Y-STRs 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. More than twenty different **Y-STR** markers have been identified, and a 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 when one is confronted with 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.

Keep in mind that STR types derived from the Y chromosome originate only from this single male chromosome. A female or XX subject does *not* contribute any DNA information. Also, unlike a conventional STR analysis 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 bands from each male. Also complicating the appearance of the DNA profile may be the pres-

ence 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, each STR type would be expected to show six bands. However, the same mixture subjected to Y-STR analysis would show only two bands (one band for each male) for each Y-STR type.

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. Significantly, about 25 percent of the DNA examinations conducted by the FBI Laboratory since 1989 have excluded suspects identified by police as the source of DNA evidence collected from the crime scene. In a number of well-publicized cases, DNA evidence has exonerated individuals who have been wrongly convicted and imprisoned for the commission of a crime (see Figure 13–16). 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. A number of well-publicized instances have occurred where the accuracy of DNA tests conducted by government-funded laboratories have been called into question.

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 twenty-three 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 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 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.

While 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 type of cases in which it will apply mtDNA technology.

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 (approximately 16,569) *A, T, G,* and *C* to make up thirty seven 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 Figure 13–17. 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 labo-

ratories simply report the number of times these sequences appear in the mtDNA database main-

tained 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 case work are unique to the

existing database, and many types are present at frequencies no greater than 1 percent in the da-

tabase. Thus it is often possible to demonstrate how uncommon a particular mitochondrial DNA

sequence is. However, even under the best of 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.

The first time mtDNA was admitted as evidence in a U.S. court was in 1996 in the case of

State of Tennessee v. *Paul Ware.* Here, mtDNA was used to link two hairs recovered from the

crime scene to the defendant. Interestingly, in this case, blood and semen evidence was absent.

Mitochondrial DNA analysis also plays a key role in the identification of human remains. An

abundant amount of mtDNA is generally found in skeletal remains. Importantly, mtDNA refer-

ence samples are available from family members sharing the same mother, grandmother, great-grandmother, and so on. One of the most publicized cases performed on human remains was the identification of the individual buried in the tomb of the Vietnam War's unknown soldier. The remains lying in the tomb were believed to belong to First Lt. Michael J. Blassie, whose A-37 warplane was shot down near An Loc, South Vietnam, in 1972. In 1984, the U.S. Army Central Identification Laboratory failed to identify the remains by physical characteristics, personal artifacts, or blood-typing results from hairs. The remains were subsequently placed in the tomb. In 1998, at the insistence of the Blassie family, the remains were disinterred for the purpose of performing mtDNA analysis and comparing the results to references from seven families thought to be associated with the case. The remains in the tomb were subsequently analyzed and confirmed to be consistent with DNA from Lt. Blassie's family.

Forensics at Work

Outrage: The O. J. Simpson Verdict

To distill this case down to its irreducible minimum (and temporarily ignoring all the other evidence pointing inexorably to Simpson's guilt), if your blood is found at the murder scene, as Simpson's was conclusively proved to be by DNA tests, that's really the end of the ball game. There is nothing more to say. (And in this case, not only was Simpson's blood found at the murder scene, but the victims' blood was found inside his car and home.) I mean, to deny guilt when your blood is at the murder scene is the equivalent of a man being caught by his wife *in flagrante* with another woman and saying to her (quoting comedian Richard Pryor), "Who are you going to believe? Me or your lying eyes?"

At the crime scene there were five blood drops leading away from the slain bodies of Nicole

Brown Simpson and Ronald Goldman toward the rear alley, four of which were immediately to the *left* of bloody size-12 shoe prints (Simpson's shoe size). This indicated, of course, that the killer had been wounded on the left side of his body. And the morning after the murders, Simpson was observed by the police to be wearing a bandage on his left middle finger. When the bandage was removed that afternoon, it was seen that he had a deep cut on the knuckle of the finger.

DNA (deoxyribonucleic acid) is the genetic material found in all human cells that carries the coded messages of heredity unique (with the exception of identical twins) to each individual. DNA, then, is our genetic fingerprint. Each of the approximately 100 trillion cells in a human body contains twenty-three pairs of chromosomes—one of each pair coming from one's father, the other from the mother—which contain DNA molecules. In criminal cases, DNA can be extracted from samples of blood, semen, saliva, skin, or hair follicles found at a crime scene and then compared to DNA drawn from a suspect to determine if there is a "match." DNA testing is a new forensic science, first used in Great Britain in 1985 and in the United States in 1987.

DNA tests on all five blood drops and on three bloodstains found on the rear gate at the crime scene showed that all of this blood belonged to Simpson. Two DNA tests were used: PCR (polymerase chain reaction) and RFLP (restrictive fragment length polymorphism). The PCR test is less precise than the RFLP, but can be conducted on much smaller blood samples as well as samples that have degenerated ("degraded") because of bacteria and/or exposure to the elements. PCR tests were conducted on four out of the five blood drops. Three showed that only one out of 240,000 people had DNA with the markers found in the sample. (A marker is a gene that makes up one portion of the DNA molecule, and the more markers in the sample, the more comparison tests can be conducted, and hence the greater the exclusion of other humans.) The fourth blood drop had markers which one out of 5200 people could have. Simpson was one of these people.

The fifth blood drop had sufficient markers for an RFLP test, and showed that only one out of 170 million people had DNA with those markers. Again, Simpson's blood did. The richest sample was on the rear gate, and an RFLP test showed that only one out of 57 billion people had those markers. Simpson was one of them. In other words, just on the blood evidence alone, there's only a one out of 57 billion chance that Simpson is innocent. Fifty-seven billion is approximately ten times the current population of the entire world.

Now I realize that Igor in Kiev, Gino in Naples, Colin down Johannesburg way, and Kartac on Pluto might have the same DNA as O. J. Simpson. If you're a skeptic I wouldn't blame you if you checked to see if Igor, Gino, Colin, or Kartac was in Brentwood on the night of the murders, used to beat Nicole within an inch of her life, had blood all over his car, driveway, and home on the night of the murders, had no alibi, and, if charged with the murders, would refuse to take the witness stand to defend himself. Who knows—maybe Simpson isn't the murderer after all. Maybe Igor or one of the others is. You should definitely check this out. And while you're checking it out, someone should be checking you into the nearest mental ward.

To elaborate on the irreducible minimum mentioned earlier, there are only three possible explanations other than guilt for one's blood being found at the murder scene, and all three are preposterous on their face. One is that Simpson left his blood there on an earlier occasion. When Simpson was interrogated by LAPD detectives on the afternoon after these murders, he said he had not cut himself the last time he was at the Bundy address a week earlier. But even without that, how can one believe that on some prior occasion Simpson bled, not just on the Bundy premises, but at the precise point on the premises where the murders occurred? In fact, so farfetched is this possibility that even the defense attorneys, whose stock-in-trade during the trial was absurdity, never proffered it to the jury.

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And here, not only was Simpson's blood found at the murder scene, but there were the four drops of Simpson's blood found just to the left of the killer's bloody shoe prints leaving the murder scene. If there is someone who isn't satisfied even by this, I would suggest that this book is perhaps not for you, that you think about pursuing more appropriate intellectual pursuits, such as comic strips. When I was a kid, one of my favorites was *Mandrake the Magician.* You might check to see if Mandrake is still doing his thing.

The second possibility is that Simpson cut himself while killing Ron Goldman and Nicole Brown in self-defense—that is, either Ron or Nicole or both together unleashed a deadly assault on Simpson, and he either took out a knife he had on his own person or wrestled Ron's or Nicole's knife away, and stabbed the two of them to death. This, of course, is just too insane to talk about. Again, even the defense attorneys, who apparently possess the gonads of ten thousand elephants, never suggested this possibility. It should be added parenthetically that if such a situation had occurred, Simpson wouldn't have had any reason to worry, since self-defense is a justifiable homicide, a complete defense to murder.

The third and final possibility is that the LAPD detectives planted Simpson's blood not just at the murder scene but to the left of the bloody shoe prints leaving the scene. This is not as insane a proposition as the first two, but only because there are degrees of everything in life. It is still an insane possibility, and if any reader is silly enough to believe that the LAPD detectives decided to frame someone they believed to be innocent of these murders (Simpson) and actually planted his blood all over the murder scene (and, of course, planted the victims' blood in Simpson's car and home), again, this book is probably not for that reader. This book is for people who are very angry that a brutal murderer is among us—with a smile on his face, no less—and want to know how this terrible miscarriage of justice could have occurred.…

Let me point out to those who believe in the "possible" existence of either of the aforementioned three innocent possibilities for Simpson's blood being found at the murder scene, that the prosecution only has the burden of proving guilt beyond a *reasonable* doubt, not beyond all possible doubt. So it isn't necessary to have all possible doubts of guilt removed from one's mind in order to reach a conclusion of guilt. Only reasonable doubts of guilt have to be removed. Of course, in this case, *no* doubt remains of Simpson's guilt....

There perhaps is no better example of the phenomenon of people seeing what they expect to see working to the prosecution's very definite disadvantage than the situation with one of the defense's expert witnesses, Dr. Henry Lee. Lee, director of the Connecticut State Forensic Science Laboratory, is reputed to be the preeminent dean of American forensic scientists, the "top forensic sleuth," as it were. But I think we all know by now how suspect reputations can be, and if Lee's testimony in the Simpson case is any indication at all of his abilities, he is nothing short of incompetent. At best, he's an example of how Mark Twain once described an expert: "Just some guy from out of town." The problem is that the jury couldn't see through the bloated reputation of Dr. Lee, and the prosecution, in its summation, never exposed Lee so the jurors could see the emperor without his clothing on.

There were two particular areas in which Lee's testimony, if believed by the jury, was very damaging to the prosecution. One, he testified that he found four small bloodstains on a paper bindle enclosing seven cotton swatches containing blood removed from one of the blood drops (Item 47) to the left of a bloody shoe print leaving the Bundy murder scene (later identified as Simpson's blood by DNA testing). Lee couldn't figure out how the blood could have leaked onto the paper when the swatches had been left out to dry overnight prior to their being packaged. The fact that there was no assurance the blood on all seven swatches had dried completely by the

time they were wrapped, or that the subsequently frozen swatches did not leak the blood later in the summer when they were thawed out for DNA testing, or that there was not some other innocent explanation (in virtually every case there are questions, the innocent answers to which are simply never learned) did not deter Lee from saying there was "something wrong," a term that resonated with the jurors during their deliberations. The implication the defense sought to convey, of course, was that the answer lay in evil LAPD conspirators who crept into the LAPD lab in the middle of the night and planted and tampered with the blood evidence.

Lee also testified that he found three key "imprints" on the terra-cotta walkway at the crime scene which he himself photographed when he went to the scene on June 25, 1994. They did not match the many size-12 Bruno Magli bloody shoe prints at the scene which the prosecutors said belonged to Simpson. One was definitely a shoe print, he testified, one was a "parallel line imprint," and the other he simply called an "imprint." The latter two "could be" shoe prints, he said, raising the inference of a second assailant. This, of course, challenged the prosecution's position that Simpson was the lone killer, and hence challenged their conception of the entire case against him.

Lee also found bloody "parallel line imprint" patterns on the envelope found at the murder scene containing the glasses belonging to Nicole's mother which she had left at the Mezzaluna restaurant earlier in the evening and which Ron Goldman was returning when he was murdered, on a small, triangular piece of paper near the bodies, and on Ron Goldman's jeans. Lee testified that all of these imprints could possibly be partial shoe prints, and since he concluded they were not from the Bruno Maglis or Ron Goldman's shoes, the defense suggested they came from the shoe of the second assailant.

But William Bodziak, the FBI's senior expert on shoe prints, and the former chairman of the

footwear and tire section of the International Association for Identification, later debunked all of Lee's conclusions. Bodziak told me he went back to the Bundy crime scene with copies of photographs Lee had taken on June 25 to examine the shoe print and the other two imprints on the walkway which Lee said "could be" shoe prints. What he found was astonishing. With blown-up color photographs, he pointed out to the jury that one of the imprints (the parallel line one) on the walkway Lee had photographed and testified to was actually tool (trowel) marks made by the workers in the laying of the cement years earlier, and the other imprint was a shoe print from one of these workers which was a permanent indentation in the concrete (ridges, depressions) that Bodziak felt with his own hands.

As to the bloody "parallel line imprint" patterns on the envelope, paper, and jeans Lee had suggested could possibly have come from the shoes of a second assailant, Bodziak said that none of them were shoe prints. The parallel line imprints on the right leg of Ron Goldman's blue jeans were too erratic to be shoe prints and also had no borders representing the edge of any heel or sole. They appeared to be consistent with having been made by a swiping or brushing motion against the jeans by a sleeve from Goldman's long-sleeved shirt, which was thick and roughly textured. Bodziak testified that he found a "striking similarity between the ribbed design on the shirt [taken from test impressions]" and the bloody imprint on the shirt. (FBI special agent Douglas Deedrick, an expert on fiber evidence, had previously testified that the bloody imprint on the jeans appeared to have come from fiber such as that on Goldman's shirt.) As to the small ("half the size of one's thumb") bloody imprint on the envelope, it too was not a part of a shoe print, again having no borders, being too erratic, and the patterns being so fine and small as to be uncharacteristic of any shoe sole or heel Bodziak had ever seen. Bodziak testified that the parallel lines were consistent with a "fabric" pattern, and could have come from the jeans or shirt of

Goldman. Bodziak also testified that the bloody imprint on the piece of paper wasn't a shoe print, and even if it had been, it would have had to come from the shoe of a tiny child.

Lee demonstrated further incompetence in the forensic technique he employed to reach his conclusions. He made no test impressions of Ron Goldman's Levi jeans and shirt (although photographs were taken of the small piece of paper, the LAPD criminalists did not collect it). This was shocking to Bodziak. He testified: "You could look at the fabric on my sleeves with a magnifying glass, but because of its three-dimensional quality, you could not determine what the exact pattern would look like in a test impression. It is absolutely essential to make test impressions for comparison purposes. It is the *only* way that you can make a valid comparison."

Lee, stung and wounded by the obvious repudiation of his conclusions by the FBI's shoe print expert (Lee's specialty is not shoe prints), told reporters from his laboratory in Connecticut that although he stuck to his conclusions, "I'm sorry I ever got involved in the Simpson case," and said he would probably resist any defense subpoena to return to Los Angeles to defend himself and his conclusions.

As it turned out, he didn't have to defend or rehabilitate himself. His reputation was enough for the jury, which should have been skeptical of every single one of his conclusions once his shoe print and imprint testimony was proved to be claptrap. The foreperson of the jury, Armanda Cooley, said in the book she coauthored on the case, *Madam Foreman:* "Dr. Henry Lee was a very impressive gentleman. Highly intelligent, world-renowned. I had a lot of respect for Dr. Lee." Lee's discredited testimony hadn't lessened his stature in Cooley's mind one iota. Juror Lionel (Lon) Cryer told the *Los Angeles Times* right after the verdict that the jury viewed Lee as "the most credible witness" of all at the trial. Cryer repeated Lee's statement that "there was something wrong," saying the jury took these words back to the jury room with them. "Dr. Lee had a

lot of impact on a lot of people," he added.

Source: Vincent Bugliosi, *OUTRAGE: The Five Reasons Why O. J. Simpson Got Away with Murder.* Copyright © 1996 by Vincent Bugliosi. Reprinted by permission of W. W. Norton & Company, Inc.

Forensic Brief

In the fall of 1979, a 61-year-old patient wandered away from a U.S. Department of Veterans Affairs medical facility. Despite an extensive search, authorities never located the missing man. More than ten years later, a dog discovered a human skull in a wooded area near the facility. DNA Analysis Unit II of the FBI Laboratory received the case in the winter of 1999. The laboratory determined that the mitochondrial DNA profile from the missing patient's brother matched the mitochondrial DNA profile from the recovered skull and provided the information to the local medical examiner. Subsequently, the remains were declared to be those of the missing patient and returned to the family for burial.

Source: *FBI Law Enforcement Bulletin* 78 (2002): 21.

THE COMBINED DNA INDEX SYSTEM (CODIS)

Perhaps the most significant investigative tool to arise from a DNA typing program allows crime laboratories to compare DNA types recovered from crime-scene evidence to those of convicted sex offenders and other convicted criminals. This capability is of tremendous value to investigators in cases in which the police have not been able to identify a suspect. All fifty states have legislatively mandated collection of DNA samples from convicted offenders of particular crimes and establishment of DNA databases for law enforcement purposes. CODIS (Combined DNA

Index System) is 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 software enables local, state, and national crime laboratories to compare DNA profiles electronically. Thousands of matches have linked serial crimes to each other and have solved crimes by allowing investigators to match crime-scene evidence to known convicted offenders. As mentioned earlier, in the United States the forensic science community has currently standardized on thirteen STRs for entry into CODIS. The CODIS concept has already had a significant impact on police investigations in various states, as shown by the following brief.

Forensic Brief

In 1990, a series of brutal attacks on elderly victims occurred in Goldsboro, North Carolina, by an unknown individual dubbed the Night Stalker. During one such attack in March, an elderly woman was brutally raped and almost murdered. Her daughter's early arrival home saved the woman's life. The suspect fled, leaving behind materials intended to ignite the residence and the victim in an attempt to conceal the crime. In July 1990, another elderly woman was brutally raped and murdered in her home. Three months later, a third elderly woman was raped and stabbed to death. Her husband was also murdered. Their house was burned in an attempt to cover up the crime, but 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 each victim enabled authorities to conclude that the same perpetrator had committed all three crimes. However, there was no suspect.

More than ten years after the commission of these crimes, 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 thus compared to thousands of convicted-offender profiles already in the database.

In April 2001, a "cold hit" was made with an individual in the convicted-offender DNA database. The perpetrator had been convicted of shooting into an occupied dwelling, an offense that requires inclusion 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, 2002, *http://www.ojp.usdoj.gov/nij/pubs-sum/194197.htm*

COLLECTION AND PRESERVATION OF BIOLOGICAL EVIDENCE FOR DNA ANALYSIS

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 eighteen DNA-bearing cells are needed to obtain an STR profile. However, modifications in the technology can readily extend the level of detection down to nine cells. A quantity of DNA that is below the normal level of detection is defined as a

low copy number. 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]

The ultimate sensitivity goal in forensic DNA analysis is profiling DNA extracted from one human cell. Such an accomplishment seems close to fruition. Researchers have reported obtaining STR profiles from one or two cells and have successfully profiled DNA from single dermal ridge fingerprints.[4] While it's premature to imply that this technology, or a comparable one, is eligible for admission in criminal trials, one cannot exclude its utilization in criminal and forensic intelligence investigations. Table 13–2 illustrates the power of DNA as a creator of physical 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, shoe covers, and possibly coveralls.

Blood has great evidential value when a transfer between a victim and suspect can be demonstrated. For this reason, all clothing from both victim and suspect should be collected and sent to the laboratory for examination. This procedure must be followed even when the presence of blood on a garment does not appear obvious to the investigator. Laboratory search procedures are far more revealing and sensitive than any that can be conducted at the crime scene. In addition, blood should also be searched for in less-than-obvious places. For example, the criminal may have wiped his or her hands on materials not readily apparent to the investigator. Investigators should look for towels, handkerchiefs, or rags that may have been used and then hidden, and should also examine floor cracks or other crevices that may have trapped blood.

Table 13–2   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,	inside	sweat, hair, dandruff

or mask		
eyeglasses	nose or ear pieces, lens	sweat, skin
facial tissue	surface area	mucus, blood, sweat, semen,
cotton swab		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, or 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	scrapings	blood, sweat, tissue

fingernail

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.** If feasible, the entire stained article 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 might 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 13–18), 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.

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. For this purpose, at least 7 cc of whole blood should be drawn from individuals by a qualified medical person. The blood sample should be collected in a sterile vacuum tube containing the preservative *EDTA* (ethylenediamine tetraacetic acid). In addition to serving as a preservative, EDTA inhibits the activity of enzymes that degrade DNA. The tubes must be kept refrigerated (not frozen) while awaiting transportation to the laboratory. In addition to blood, other options exist for obtaining standard/reference DNA specimens. 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 13–19).

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, 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.

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. Change gloves before handling each new piece of evidence.

2. Collect a substrate control for possible subsequent laboratory examination.

3. 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.

4. Always package each item of evidence in its own well-ventilated container.

Forensic Brief

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 due to fear from 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 six to twenty-four hours. As such, 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
THO1	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

Source: 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.**

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 (see p. 354). Interestingly, luminol does not inhibit the ability to detect and characterize STRs.[5] Therefore, luminol 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.

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 associated with relatively long repeating DNA strands are called restriction fragment length polymorphisms (RFLPs) and form the basis for one of the first DNA-typing procedures. Once the DNA molecules have been cut up by a restriction enzyme, the resulting fragments are sorted out by electrophoresis. A typical DNA fragment pattern shows two bands (one RFLP from each chromosome). When comparing the DNA fragment patterns of two or more specimens, one merely looks for a match between the band sets.

Polymerase chain reaction (PCR) is the outgrowth of knowledge gained from an understanding of how DNA strands naturally replicate within a cell. PCR offers a distinct advantage in that it can amplify minute quantities of DNA. PCR technology cannot be applied to RFLP DNA typing. The RFLP strands are too long, often numbering in the thousands of bases. PCR is best used with DNA strands that are no longer than a couple of hundred bases. Another advantage in mov-

ing 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 the adverse conditions not uncommon at crime scenes.

The latest method of DNA typing, short tandem repeat (STR) analysis, has emerged as the most successful and widely used DNA-profiling procedure. STRs are locations on the chromosome that contain short sequences that repeat themselves within the DNA molecule. They serve as useful markers for identification because they are found in great abundance throughout the human genome. STRs normally consist of repeating sequences three to seven bases long, and the entire strand of an STR is also very short, less than 400 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 extreme decomposition. Also, because of their shortness, STRs are ideal candidates for multiplication by PCR, thus overcoming the limited-sample-size problem often associated with crime-scene evidence. What makes STRs so attractive to forensic scientists is that hundreds of different types of STRs are found in human genes. 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 thirteen STRs. With STR, as few as 125 picograms of DNA are required for analysis. This is one-hundredth the amount normally required for RFLP analysis.

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 twenty 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. Recombinant DNA relies on the ability of chemicals known as _____ to cut DNA into fragments.

16. True or False: All of the letter sequences in DNA code for the production of proteins.

_____

17. In RFLP DNA typing, restriction enzymes are used to cut out (repeating, random) sequences from the DNA molecule.

18. In RFLP DNA typing, restriction enzymes are used to cut out sequences of DNA with different (widths, lengths).

19. DNA fragments can be sorted according to their size by the technique of _____.

20. In the RFLP DNA typing process, DNA fragments are transferred to a nylon membrane by a process called _____ blotting.

21. True or False: In the RFLP DNA typing process, a radioactively labeled probe is used to visualize the separated DNA fragments. _____

22. The probe complementary to the base sequence *T–A–G* has the letter sequence _____.

23. In RFLP DNA typing, a typical DNA pattern shows (two, three) bands.

24. True or False: Specimens amenable to DNA typing are blood, semen, body tissues, and hair. _____

25. Short DNA segments containing repeating sequences of three to seven bases are called _____.

26. True or False: The longer the DNA strand, the less susceptible it is to degradation. _____

27. The short length of STRs allows them to be replicated by _____.

28. The concept of (CODIS, multiplexing) involves simultaneous detection of more than one DNA marker.

29. DNA fragments can be separated and identified by (gas chromatography, capillary electro-phoresis).

30. The amelogenin gene shows two bands for a (male, female) and one band for a (male, female).

31. Y-STR typing is useful when one is confronted with a DNA mixture containing more than one (male, female) contributor.

32. Mitochondrial DNA is inherited from the (mother, father).

33. True or False: Mitochondrial DNA is more plentiful in the human cell than is nuclear DNA. _____

34. (Two, Four) regions of mitochondrial DNA have been found to be highly variable in the hu-

man population.

35. True or False: Polymerase chain reaction is a part of the process used in the forensic analysis of RFLP, STRs, and mitochondrial DNA. _____

36. The national DNA database in the United States has standardized on _____ STRs for entry into the database.

37. True or False: Y-STR data is normally entered into the CODIS database collection. _____.

38. Small amounts of blood are best submitted to a crime laboratory in a (wet, dry) condition.

39. True or False: Airtight packages make the best containers for blood-containing evidence. _____

40. Whole blood collected for DNA typing purposes must be placed in a vacuum containing the preservative _____.

41. A typical STR DNA type emanating from a single individual shows a (one, two, three)-band pattern.

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Case Study

The Forensic Community's Response to September 11

Wilder Damian Smith

Staff editor for *Analytical Chemistry*

United Flight 175, hijacked by terrorists, crashes into 2 World Trade Center.

Courtesy Robert Clark, Aurora & Quanta Productions Inc.

On September 11, Brion Smith was home in Frederick, Md., enjoying a vacation day when the news flashed across his television screen—the World Trade Center (WTC) buildings had been struck by two hijacked airplanes. Minutes later, a third plane struck the Pentagon while a fourth later crashed in a field in Somerset County, Pa. Acting on impulse, Smith, chief deputy medical examiner for the DNA Division of the Office of the Armed Forces Medical Examiner (OAFME), immediately gathered his things and headed in to work. "Your first inclination, of course, is to go to the crash site," he says, "however, a DNA person has little utility outside of the labora-

tory."…

World Trade Center

Marie Samples, an assistant director in the Department of Forensic Biology in the Office of Chief Medical Examiner (OCME) in New York, was sitting in a management meeting when a co-worker poked his head in and delivered the horrific news. "When someone tells you that the WTCs have just collapsed, you don't ever fathom that happening," says Samples. "I don't think it sunk in with me until I got home."

Overseeing mass tragedies is nothing new to the OCME. In 1990, the lab handled the Happy Land Social Club fire in which 87 people died; then in 1993, they handled the *Golden Venture* tragedy in which 286 Chinese immigrants drowned when their boat went aground off the coast of New Jersey. The OCME has about 90 experienced technicians on staff who perform various tasks, including examining physical evidence; conducting DNA extractions; and overseeing DNA quantitation, amplification, and finally, DNA typing.

"Our first thought was that we would be able to handle the samples in-house," says Samples. But as the estimates of the number of bodies at the WTC site continued to pile up—initial reports were as high as 7000—those plans quickly changed. "We've handled big disasters in the past, but this was nothing like we've ever seen," exclaims Samples. Although the OCME has the largest DNA analysis lab in the country, the thought of tackling a project with that many unknown and presumed dead presented other problems for Samples and her lab. "We had two big responsibilities that were clashing," she says, "our commitment to serve the criminal justice system in New York for the cases we normally handle—sexual assaults, homicides, etc.—and our role as a support lab for the medical examiners in determining the cause of death." She goes on to say,

"We knew we couldn't do both of them well at the same time, so we decided to contract out the DNA typing work."

As the search and rescue mission for the victims of the WTC collapse became a recovery mission, more problems faced the forensic community. At Ground Zero—the name given to the site of the collapsed WTC buildings—literally millions of human remains were lying scattered deep beneath the tons of twisted metal and shattered cement. "We have to remember that when those towers fell, they turned into giant shredders," says Kevin McElfresh, a senior scientist with BODE Technologies. The smoldering fires, the exposure to the outside weather, and the estimated amount of time it would take to remove the debris—a year, by some calculations—also presented problems for the chemists. "At least in the [1999] Swiss Air crash, the remains were in the water at the bottom of the ocean in four degree water; even though they were down there for three months, we were still able to extract DNA to generate full profiles," says Benoit Leclair, senior scientist with Myriad Genetics.…

Some 7 months later, remains were still being collected and taken to Fresh Kills, an abandoned 3000-acre landfill on Staten Island, N.Y., that has been reincarnated as the country's largest "rake-and-sift" DNA lab. There, workers meticulously pick through bits of concrete, rocks, and other rubble in search of the tiniest remnants of human tissue, teeth, and even hair to aid in the identification process. The OCME extracts the DNA from each of the remains recovered, and those extracts are then shipped off to the respective companies for DNA typing and profiling.

To handle the profiling, the OCME contracted the services of three companies: Myriad Genetics, Celera Genomics, and BODE Technologies. Blood and tissue samples recovered from the site are being sent to Myriad Genetics, based in Salt Lake City, Utah. The company is using the

technique of short tandem repeats (STR) on the recovered tissues. "I would refer to this as data mining," says Leclair. STR is a technique that focuses on 13 loci found over the 23 pairs of chromosomes that make up a human's genome. After polymerase chain reaction (PCR) amplification, the newly formed DNA fragments are separated by capillary electrophoresis. Myriad had worked with the New York State Police prior to September 11, performing similar analysis on New York's rape kits.

When blood and tissue samples are not available from the WTC site for DNA typing, forensic scientists often have to turn to another source: bones. BODE Technical Group, a Virginia-based company, is overseeing the bone analysis of the victims. Its lab has about 70 employees, and one of its two specialized units is devoted to forensic analysis. The company participated in the forensic identification of the 88 victims in the Alaska Air crash in 2000. Currently, it has received over 7000 samples from the WTC site.

When the tissue samples are severely burned or degraded, a process called mitochondrial DNA (mtDNA) analysis often has to be done; because this was the case with some of the recovered remains from Ground Zero, the OCME contracted the Rockville, Md., company Celera Genomics to oversee the mtDNA analyses of the WTC victims. However, Celera, a business under the Applera Corp., was an unusual choice. "Unlike BODE and Myriad," says Heather Kowalski, Celera's director of corporate communications, "[Celera] didn't have a forensic part to our business before this tragedy occurred." Celera is known mainly for its work on the Human Genome Project and as a high-throughput sequencing company.

In preparation for the incoming samples, Celera built four new forensic laboratories in the span of two months and hired Rhonda Roby to oversee the forensics program. Before that, Roby worked as a forensic scientist for Applied Biosystems (AB), which is also a business unit of Ap-

plera, and which has a Human Identification Group and experience working with the forensic community. Despite the novelty of the project, Roby wasn't intimidated. "As scientists, we knew we had something to offer, and Applera wanted to do it right," says Roby. "Celera offers expertise in high-throughput sequencing and bioinformatics capabilities, and Applied Biosystems offers expertise as the inventors of the sequencing chemistry, software and instruments, and a team of forensic scientists from the Human Identification Group."

Quality Control

After DNA profiles are obtained, the results are then shipped back to the New York State Police Laboratory in Albany, N.Y. There, the information is stored in a specially modified version of the FBI's Combined DNA Index System (CODIS) database.

The original CODIS database stores the DNA information of convicted felons and is used to match that information in prosecution cases. This new version of the database uses the same DNA comparison software, but only for purposes of matching the September 11 DNA profiles to those of the recovered victims' blood samples submitted by their relatives, and to the DNA information obtained from the victims' toothbrushes, hair, soiled laundry, and used cigarette butts. Also, as part of the OCME's quality control efforts, BODE is also repeating 5% of the mtDNA analysis that Celera conducts.

Sample Analysis

Medical and legal issues have to be considered when determining how much of a sample is needed to yield identifying information. The condition of the recovered body parts determines which type of analysis—nuclear (nucDNA) or mtDNA—is performed.

NucDNA analysis is the most commonly used because it's faster, the genome is found in the

cell's nucleus, and the DNA has alleles from each parent. In nucDNA analysis, the DNA fragments are analyzed and amplified using PCR. The profile from the nucDNA is then obtained and used to match and verify a victim. However, this type of analysis usually requires a lot of sample. "The problem," says Smith, "is that despite having a large amount of sample, sometimes there are only three grams of usable tissue available for analysis; and that is often totally exhausted during analysis."

MtDNA analysis is somewhat different. Mitochondria are abundant in the cell's cytoplasm, but the mtDNA only comes from the mother. The high number of mtDNA genomes in the cell increases the likelihood of successful PCR amplification. However, mtDNA analysis is more difficult to perform than nucDNA, more time consuming, and very expensive.

The second issue facing analysts is more humanitarian. "We can't tell someone, 'The good news was that it was him; the bad news is that he's all gone'," says Smith. Most families would rather have some remains of their loved one to take home, even if it is just a small piece. "There isn't one of our people that doesn't understand that there is a family in dire need to know what happened to their loved one," says McElfresh. The scientists use every piece of information they can to find answers. "What gets to me the most is that when it's late and you have a set of records in front of you and what you are looking at is the reconstruction of a person's life through the eyes and the contributions of their family," he says. The resolve of the scientists, says Smith, is evident every day. "With this project, I've seen people so driven that they are standing with their eyes shut and you have to tell them to go home," he says.

Courtesy AP/Wide World Photos

The Pentagon and Somerset

Civilian plane crashes are normally assigned to the Federal Aviation Administration (FAA) and the National Transportation Safety Board (NTSB). However, the DNA Division of the OAFME—a federal lab based in Rockville, Md., with two forensic facilities—handled the remains from the Pentagon and the Somerset County, Pa., sites. "We [NTSB] don't have DNA analysis capability," says Frank Ciaccio, chief of forensic sciences and a forensic anthropologist for the NTSB.

In 1996, the NTSB established a memorandum of understanding with the Department of Defense that recommends to local coroners that they use the services of the Armed Forces DNA Identification Laboratory (AFDIL)—the first facility of the OAFME—for DNA analysis. Under the terms of that agreement, the Armed Forces Institute of Pathology (AFIP) simply had to be invited to perform the DNA identifications of the crash victims by the local coroner, and that is what happened in the Pentagon and Somerset cases.

Previously, the AFDIL had performed DNA casework on the victims of the 1999 Egypt Air and the 2000 Alaska Air plane crashes. The AFDIL has 30 technicians divided into 6 teams that specialize in mtDNA analysis and a smaller group that handles all the nucDNA casework. The mtDNA section of the AFDIL was created in 1991 and has been instrumental in the identification of the recovered remains of American servicemen from the Korean War. To date, the AFDIL has received over 2000 samples for typing from the Pentagon and Somerset crashes.

The second OAFME facility, called the Armed Forces Repository of Specimen Samples for the Identification of Remains, houses blood-stained cards for all active duty, reserve, and National Guard military personnel. These filter paper cards are refrigerated and contain each person's name, social security number, date of birth, and two quarter-size spots of blood. In the Pentagon and Somerset crashes, about 50 of the victims were active duty American servicemen.

Were They Prepared?

"We have the largest DNA lab in the country, and even we couldn't handle [the WTC] caseload," says Samples. "It's hard for a lab to try to prepare for something along the lines of New York when it isn't likely to happen." (Sample's lab also had 12 new lab technicians start work on September 10.)

Learning from previous crashes, Smith's lab implemented a few critical changes. "We found that by having a DNA collection team [at the crash site], they could collect the tissue sample after it has been taken from the body and tag it with a number and bar code onsite before it gets back to the lab," he says. Creating better software in the chain of custody was also important. "For the Pentagon and Somerset crashes we set up two computer systems, one at the Dover Air Force Base and the other in Somerset County for the Pennsylvania crash."

Smith believes experience is the best teacher. "The thing we were missing in the Egypt and Alaska Air crashes was how to compare hundreds of DNA profiles obtained from the evidence with hundreds of DNA reference profiles," he says. "The hardest part of the project was sorting, comparing, matching, and reporting the data, so there was clearly a role for automation."

Need for New Technology

Although forensic technology has advanced, Leclair believes it still is always a step behind the last disaster. "We have the DNA typing tools to tackle a disaster such as Swiss Air only as a result of the TWA 800 disaster," states Leclair. Back in the late 1980s, DNA analysis was all done using a variable number of tandem repeats or restriction fragment-length polymorphisms, which required an enormous amount of sample and analysis time.

Today, most labs are using PCR to analyze DNA. "PCR has been the biggest addition to forensic

technology," says McElfresh. "I can't imagine dealing with something the magnitude of this [WTC buildings and Pentagon] with that old technology."

But the tragedies of September 11, say some forensic scientists, should be a wake-up call to the community. "Considering the magnitude of the New York disaster, one can readily see the need for powerful bioinformatics tools," states Leclair.

Roby believes that robotics and more advanced automation are the technologies of the future for forensics. "What may come out of [September 11] is that we are using robotic systems and automation, and we may be able to advance forensic sciences with the scientific technology that is available in other areas with these systems," she says. Samples agrees. "We had thought about using the robotics system before, but our caseload had never justified using them."

Justifying new technology, and finding the time to develop it, seem to be the biggest obstacles. "I think the forensic community could have handled the caseload of the WTC buildings without robotics and automation," says Roby, "but the question is how long would it have taken?"

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DNA

Abbreviation for deoxyribonucleic acid—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.

Complementary Base Pairing

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

WebExtra 13.1

What Is DNA?

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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.

Human Genome

The total DNA content found within the nucleus of a cell. In humans, it is composed of approxi-

mately 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.

Restriction Enzymes

Chemicals that act as scissors to cut DNA molecules at specific locations.

Tandem Repeat

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

Restriction Fragment Length Polymorphisms (RFLP)

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.

Hybridization

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

Primer

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

WebExtra 13.2

Click on "Amplification" and Follow the PCR Process Through Five Cycles

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Short Tandem Repeat (STR)

A region of a DNA molecule that contains short segments consisting of three to seven repeating base pairs.

Multiplexing

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

WebExtra 13.3

See the 13 CODIS STRs and Their Chromosomal Positions

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WebExtra 13.4

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

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WebExtra 13.5

Understand the Operational Principles of Capillary Electrophoresis

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Amelogenin Gene

A genetic locus useful for determining gender.

WebExtra 13.6

See the Electropherogram Record from One Individual's DNA

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Y-STRs

Short tandem repeats located on the human Y chromosome. Their utility in forensic science is that they originate only from a male donor of DNA.

WebExtra 13.7

An Animation Depicting Y-STRs

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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.

WebExtra 13.8

See How We Inherit Our Mitochondrial DNA

www.prenhall.com/Saferstein

Sequencing

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

WebExtra 13.9

Look Into the Structure of Mitochondrial DNA and See How It Is Used for DNA Typing

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Picogram

One-trillionth of a gram, or 0.000000000001 gram.

Low Copy Number

Fewer than eighteen 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.

Substrate Control

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

Buccal Cells

Cells derived from the inner cheek lining.

WebExtra 13.10

DNA Forensics

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Figure 13–1 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.

Figure 13–2 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.

Figure 13–3 (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.

Figure 13–4 Replication of DNA. The strands of the original DNA molecule are separated, and two new strands are assembled.

Figure 13–5 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. *Courtesy Applied Biosystems, Foster City, Calif.*

Figure 13–6 The joining of DNA from two different sources via recombinant DNA technology.

Figure 13–7 A DNA segment consisting of a series of repeating DNA units. In this illustration, the fifteen-base core can repeat itself hundreds of times. The entire DNA segment is typically hundreds to thousands of bases long.

Figure 13–8 Intertwined strands of DNA representing segments of two chromosomes. Note that the chromosome segment on the left contains three repeating sequences of *T–A–G,* while the chromosome segment on the right has two repeating sequences of *T–A–G.*

Figure 13–9 The DNA RFLP typing process.

Figure 13–10 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. *Courtesy Edvotek, The Biotechnology Education Company*

Figure 13–11 The dress and the FBI Report of Examination for a semen stain located on the dress.

Figure 13–12 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*.

Figure 13–13 Triplex system containing three loci: FGA, vWA, and D3S1358, indicating a match between the questioned and the standard/reference stains.

Figure 13–14 Appropriate primers flanking the repeat units of a DNA segment must be selected and put in place in order to initiate the PCR process.

Figure 13–15 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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Figure 13–17 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.

Figure 13–18 Air-dried swabs are placed in a swab box for delivery to the forensic labora-tory. *Courtesy Tri-Tech, Inc., Southport, N.C., www.tritechusa.com*

Figure 13–19 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 transfer of buccal cells onto the cotton bulb of the swab. The kit is then delivered to the forensic laboratory. *Courtesy Tri-Tech, Inc., Southport, N.C., www.tritechusa.com*

[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 utilizing the genetic code it received from DNA.

[2] Applied Biosystems, 850 Lincoln Centre Drive, Foster City, Calif. 94404.

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

[4] E. K. Hanson and J. Ballantyne, "Whole Genome Amplification Strategy for Forensic Genetic Analysis Using Single or Few Cell Equivalents of Genomic DNA," *Analytical Biochemistry* 346 (2005): 246.

[5] 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.