Learning Objectives

After studying this chapter you should be able to:

- Name the parts of a nucleotide and explain how nucleotides are linked to form DNA
- Understand the concept of base pairing as it relates to the double-helix structure of DNA
- Contrast DNA strands that code for the production of proteins with strands that contain repeating base sequences
- Explain the technology of polymerase chain reaction (PCR) and how it applies to forensic DNA typing
- 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 proper preservation of biological evidence for laboratory DNA analysis

National Science Content Standards

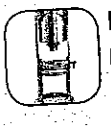

Scientific Inquiry


Science and Technology


Physical Science


Science in Personal and Social Perspective


Life Science


History and Nature of Science

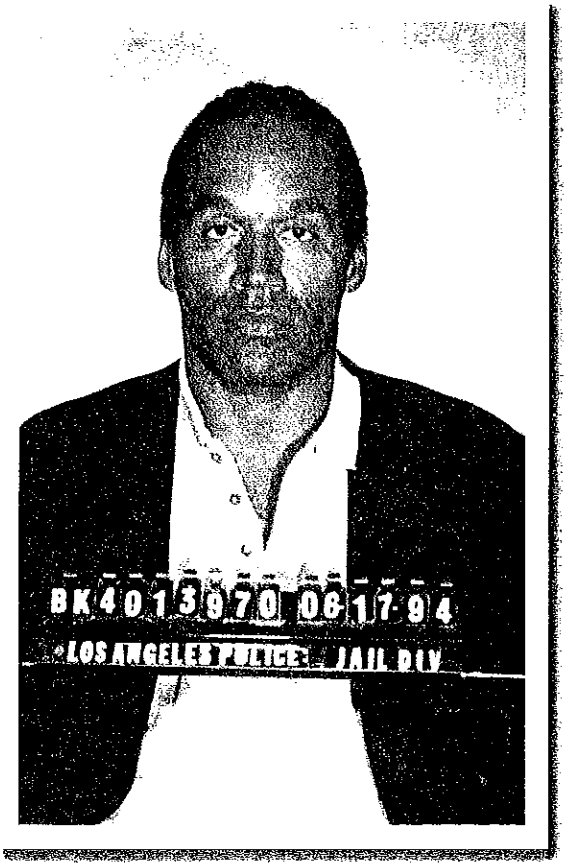

Earth/Space Science

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

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
touch DNA
Y-STRs

Understanding DNA

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

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

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

This discovery caught the imagination of the forensic science community, because forensic scientists have long searched for ways to definitively link biological evidence such as blood, semen, hair, and 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. DNA typing has allowed forensic scientists to accomplish this goal. Although the technique is still relatively new, DNA typing has become routine in public crime laboratories. It also 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.

chromosomes
A threadlike structure in the cell nucleus composed of DNA, along which the genes are located

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 body cells to make proteins that determine everything including hair color and susceptibility to diseases. Each gene is composed of DNA designed to carry out a single body function.

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. However, the first 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 (see Figure 9–1). It turns out that DNA is an extraordinary molecule skillfully designed to control the genetic traits of all living cells, plant and animal.

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

The Structure of DNA Before examining the implications of Watson and Crick's discovery, let's see how DNA is constructed. DNA is a **polymer**. A polymer is a

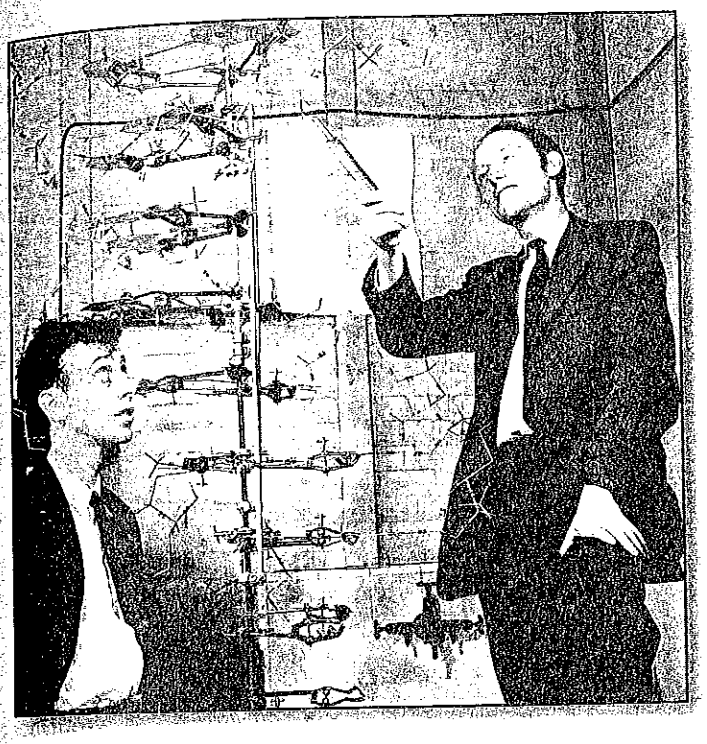


FIGURE 9–1
The discoverers of the structure of DNA. James Watson at left and Francis Crick, seen with their model of part of a DNA molecule.
Courtesy Photo Researchers Inc.

very large molecule made by linking a series of repeating units, or monomers. In this case, the units are known as **nucleotides**.

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

Nucleotides. A nucleotide is composed of a sugar molecule, a phosphorus atom surrounded by four oxygen atoms, and a nitrogen-containing molecule called a base. Figure 9–2 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.

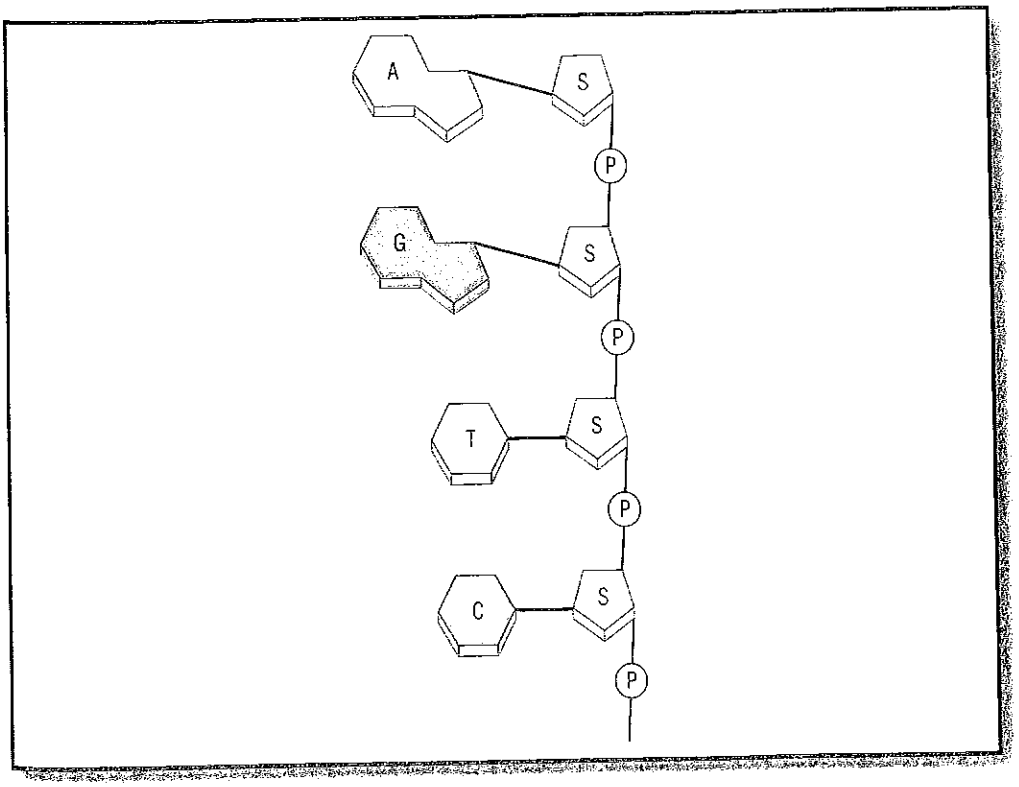


FIGURE 9–2
How nucleotides can be linked to form a DNA strand. S designates the sugar component, which is joined with phosphate groups (P) to form the backbone of DNA. Projecting from the backbone are four bases: A, adenine; G, guanine; T, thymine; and C, cytosine.

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 for cytosine, G for guanine, and T for thymine.

Again, notice in Figure 9–2 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; a DNA strand can be composed of a long chain with millions of bases. This information was well known to Watson and Crick by the time they set about to detail the structure of DNA. Their efforts led them to discover that the DNA molecule is composed of two DNA strands coiled into a double helix. This can be thought of as resembling two wires twisted around each other.

As Watson and Crick 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 9–3).

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

Complementary Base Pairing. The only arrangement possible in the double-helix configuration is 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 sequenced on a DNA strand. Thus, one can observe the sequences T–A–T–T or G–T–A–A or G–T–C–A. When these sequences are joined with their complements in a double-helix configuration, they pair as follows:

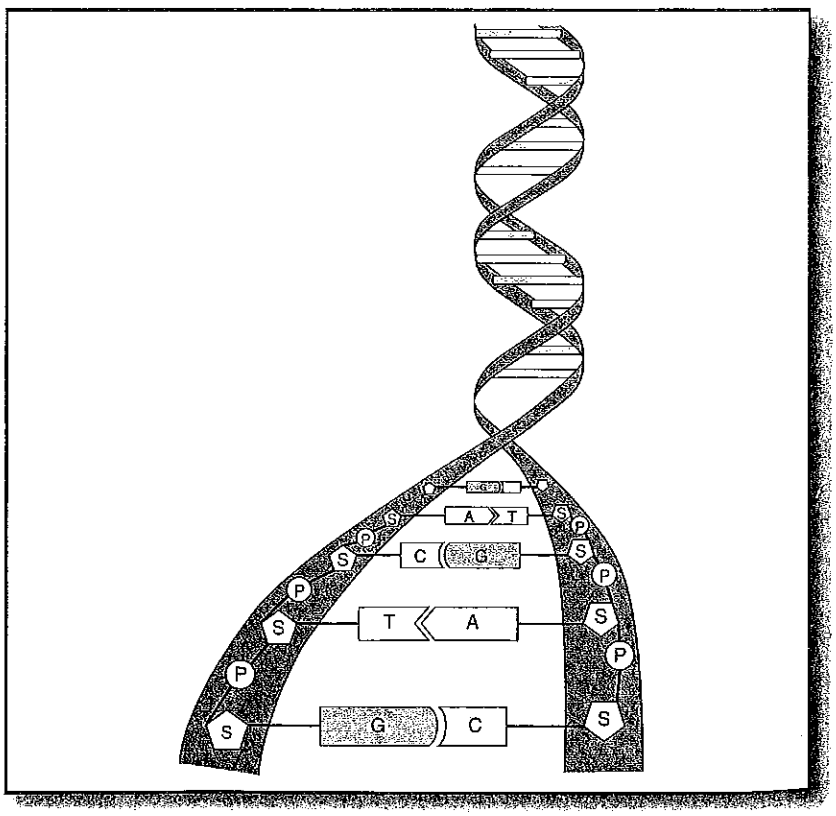


FIGURE 9–3
A representation of a DNA double helix. Notice how bases G and C pair with each other, as do bases A and T. This is the only arrangement in which two DNA strands can align with each other in a double-helix configuration. *Courtesy of Photo Researchers, Inc.*

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

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What Is DNA?
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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 made by linking a combination of **amino acids.** Although thousands of proteins exist, they can all be derived from a combination of up to 20 known amino acids. The sequence of amino acids in a protein chain determines the shape and function of the protein.

proteins
Polymers of amino acids that play basic roles in the structure and function of living things

amino acids
The building blocks of proteins

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 9–4(a). Studies of individuals who have 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 9–4(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 (see Figure 9–5).

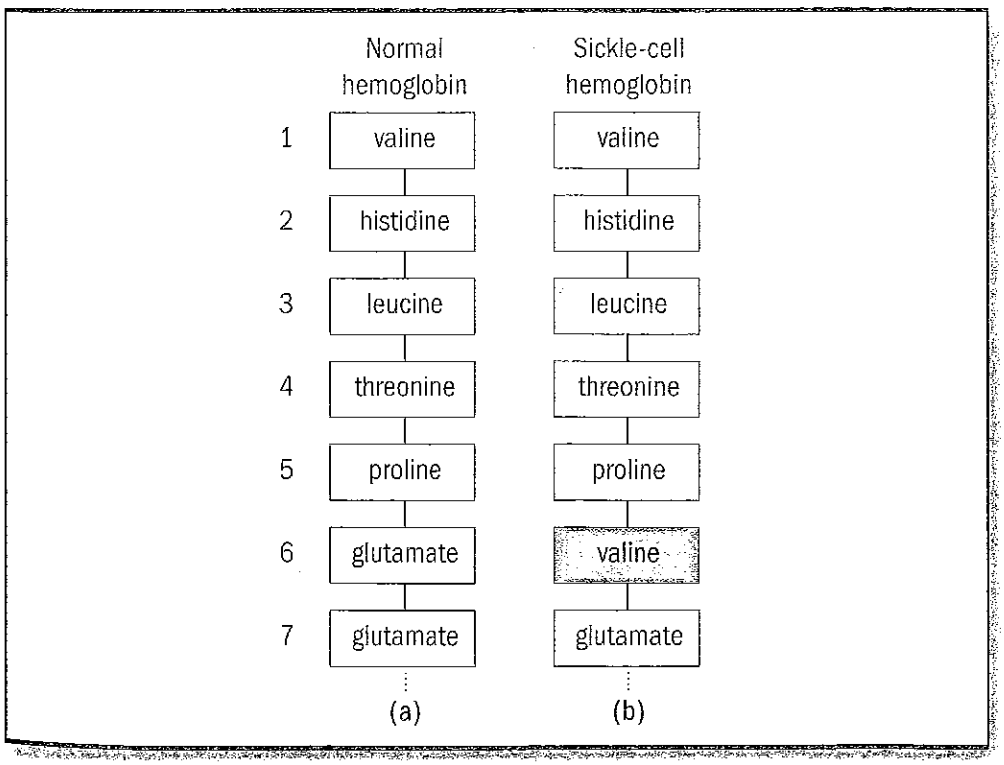


FIGURE 9–4 (a) A string of amino acids composes one of the protein chains of hemoglobin. (b) Substitution of just one amino acid for another in the protein chain results in sickle-cell hemoglobin.

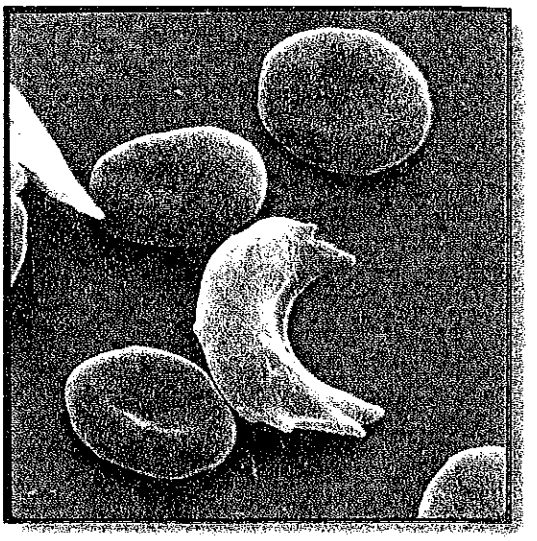


FIGURE 9–5
Sickle cell anemia is an inherited anomaly in which the red blood cells contain an abnormal form of hemoglobin. Decreased oxygen supply causes normal red blood cells to change their form into sickles. *Courtesy Photo Researchers, Inc.*

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 in this segment translates into

[C–G–T] – [C–T–A] – [A–A–A] – [C–G–T]

or the protein chain

alanine — aspartate — phenylalanine — alanine

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

If we look at the difference between "normal" and sickle-cell hemoglobin (see Figure 9–4), we see that the latter is formed by substituting one amino acid (valine) for another (glutamate). Within the DNA segment that codes for the production of normal hemoglobin, the letter sequence is

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

Individuals with sickle-cell disease carry the sequence

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

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

As scientists unravel the base sequences of DNA, they obtain a greater appreciation for the roles of proteins 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 13-year project to determine the order of bases on all 23 pairs of human chromosomes (also called the **human genome**) is now complete. Knowing the location on a specific chromosome at which DNA codes for production of a particular protein is useful for diagnosing and treating genetic diseases. This information is also crucial for understanding the underlying causes of cancer. Also, comparing the human genome with that of other organisms will help us understand the role and implications of evolution.

human genome
The order of bases on all 23 pairs of human chromosomes

Quick Review

- The gene is the fundamental unit of heredity. Each gene is 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 of DNA are aligned in a double-helix configuration so that adenine pairs with thymine and guanine pairs with cytosine. This concept is known as complementary base pairing.
- The order in which the base pairs are arranged defines the role and function of a DNA molecule.

Replication of DNA

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

DNA **replication**—the synthesis of new DNA from existing DNA—begins with the unwinding of the DNA strands in the double helix. Each strand is then exposed to a collection of free nucleotides. Letter by letter, the double helix is re-created as the nucleotides are assembled in the proper order, as dictated by the principle of base pairing (*A* with *T* and *G* with *C*). The result is the emergence of two identical copies of DNA where before there was only one (see Figure 9–6). A cell can now pass on its genetic identity when it divides.

replication
The synthesis of new DNA from existing DNA

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

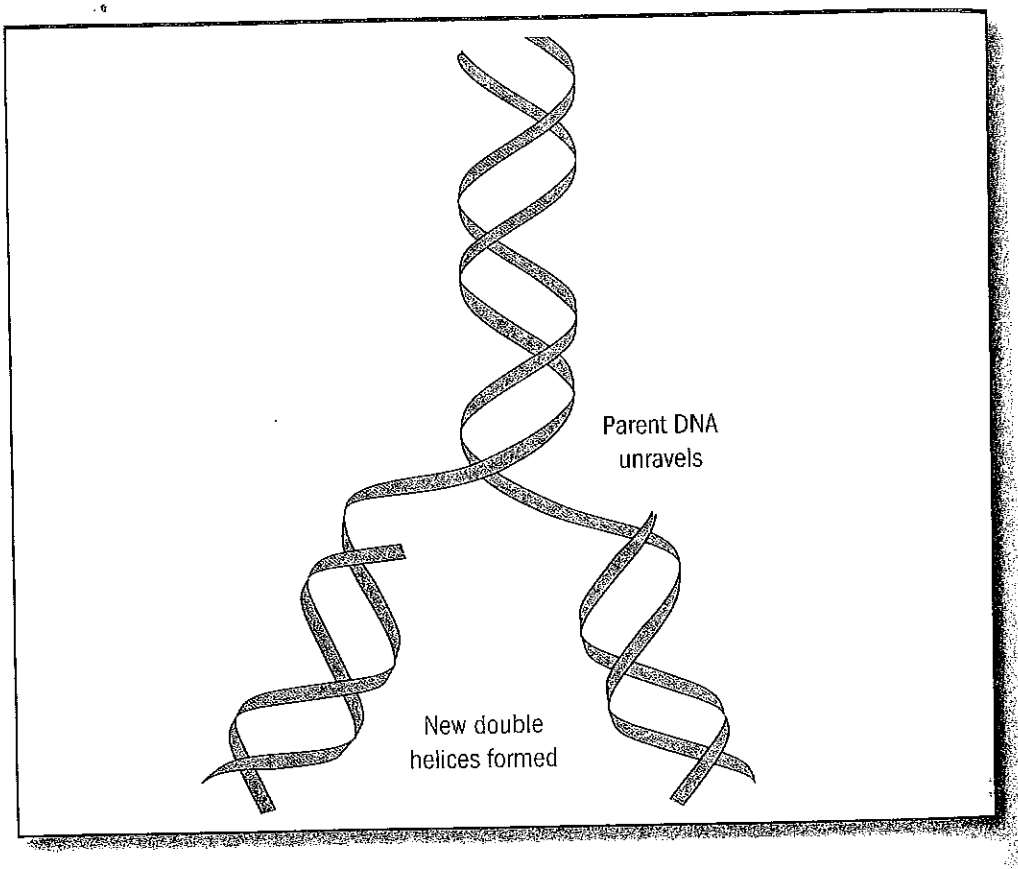


FIGURE 9–6
Replication of DNA. The strands of the original DNA molecule are separated, and two new strands are assembled.

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

polymerase chain reaction (PCR)
A technique for replicating or copying a portion of a DNA strand outside a living cell

In PCR, small quantities of DNA or broken pieces of DNA found in crime-scene evidence can be copied with the aid of a DNA polymerase. The copying process can be accomplished in an automated fashion using a DNA Thermal Cycler (see Figure 9–7). Each cycle of the PCR technique results in a doubling of the DNA. Within a few hours, 30 cycles can multiply DNA a billionfold. Once DNA copies are in hand, they can be analyzed by any of the methods of modern molecular biology. The ability to multiply small bits of DNA opens new and exciting avenues for forensic scientists to explore. It means that sample size is no longer a limitation in characterizing DNA recovered from crime-scene evidence.

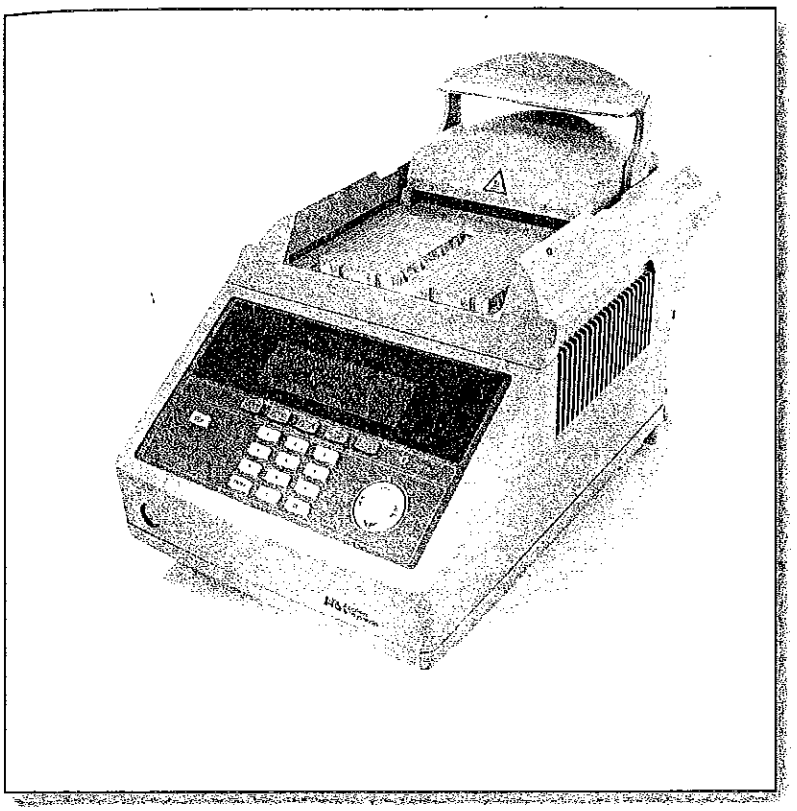


FIGURE 9–7
The DNA Thermal Cycler, an instrument that automates the rapid and precise temperature changes required to copy a DNA strand. Within a matter of hours, DNA can be multiplied a billionfold. *Courtesy Applied Biosystems, Foster City, Calif.*

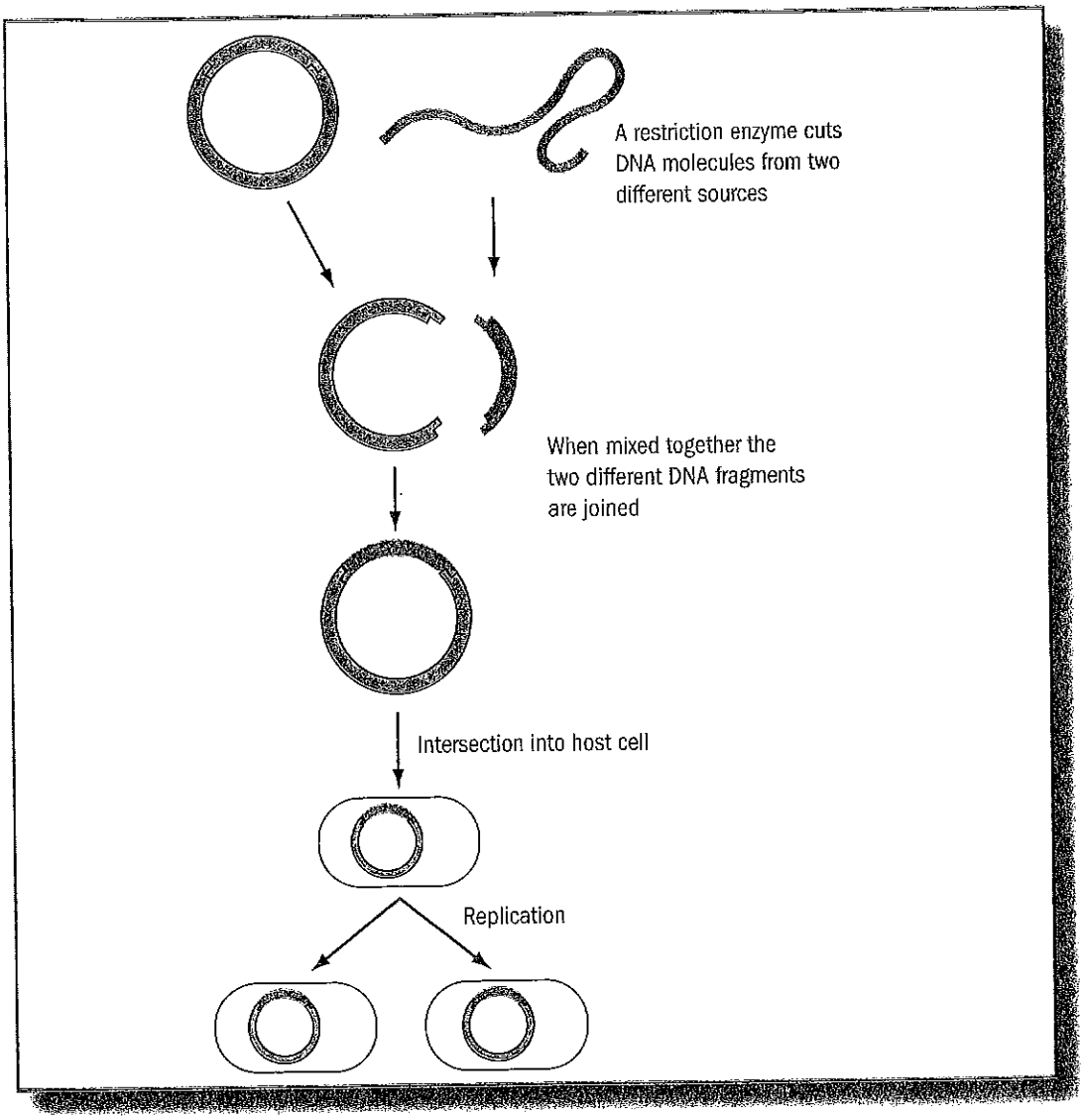
Quick Review

- DNA replication begins with the unwinding of the DNA strands in the double helix. The double helix is re-created as the nucleotides are assembled in the proper order (A with T and G with C). Two identical copies of DNA emerge from the process.
- PCR (polymerase chain reaction) is a technique for replicating or copying a portion of a DNA strand outside a living cell.
- Recombinant DNA is a process by which the DNA of an organism is altered by inserting into it fragments of DNA from another organism (see Inside the Science on the following pages).

Inside the Science

Recombinant 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.



Inside the Science (CONTINUED)

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 they recognize 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). 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. 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 with Tandem Repeats

Geneticists have discovered that portions of the DNA molecule contain sequences of letters that are repeated numerous times. In fact, more than 30 percent of the human genome is composed of repeating segments of DNA. These repeating sequences, or **tandem repeats**, seem to act as filler or spacers between the coding regions of DNA. Although these repeating segments do not seem to affect our outward appearance or control any other basic genetic function, they are nevertheless part of our genetic makeup, inherited from our parents in the manner illustrated by the Punnett square (page 301). 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.

tandem repeat
Region of a chromosome that contains multiple copies of a core DNA sequence arranged in a repeating fashion

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 9–8). All humans have the same type of repeats, but there is tremendous variation in the number of repeats that each of us has.

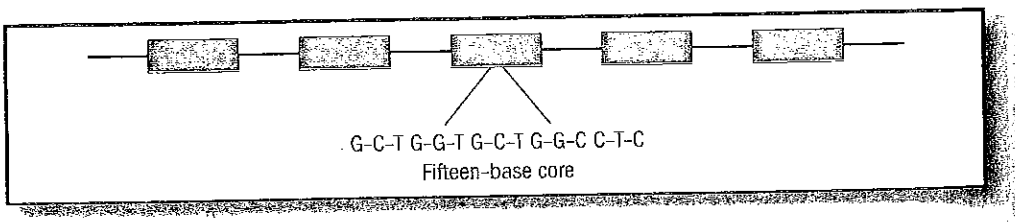


FIGURE 9–8
A DNA segment consisting of a series of repeating DNA units. In this illustration, the fifteen-base core can repeat itself hundreds of times. The entire DNA segment is typically hundreds to thousands of bases long.

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. The forensic science community selected a number of different RFLPs for performing DNA typing. Typically a core sequence is 15 to 35 bases long and repeats itself up to a thousand times.

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

restriction enzyme
Chemical that acts as scissors to cut DNA molecules at specific locations

Let's examine some DNA strands with regions of repeating base sequences to see how this process works. Figure 9–9 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 with a sequence of *T–A–G*.

Note an important distinction between the two chromosomes: the chromosome on the left has three repeating sequences of *T–A–G*, whereas 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

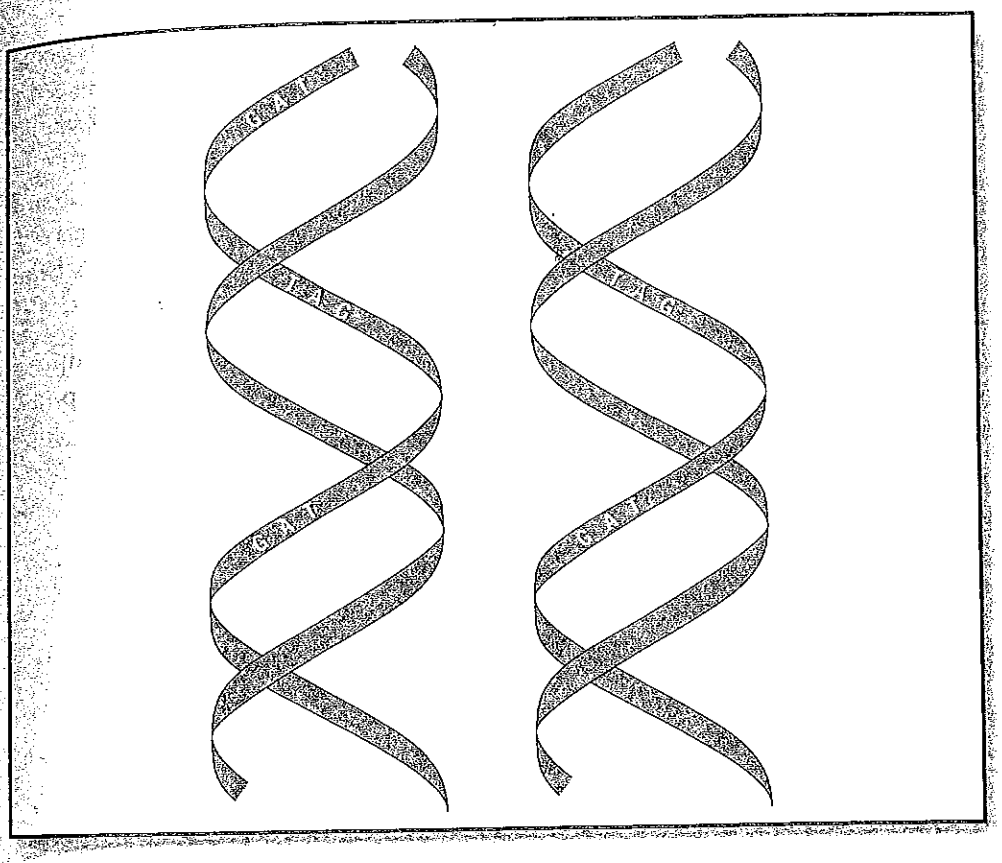


FIGURE 9–9
Interwined strands of DNA representing segments of two chromosomes. Note that the chromosome segment on the left contains three repeating sequences of *T-A-G*, while the chromosome segment on the right has two repeating sequences of *T-A-G*.

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 9–9, the chromosome pair, when cut, will yield two different fragment lengths of *T–A–G*.

Electrophoresis 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**.

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

During the electrophoretic process, DNA from various sources, cut up by restriction enzymes, is placed on a plate coated with a gel medium. When the gel is subjected to an electric potential, the DNA fragments migrate across the plate. Because smaller DNA fragments move faster along the plate than do larger fragments, the process separates the fragments according to size.

Hybridization 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**).

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

DNA Typing with RFLP 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.)

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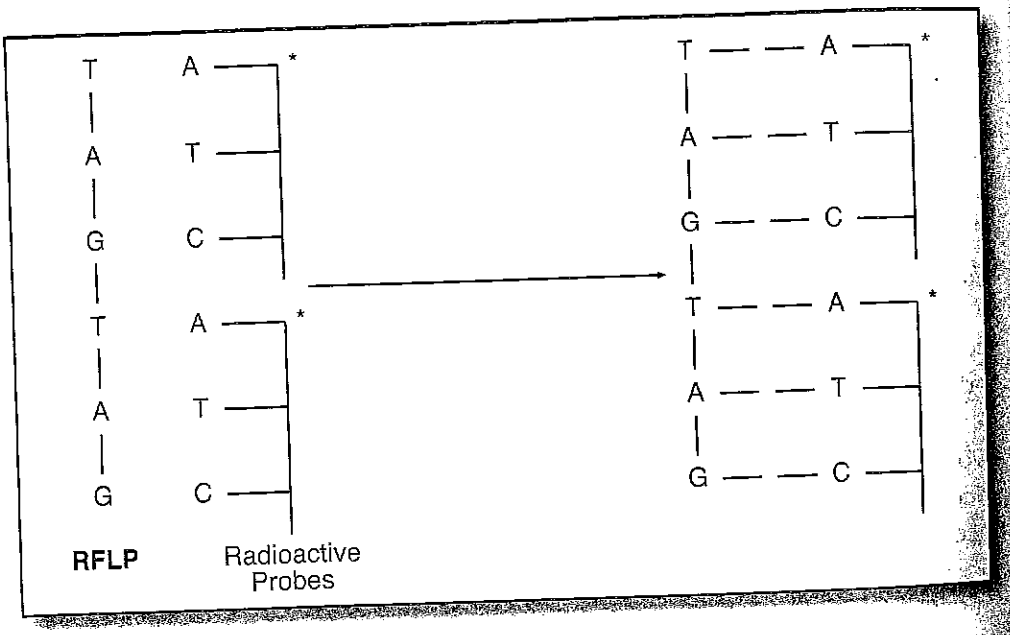


Next, the nylon sheet is placed against X-ray film and exposed for several days. The radioactive decay products strike the film. When the film is processed, bands appear where the radioactive probes stuck to the fragments on the nylon sheet. The length of each fragment is determined by running known DNA fragment lengths alongside the test specimens and comparing the distances they migrated across the plate. The entire DNA-typing process is depicted in Figure 9–10.

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 9–11, 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. However,

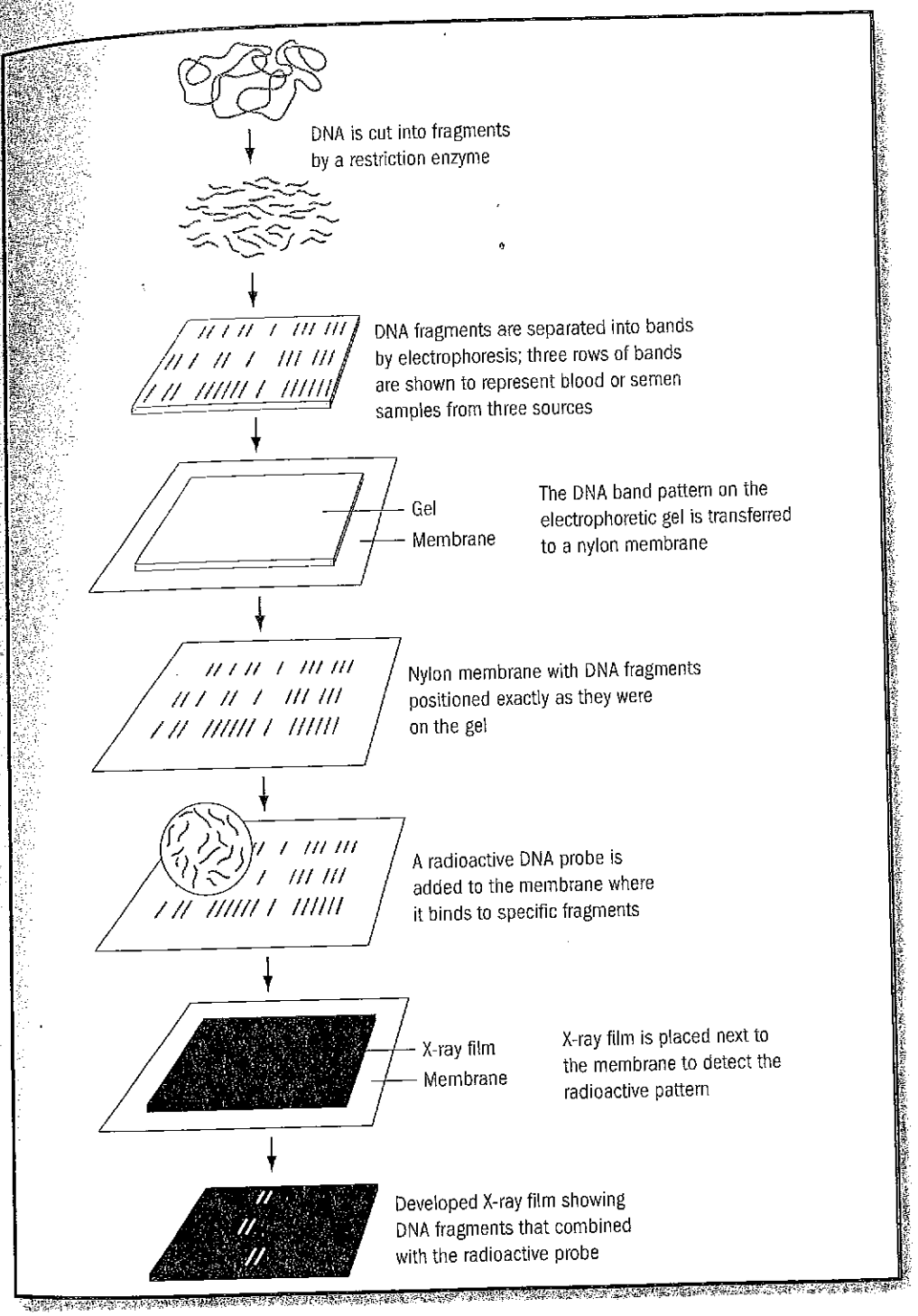


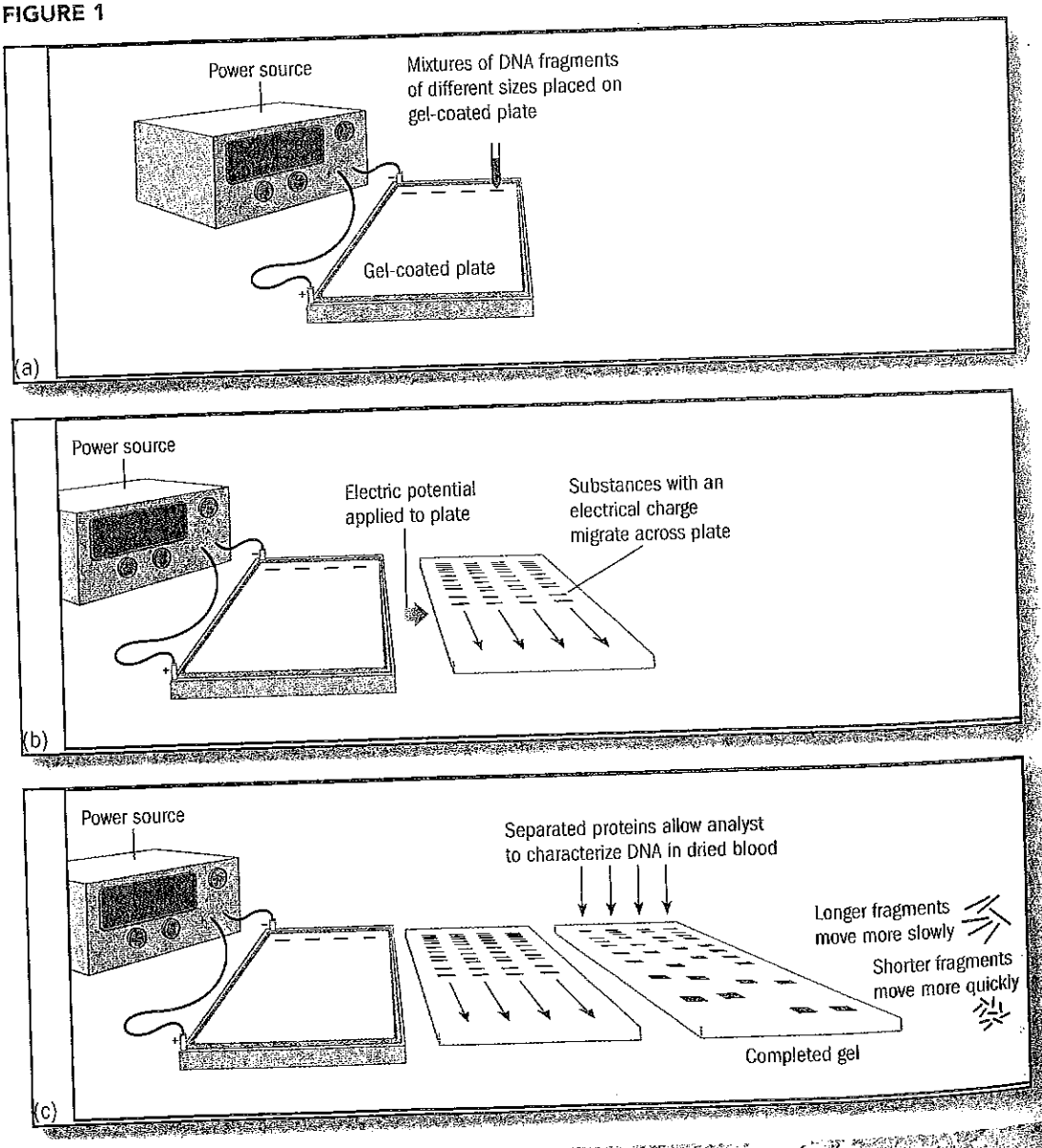
FIGURE 9–10
The DNA RFLP typing process.

Inside the Science

Electrophoresis

Electrophoresis is somewhat related to thin-layer chromatography (discussed in Chapter 5) in that it separates materials according to their migration rates on a stationary solid phase. However, electrophoresis does not use a moving liquid phase to move the material; instead, an electrical potential is placed across the stationary medium.

FIGURE 1



Inside the Science (CONTINUED)

The nature of the medium can vary; most forensic applications call for a starch or agar gel coated onto a glass plate. Under these conditions, only substances that possess an electrical charge migrate across the stationary phase (see Figure 1). Because many substances in blood carry an electrical charge, they can be separated and identified by electrophoresis. The technique is particularly useful for separating and identifying complex biochemical mixtures. In forensic science, electrophoresis is most useful for characterizing proteins and DNA in dried blood (see Figure 2).

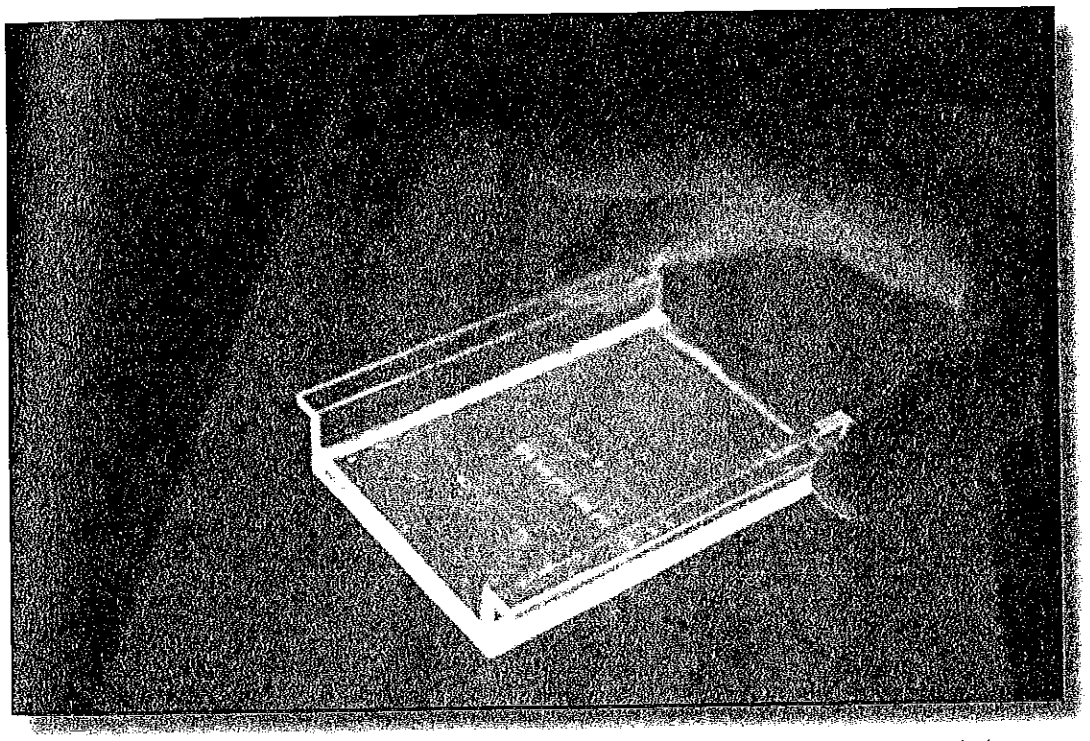


FIGURE 2 DNA fragments separated by gel electrophoresis are visualized under a UV light. *Courtesy Cytographics, Visuals Unlimited*

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

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 a hundred in a population, then four different probes would have a combined frequency of one in 100 million (1/100 × 1/100 × 1/100 × 1/100).

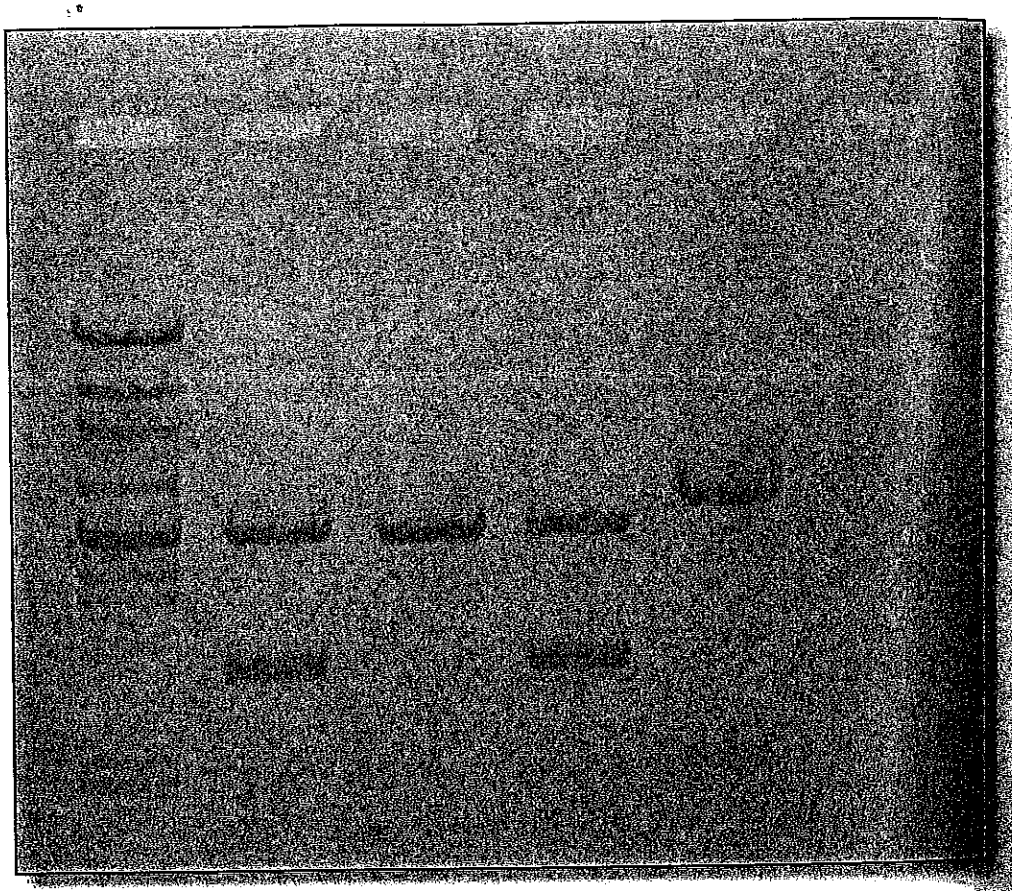
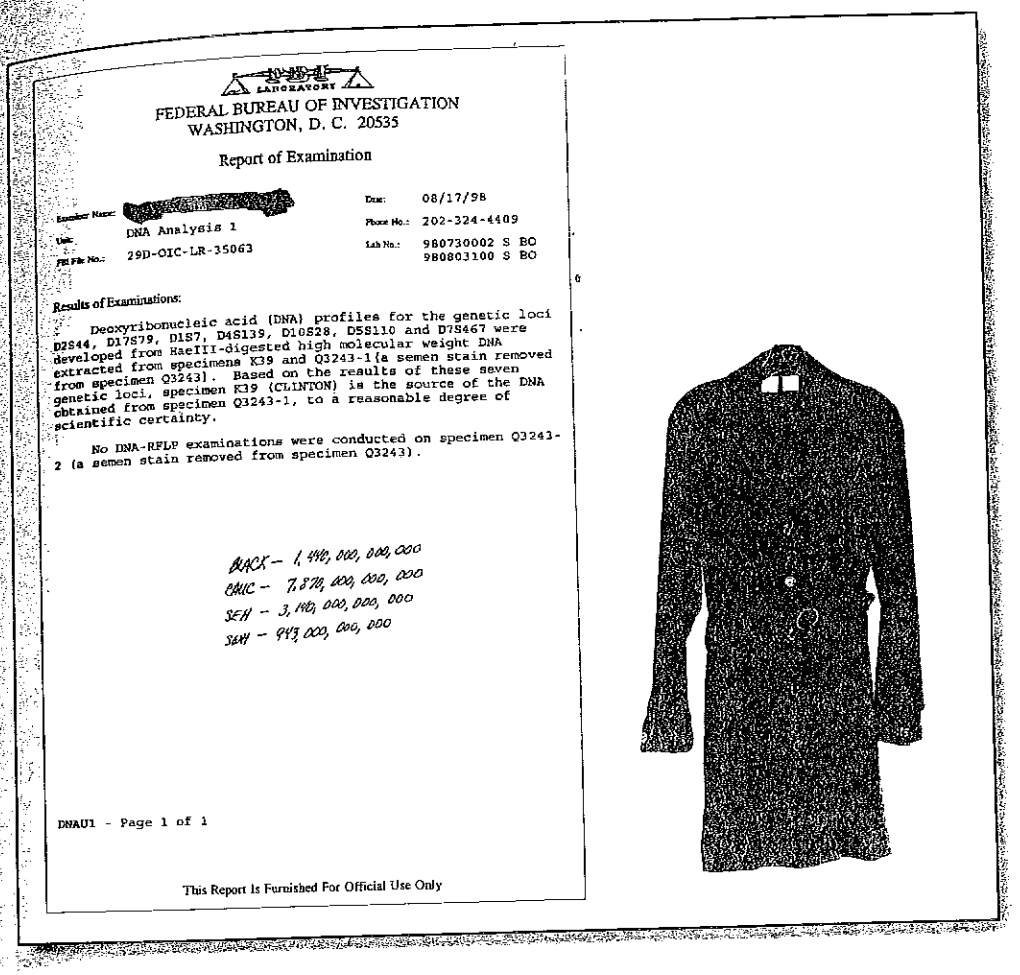


FIGURE 9–11
A DNA profile pattern of a suspect and its match to crime-scene DNA. From left to right, lane 1 is a DNA standard marker; lane 2 is the crime-scene DNA; and lanes 3 to 5 are control samples from suspects 1, 2, and 3, respectively. Crime-scene DNA matches suspect 2. *Courtesy Edvotek, The Biotechnology Education Company, www.edvotek.com*

RFLP DNA typing has the distinction of being the first scientifically accepted protocol in the United States used for forensic characterization of DNA. However, its utility has been short lived. New technology incorporating polymerase chain reaction (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 9–12.



LABORATORY
FEDERAL BUREAU OF INVESTIGATION
WASHINGTON, D. C. 20535

Report of Examination

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

Results of Examinations:

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

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

BLACK – 1,440,000,000,000
CAUC – 7,870,000,000,000
SEH – 3,140,000,000,000
SEW – 943,000,000,000

DNAU1 - Page 1 of 1

This Report Is Furnished For Official Use Only

FIGURE 9–12
The dress and the FBI Report of Examination for a semen stain located on the dress.

Quick Review

- Portions of the DNA molecule contain sequences of bases that are repeated numerous times. These tandem repeats offer a means of distinguishing one individual from another through DNA typing.
- Length differences associated with relatively long repeating DNA strands—called restriction fragment length polymorphisms (RFLPs)—formed the basis for one of the first DNA-typing procedures.
- In the laboratory, DNA molecules are cut up by a restriction enzyme, and the resulting fragments are sorted out by electrophoresis.
- Materials undergoing electrophoresis are forced to move across a gel-coated plate under the influence of an electrical potential. Substances such as DNA can be separated and characterized using electrophoresis.
- A typical DNA fragment pattern shows two bands (one RFLP from each chromosome).

Polymerase Chain Reaction (PCR)

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

–G–T–C–T–C–A–G–C–T–T–**C–C–A–G**–

–**C–A–G–A**–G–T–C–G–A–A–G–G–T–C–

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

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

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

–G–T–C–T–C–A–G–C–T–T–C–C–A–G–

–C–A–G–A–G–T–C–G–A–A–G–G–T–C–

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

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

The third step is to add the DNA polymerase and a mixture of free nucleotides (A, C, G, T) to the separated strands (see Figure 9–13). When the test tube is heated to 72°C, the polymerase enzyme directs the rebuilding of a double-stranded DNA molecule, extending the primers by adding the appropriate bases, one at a time, resulting in the production of two complete pairs of double-stranded DNA segments:

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

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

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

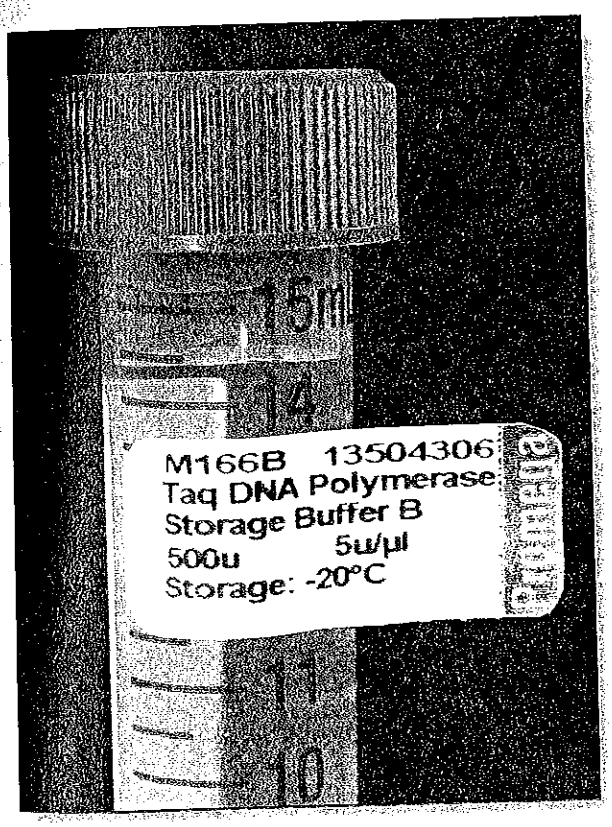


FIGURE 9-13
Vial of DNA Polymerase used in PCR for replication of DNA. *Courtesy Beth Plowes - Proteapix*

MyCrimeKit: WebExtra 9.3
View a Polymerase Chain Reaction
www.mycrimekit.com

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 a third 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.

Quick Review

- Polymerase chain reaction (PCR) can amplify minute quantities of DNA. The technique evolved from an understanding of how DNA strands naturally replicate within a cell.
- PCR technology cannot be applied to RFLP DNA typing because 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.
- Long RFLP strands tend to break apart under the adverse conditions at many crime scenes. The shorter DNA strands used in PCR are more stable and less subject to degradation caused by adverse environmental conditions.

Short Tandem Repeats (STRs)

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

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 are often recovered from bodies or stains that have been subject to extreme decomposition. Also, because

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 required—1/50 to 1/100 the amount 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 5 to 11 repeats of *A–A–T–G*. Figure 9–14 illustrates two such TH01 variants, one containing 6 repeats and the other containing 8 repeats of *A–A–T–G*.

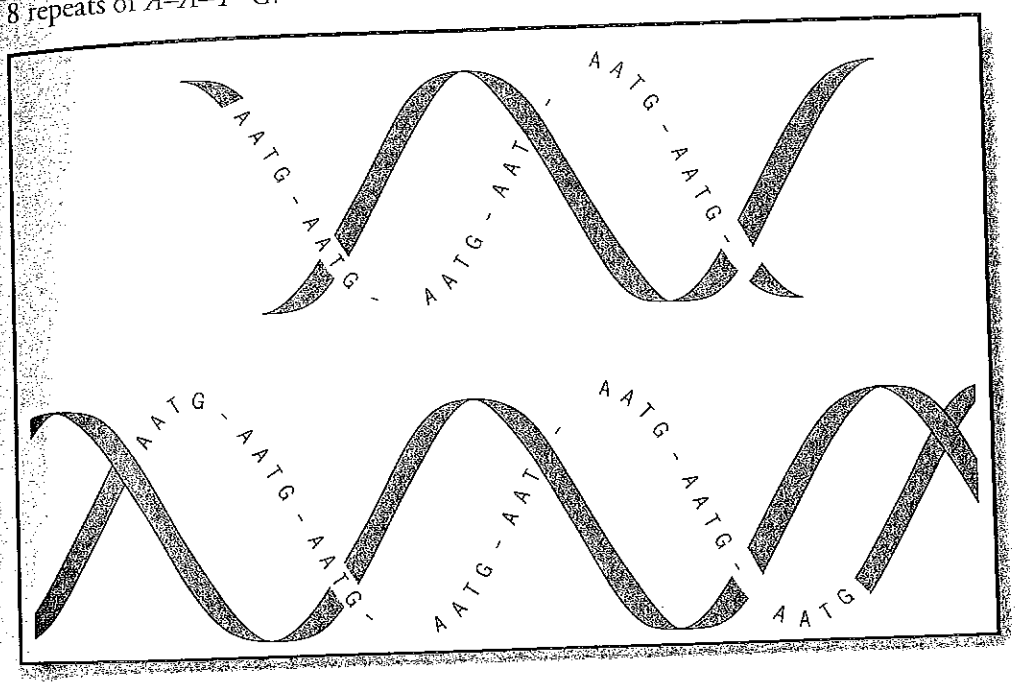


FIGURE 9–14 Variants of the short tandem repeat TH01. The upper DNA strand contains six repeats of the sequence A-A-T-G; the lower DNA strand contains eight repeats of the sequence A-A-T-G. This DNA type is known as TH01 6, 8.

During a forensic examination, TH01 is extracted from biological materials and amplified by PCR as described earlier. The ability to copy an STR means that extremely small amounts of the molecule can be detected and analyzed. Once the STRs have been copied or amplified, they are separated by electrophoresis. 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 the percentage of the population from which these STRs can emanate. This gives rise to the concept of **multiplexing**. Using PCR technology, one can simultaneously extract and amplify a combination of different STRs.

One STR system on the commercial market is the STR Blue Kit.[2] This kit provides the necessary materials for amplifying and detecting three STRs (a process

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

called *triplexing*)—D3S1358, vWA, and FGA. The design of the system ensures that the size of the STRs does not overlap, thereby allowing each marker to be viewed clearly on an electrophoretic gel, as shown in Figure 9–15. In the United States, the forensic science community has standardized on 13 STRs for entry into a national database known as the Combined DNA Index System (CODIS).

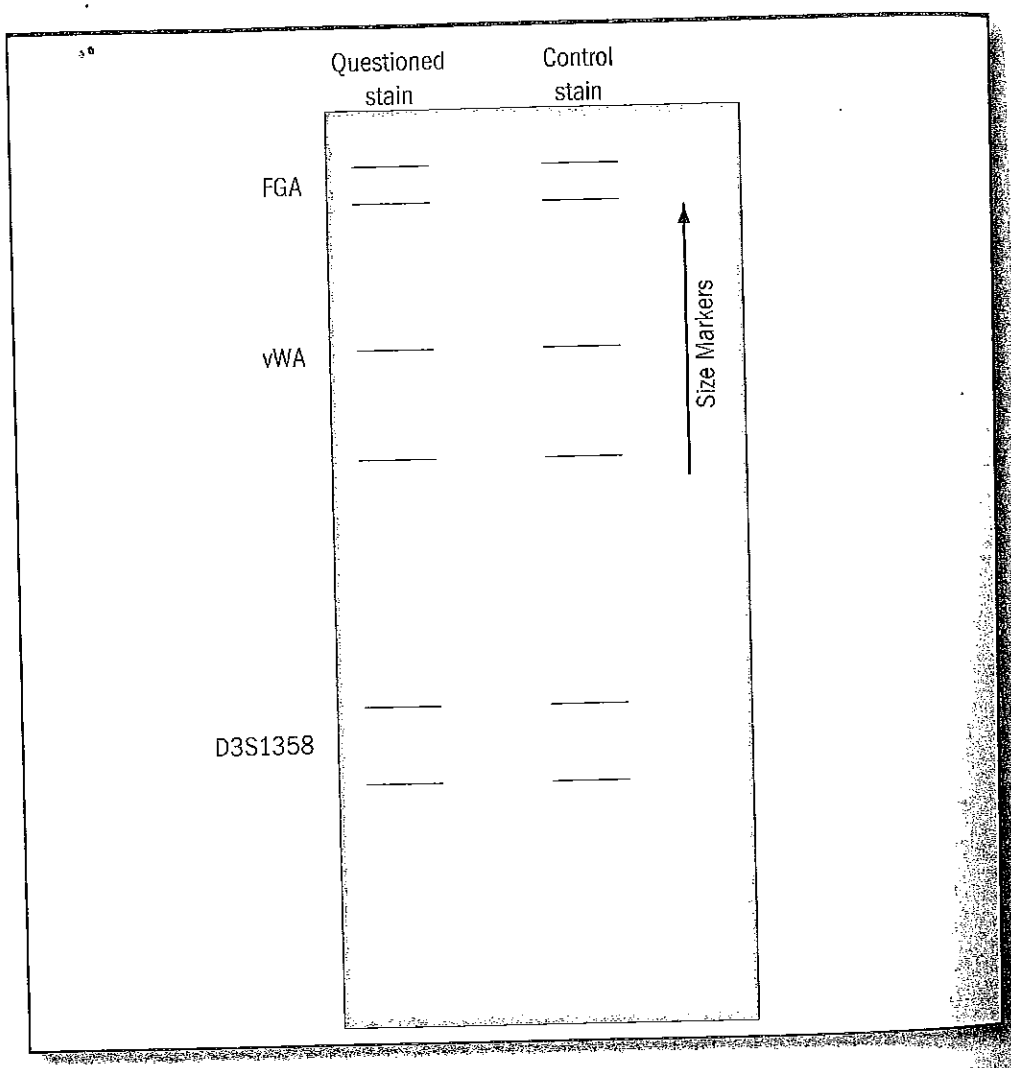


FIGURE 9-15
Triplex system containing three loci: FGA, vWA, and D3S1358, indicating a match between the questioned and the standard/reference stains.

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 9–16 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 15 different STRs (see Figure 9–17).

MyCrimeKit: WebExtra 9.4
See the 13 CODIS STRs and Their Chromosomal Positions
www.mycrimekit.com

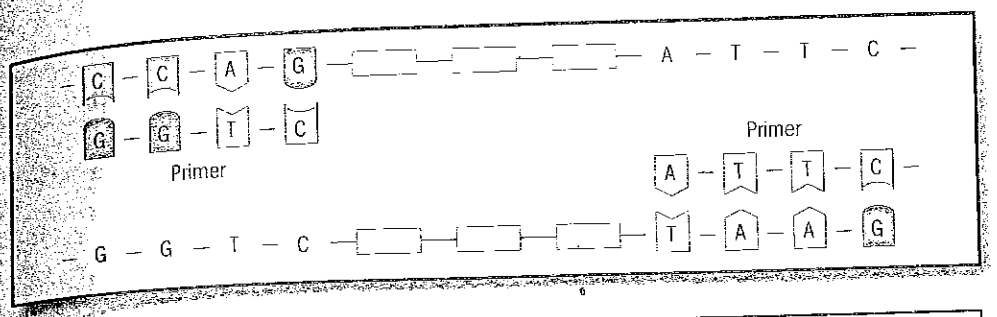


FIGURE 9–16
Appropriate primers flanking the repeat units of a DNA segment must be selected and put in place to initiate the PCR process.

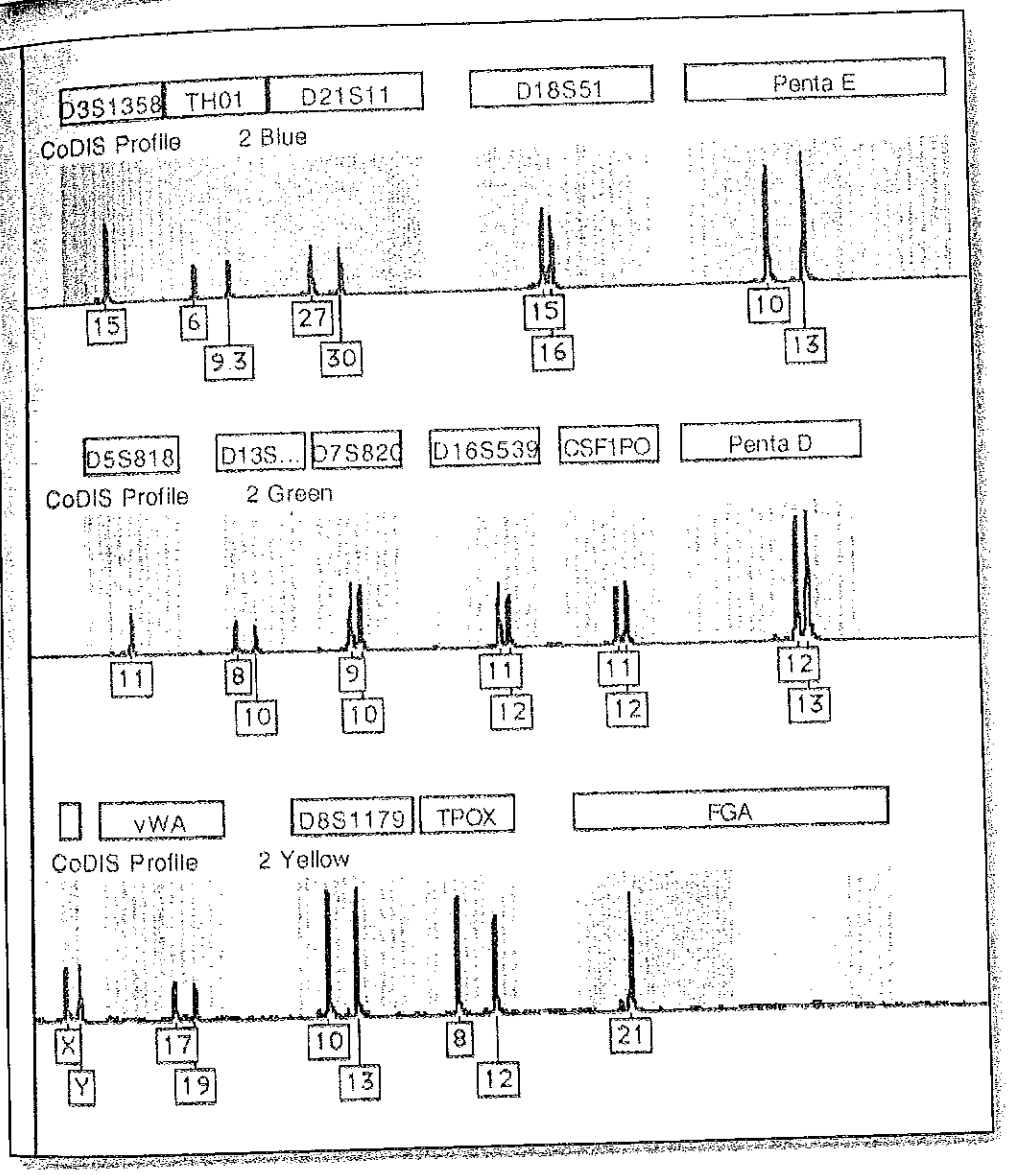


FIGURE 9–17
STR profile for 15 loci. *Courtesy H. Edward Grotjan, Ph.D.*

The 13 CODIS STRs are listed in Table 9–1 along with their probabilities of identity. The probability of identity is a measure of the likelihood that two individuals selected at random will have an identical STR type. The smaller the value of this probability, the more discriminating the STR. A high degree of discrimination and even individualization can be attained by analyzing a combination of STRs (multiplexing). Because STRs occur independently of each other, the

probability of biological evidence having a particular combination of STR types is determined by the product of their frequency of occurrence in a population. This combination is referred to as the *product rule*. Hence, the greater the number of STRs characterized, the smaller the frequency of occurrence of the analyzed sample in the general population.

The combination of the first three STRs shown in Table 9–1 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 13 STRs shown in Table 9–1 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, several commercially available kits allow forensic scientists to profile STRs in the kinds of combinations cited here.

Table 9–1
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

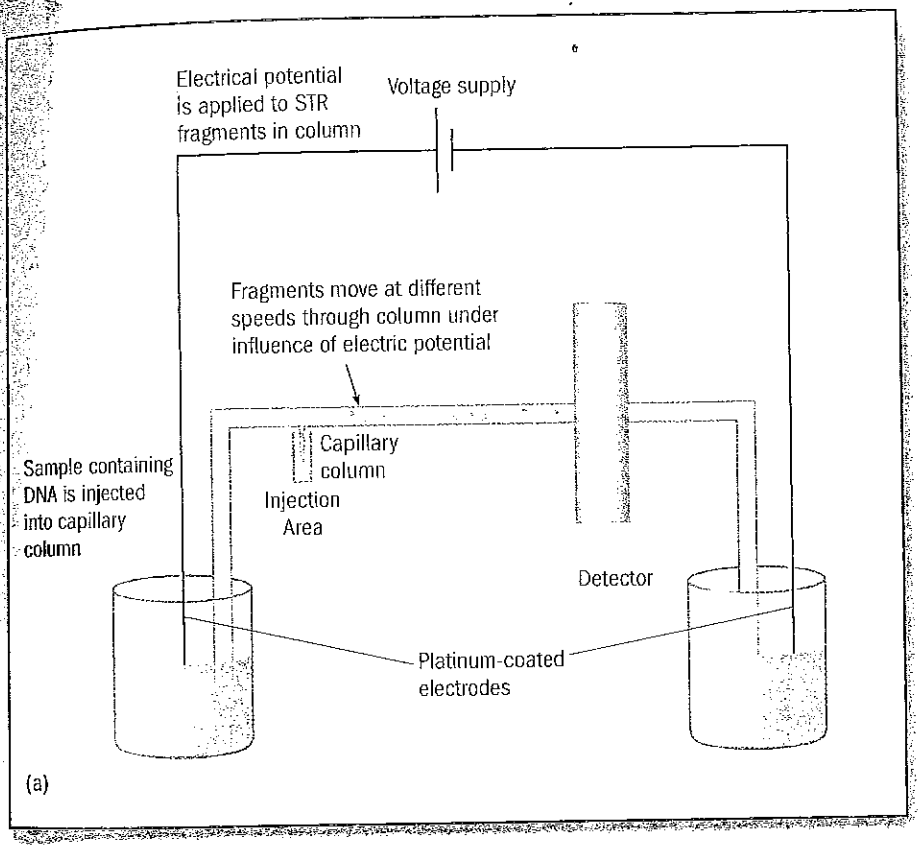
Source: The Future of Forensic DNA Testing: Predictions of the Research and Development Working Group. Washington, D.C.: National Institute of Justice, Department of Justice, 2000, p. 41.

MyCrimeKit: WebExtra 9.5
Calculate the Frequency of Occurrence of a DNA Profile
www.mycrimekit.com

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

As illustrated in Figure 9–18, 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.



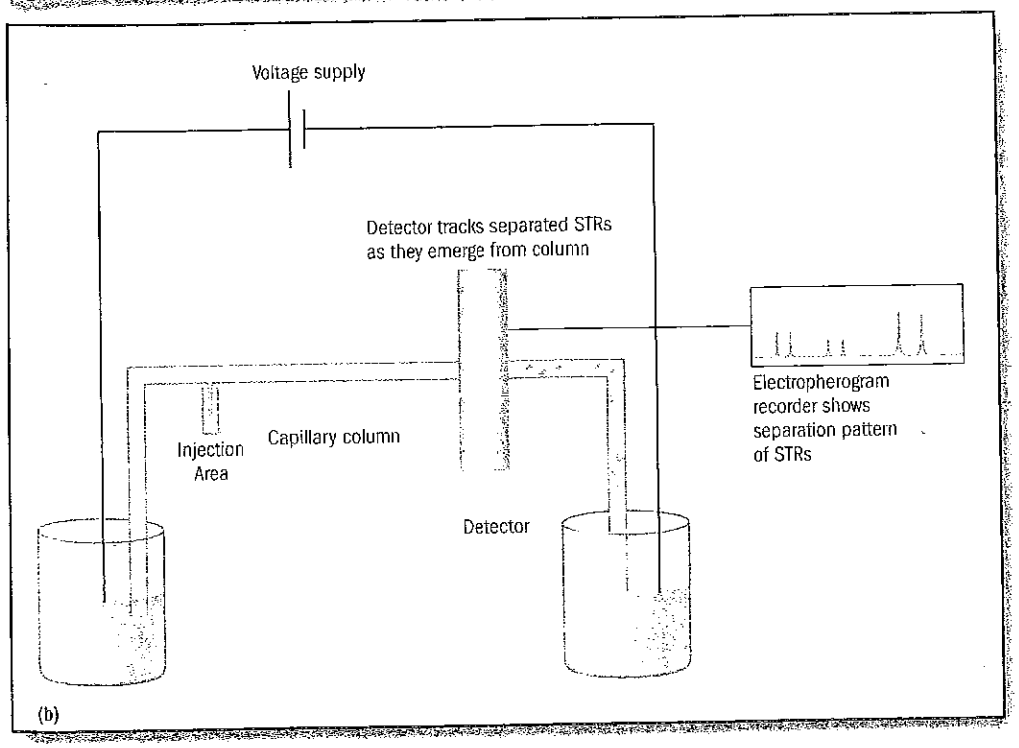


FIGURE 9–18
Capillary electrophoresis technology has evolved from the traditional flat gel electrophoresis approach. The separation of DNA segments is carried out on the interior wall of a glass capillary tube coated with a gel polymer and kept at a constant voltage. The size of the DNA fragments determines the speed at which they move through the column. This figure illustrates the separation of three sets of STRs (triplexing).

amelogenin gene
A genetic locus useful for determining sex

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

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 20 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 for analyzing blood, saliva, or a vaginal swab that is a mix originating from more than one male. For example, Y-STRs prove useful when multiple males are involved in a sexual assault.

Keep in mind that STR types derived from the Y chromosome originate only from this single male chromosome. A female subject, or one with an XX chromosome pattern, does not contribute any DNA information. Also, unlike a conventional STR 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.

MyCrimeKit: WebExtra 9.6
Understand the Operational Principles of Capillary Electrophoresis
www.mycrimekit.com

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

MyCrimeKit: WebExtra 9.7
See the Electropherogram Record from One Individual's DNA
www.mycrimekit.com

MyCrimeKit: WebExtra 9.8
View an Animation Depicting Y-STRs
www.mycrimekit.com

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. The importance of DNA analyses in criminal

investigations has also placed added burdens on crime laboratories to improve their quality-assurance procedures and to ensure the correctness of their results. In several well-publicized instances, the accuracy of DNA tests conducted by government-funded laboratories has been called into question.

Quick Review

- 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.
- The entire strand of an STR is very short, less than 450 bases long. This makes STRs much less susceptible to degradation, and they are often recovered from bodies or stains that have been subjected to extreme decomposition.
- 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, in which the forensic scientist can simultaneously extract and amplify a combination of different STRs.
- With STR, as little as 125 picograms of DNA is required for analysis—1/100 the amount normally required for RFLP analysis.

Mitochondrial DNA

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

Mitochondria are cell structures found in all human cells (see Figure 9–19). 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.

mitochondria
Small structures outside the nucleus that supply energy to the cell

Forensic scientists rely on mtDNA to identify a subject when nuclear DNA is significantly degraded, such as in charred remains, or when nuclear DNA may be present in only very small quantities (such as in a hair shaft). Interestingly, when authorities cannot obtain a reference sample from an individual who may be long deceased or missing, an mtDNA reference sample can be obtained from any maternally related relative. However, all individuals of the same maternal lineage will be indistinguishable by mtDNA analysis.

Although mtDNA analysis is significantly more sensitive than nuclear DNA profiling, forensic analysis of mtDNA is more rigorous, time consuming, and

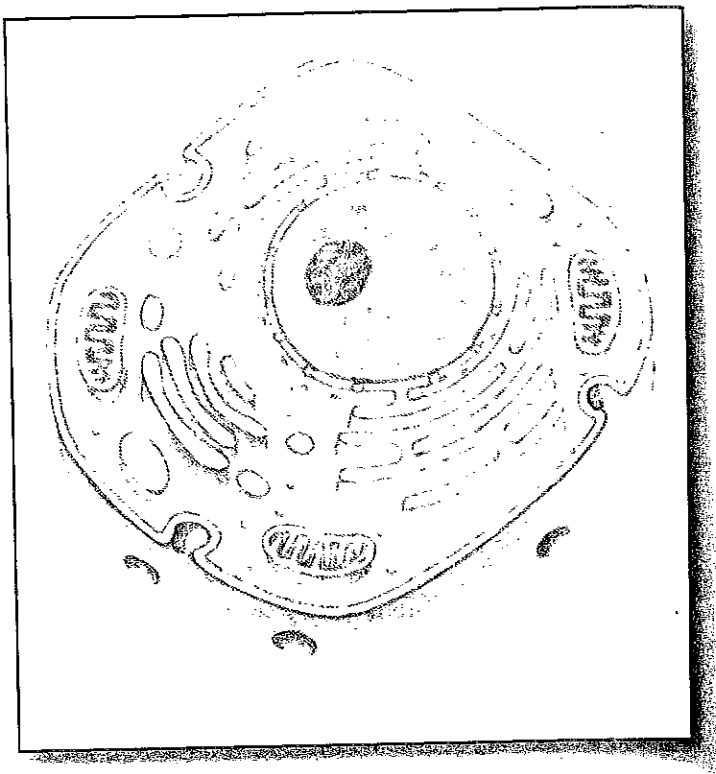


FIGURE 9–19
Illustration of a human cell containing a nucleus and cell structures involved in cell functions within the cell's cytoplasm. Mitochondria units in the shape of a loop make the energy for the cell. *Courtesy Dorling Kindersley Media Library*

costly than nuclear DNA profiling. For this reason, only a handful of public and private forensic laboratories receive evidence for mtDNA determination. The FBI Laboratory strictly limits the types 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, C, G,* and *T*). By contrast, mtDNA is constructed in a circular or loop configuration. Each loop contains enough *A, C, G,* and *T* (approximately 16,569 total nucleotides) to make up 37 genes involved in mitochondrial energy generation.

Two regions of mtDNA have been found to be highly variable in the human population. These two regions have been designated hypervariable region I (HV1) and hypervariable region II (HV2), as shown in Figure 9–20. 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–C–G* 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.

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

Once the sequences of the hypervariable regions from a case sample are obtained, most laboratories simply report the number of times these sequences appear in the mtDNA database maintained by the FBI. The mtDNA database contains about five thousand sequences. This approach permits an assessment of how common or rare an observed mtDNA sequence is in the database.

Interestingly, many of the sequences that have been determined in casework are unique to the existing database, and many types are present at frequencies of no greater than 1 percent in the database. Thus it is often possible to demonstrate

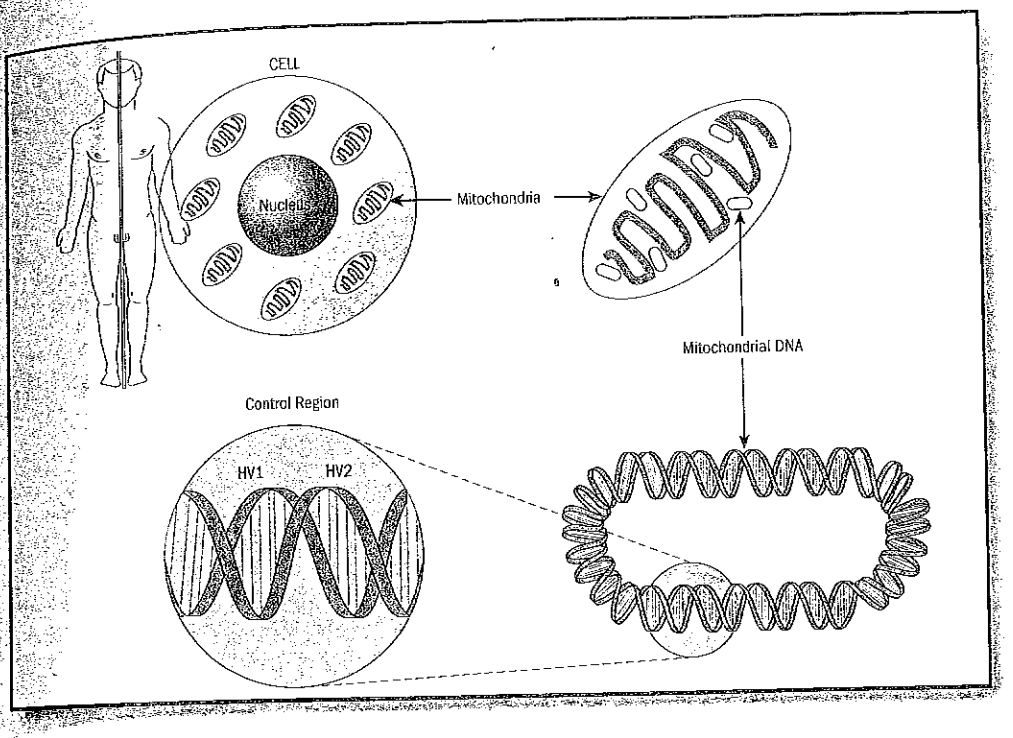


FIGURE 9–20 Every cell in the body contains hundreds of mitochondria, which provide energy to the cell. Each mitochondrion contains numerous copies of DNA shaped in the form of a loop. Distinctive differences between individuals in their mitochondrial DNA makeup are found in two specific seqments of the control region on the DNA loop known as HV1 and HV2.

how uncommon a particular mtDNA sequence is. However, even under the best circumstances, mtDNA typing does not approach STR analysis in its discrimination power. Thus, mtDNA analysis is best reserved for samples for which nuclear DNA typing is simply not possible.

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 reference 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 mtDNA analysis, and the results were compared 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.

MyCrimeKit: WebExtra 9.9
See How We Inherit Our Mitochondrial DNA
www.mycrimekit.com

MyCrimeKit: WebExtra 9.10
Look into the Structure of Mitochondrial DNA and See How It's Used for DNA Typing
www.mycrimekit.com

Quick Review

- Mitochondrial DNA is located outside the cell's nucleus and is inherited from the mother.
- Two regions of mitochondrial DNA, HV1 and HV2, are sequenced for forensic typing purposes.
- 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.

The Combined DNA Index System (CODIS)

Perhaps the most significant investigative tool to arise from a DNA-typing program is CODIS (Combined DNA Index System), a computer software program developed by the FBI that maintains local, state, and national databases of DNA profiles from convicted offenders, unsolved crime-scene evidence, arrestees, and profiles of missing people. CODIS allows crime laboratories to compare DNA types recovered from crime-scene evidence to those of convicted sex offenders and other convicted criminals. This capability is of tremendous value to investigators in cases in which the police have not been able to identify a suspect. All 50 states have legislatively mandated collection of DNA samples from convicted offenders of particular crimes and establishment of DNA databases for law enforcement purposes. Thousands of CODIS matches have linked serial crimes to each other and have solved crimes by allowing investigators to match crime-scene evidence to known convicted offenders. The CODIS concept has already had a significant impact on police investigations in various states, as shown by the following case.

Currently, more than 170 public law enforcement laboratories across the United States participate in CODIS. In addition, CODIS is used by more than 40 law enforcement laboratories in more than 25 countries for their own DNA database inquiries. As previously mentioned, state laws now require the acquisition of DNA profiles from all convicted offenders. These profiles are continually added to state and national DNA data banks and have proven to be invaluable investigative resources for law enforcement. CODIS has produced more than 95,000 hits.

Quick Review

- CODIS 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.

Case Files

Using Mitochondrial DNA

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.

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 18 DNA-bearing cells are needed to obtain an STR profile. However, modifications in the technology can readily extend the level of detection down to 9 cells. A quantity of DNA that is below the normal level of detection is defined as a **low copy number**. 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 phenomenon of transferring DNA via skin cells onto the surface of an object has come to be called **touch DNA**. Again, keep in mind that, in theory, only 18 skin cells deposited on an object are required to obtain a DNA profile.

picogram
One-trillionth of a gram, or 0.000000000001 gram

low copy number
Fewer than 18 DNA-bearing cells

epithelial cells
The outer layer of skin cells

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

Case Files

Cold-Hit Identification

In 1990, a series of attacks on elderly victims were committed 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 burn the residence and the victim in an attempt to conceal the crime.

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

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

In April 2001, a "cold hit" was made 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.

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 profiled DNA from single dermal ridge fingerprints.[4] Although it's premature to imply that this technology, or a comparable one, is eligible for admission in criminal trials, one cannot exclude its use in criminal and forensic intelligence investigations (see Figure 9–21). Table 9–2 illustrates the power of DNA as a creator of physical evidence.

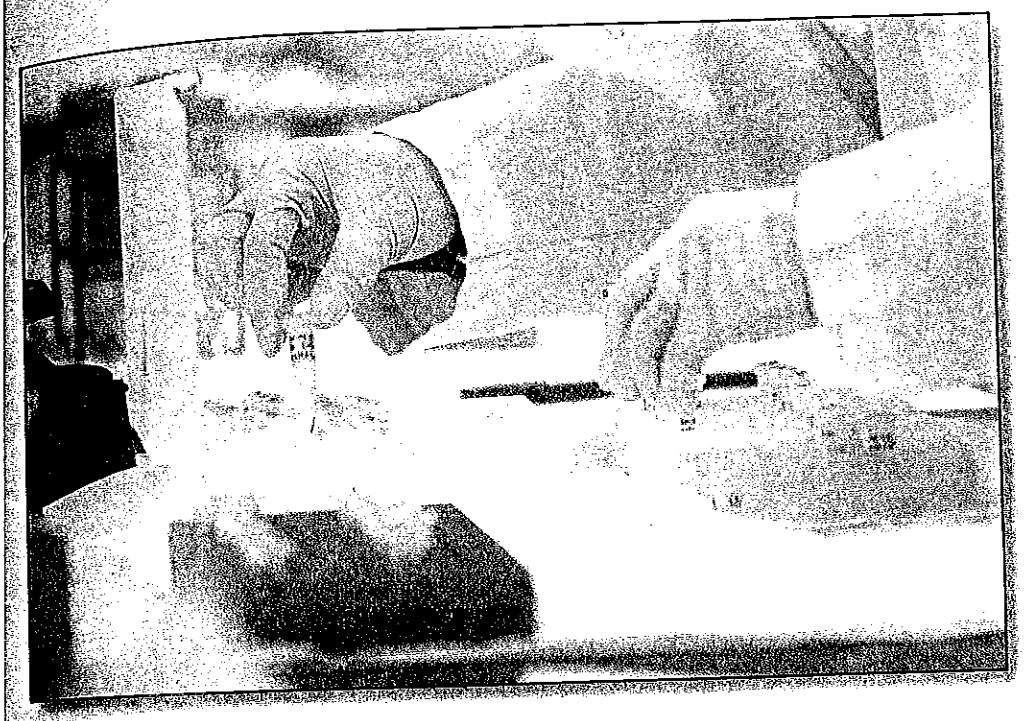


FIGURE 9–21
A criminalist inspects and extracts DNA from crime scene evidence at a DNA crime lab. *Courtesy Corbis*

Table 9–2
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, or mask	Inside	Sweat, hair, dandruff
Eyeglasses	Nose or ear pieces, lens	Sweat, skin
Facial tissue, cotton swab	Surface area	Mucus, blood, sweat, semen, ear wax
Dirty laundry	Surface area	Blood, sweat, semen
Toothpick	Tips	Saliva
Used cigarette	Cigarette butt	Saliva
Stamp or envelope	Licked area	Saliva
Tape or ligature	Inside/outside surface	Skin, sweat
Bottle, can, 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 fingernail	Scrapings	Blood, sweat, tissue

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

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 evaluate 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. The blood pattern should not be disturbed before this phase of the investigation is completed.

The evidence collector must handle all body fluids and biologically stained materials with a minimum 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.

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 may use the substrate swab to confirm that the results of the tests performed were brought about by the stain and not by the material on which it was deposited. However, this practice is normally not necessary when DNA determinations are carried out in the laboratory. One point

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

is critical: the collected swabs must not be packaged in a wet state. After collection, 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 9–22), which has a circular hole to allow air circulation. The swab box can then be placed in a paper or manila envelope.

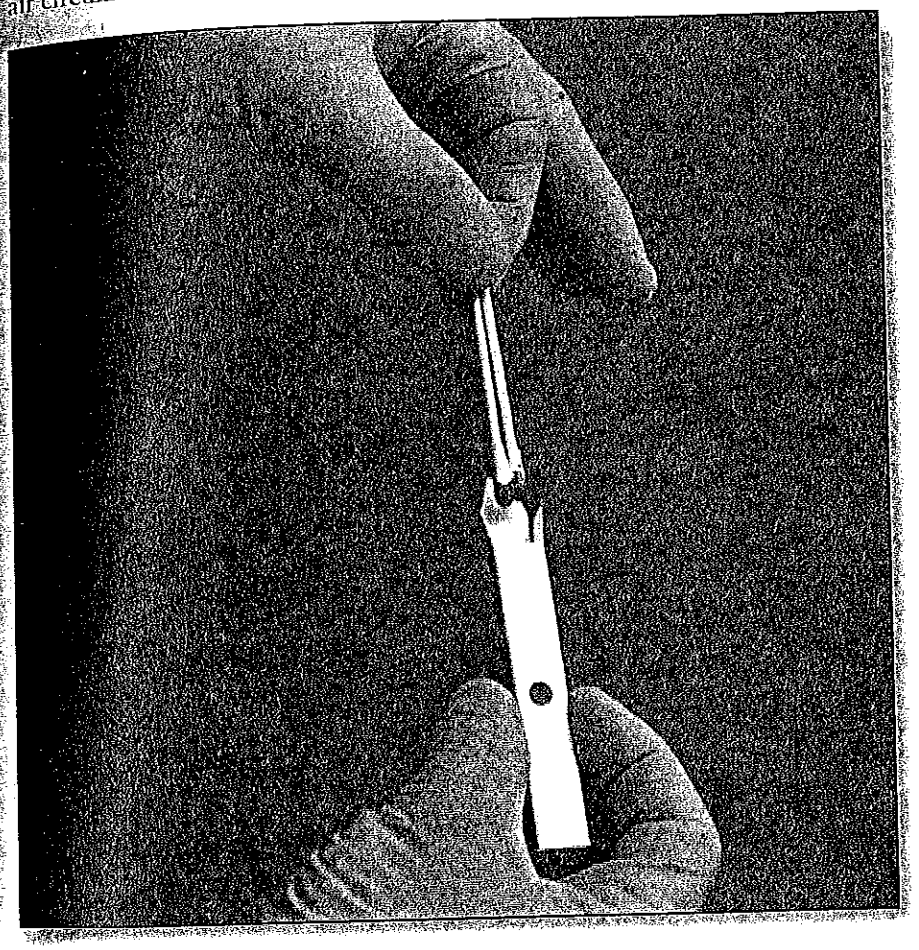


FIGURE 9–22
Air-dried swabs are placed in a swab box for delivery to the forensic laboratory. *Courtesy Tri-Tech, Inc., Southport, N.C., www.tritechusa.com*

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.

Collection of Reference Samples

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 milliliters 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/

buccal cells
Cells from the inner cheek lining

reference, one that nonmedical personnel can readily use, is the *buccal swab*. A swab is 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.

With the increasing need for collection and analysis of DNA samples in forensic investigations, long-term storage of DNA has become an important consideration. FTA paper is a type of commercially available filter paper on which DNA-containing specimens are placed. The card is fixed with bacteriocidal and fungicidal materials that will protect the DNA from microbial and environmental degradation. The card allows the entrapped DNA to be stored without refrigeration for many years. Figure 9–23 illustrates the collection of a buccal swab and its transfer onto an FTA card for storage.

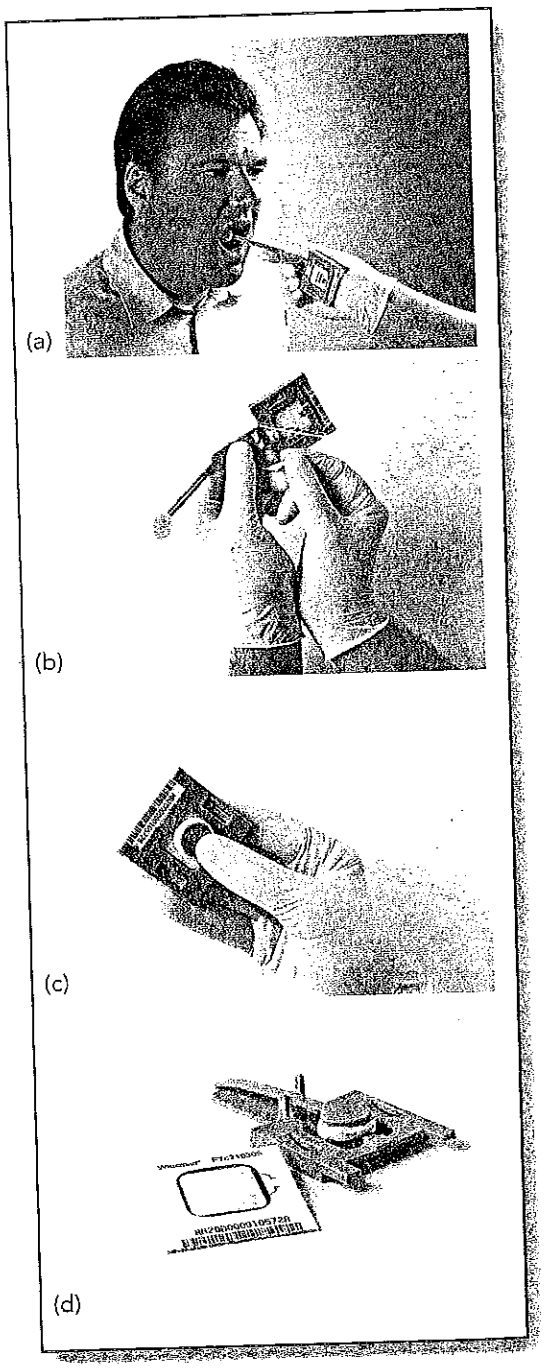


FIGURE 9–23
(a) A buccal swab is collected by rubbing each cheek for 15 seconds. (b) A protective film is peeled of the FTA card. (c) The swab is snapped in place against the FTA paper. (d) The FTA card is removed from the collection device and stored. *Courtesy GE Healthcare Bio-Sciences Corp. (GEHC) Piscataway, N.J., www.whatman.com*

If an individual is not available to give a DNA standard/reference sample, some interesting alternatives are available, 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. 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 asked to supply the New York City DNA Laboratory with these types of items in an effort to match recovered DNA with human remains.

MyCrimeKit: WebExtra 9.11
View DNA Forensics
www.mycrimekit.com

Contamination

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 suggests 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.**

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 (discussed in Chapter 8). 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.

Case Files

Contact Lens Evidence

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

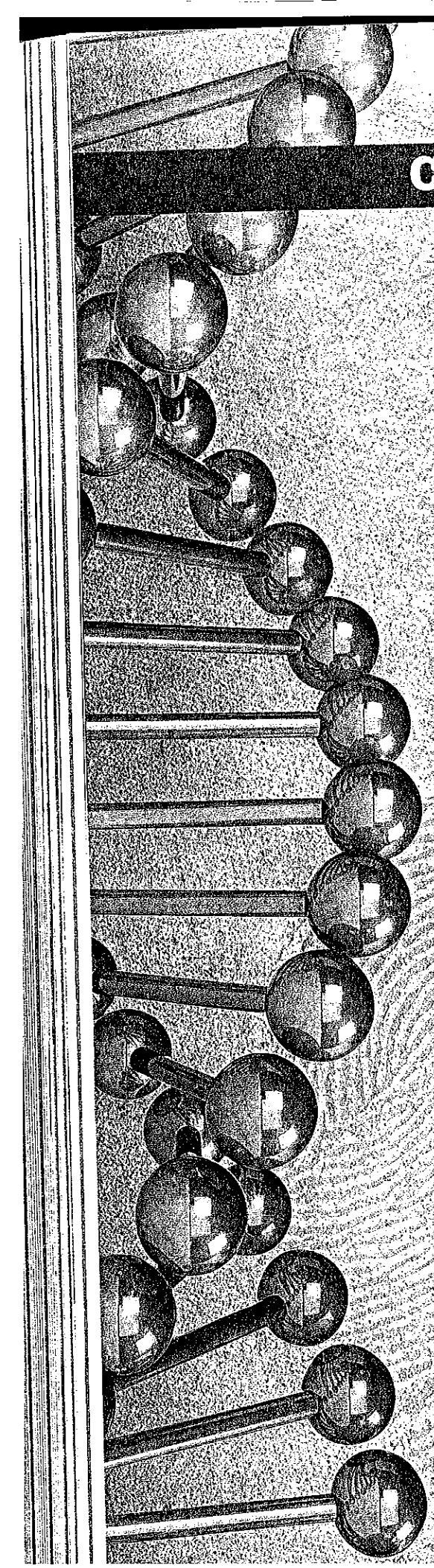
In the laboratory, approximately 20 nanograms of human DNA was recovered from the contact lens fragments. Cells from both the eyeball and the interior of the eyelids are naturally replaced every 6 to 24 hours. Therefore, both are potential sources for the DNA found. The DNA profile originating from the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

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

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.

Quick Review

- Biological evidence should not be packaged in plastic or airtight containers because the accumulation of residual moisture could contribute to the growth of blood-destroying bacteria and fungi. Each article should be packaged separately in a paper bag or in a well-ventilated box.
- The least intrusive method for obtaining a DNA standard/reference is the buccal swab. In this procedure, cotton swabs are placed in the subject's mouth and the inside of the cheek is vigorously swabbed, resulting in the transfer of cells from the inner cheek lining onto the swab.



Chapter Review

- The gene is the fundamental unit of heredity. Each gene is 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 of DNA are aligned in a double-helix configuration so that adenine pairs with thymine and guanine pairs with cytosine. This concept is known as complementary base pairing.
- The order in which the base pairs are arranged defines the role and function of a DNA molecule.
- DNA replication begins with the unwinding of the DNA strands in the double helix. The double helix is re-created as the nucleotides are assembled in the proper order (*A* with *T* and *G* with *C*). Two identical copies of DNA emerge from the process.
- PCR (polymerase chain reaction) is a technique for replicating or copying a portion of a DNA strand outside a living cell.
- Recombinant DNA is a process by which the DNA of an organism is altered by inserting into it fragments of DNA from another organism.
- Portions of the DNA molecule contain sequences of bases that are repeated numerous times. These tandem repeats offer a means of distinguishing one individual from another through DNA typing.
- Length differences associated with relatively long repeating DNA strands—called restriction fragment length polymorphisms (RFLPs)—formed the basis for one of the first DNA-typing procedures.
- In the laboratory, DNA molecules are cut up by a restriction enzyme and the resulting fragments are sorted out by electrophoresis.
- Materials undergoing electrophoresis are forced to move across a gel-coated plate under the influence of an electrical potential. Substances such as DNA can be separated and characterized using electrophoresis.
- A typical DNA fragment pattern shows two bands (one RFLP from each chromosome).
- Polymerase chain reaction (PCR) can amplify minute quantities of DNA. The technique evolved from an understanding of how DNA strands naturally replicate within a cell.
- PCR technology cannot be applied to RFLP DNA typing because 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.

- Long RFLP strands tend to break apart under the adverse conditions at many crime scenes. The shorter DNA strands used in PCR are more stable and less subject to degradation caused by adverse environmental conditions.
- 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.
- The entire strand of an STR is very short, less than 450 bases long. This makes STRs much less susceptible to degradation, and they are often recovered from bodies or stains that have been subjected to extreme decomposition.
- 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, in which the forensic scientist can simultaneously extract and amplify a combination of different STRs.
- With STR, as little as 125 picograms of DNA is required for analysis—1/100 the amount normally required for RFLP analysis.
- Mitochondrial DNA is located outside the cell's nucleus and is inherited from the mother.
- Two regions of mitochondrial DNA, HV1 and HV2, are sequenced for forensic typing purposes.
- 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.
- CODIS 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.
- Biological evidence should not be packaged in plastic or airtight containers because the accumulation of residual moisture could contribute to the growth of blood-destroying bacteria and fungi. Each article should be packaged separately in a paper bag or in a well-ventilated box.
- The least intrusive method for obtaining a DNA standard/reference is the buccal swab. In this procedure, cotton swabs are placed in the subject's mouth and the inside of the cheek is vigorously swabbed, resulting in the transfer of cells from the inner cheek lining onto the swab.

Review Questions

1. New technology incorporating _____ has supplanted RFLP.
   a. PCR
   b. STR
   c. mtDNA
   d. CODIS

2. A/An _____ is composed of a sugar molecule, a phosphorus-containing group, and a base
   a. amino acid
   b. protein
   c. chromosomes
   d. nucleotide

3. DNA _____ is the synthesis of new DNA from existing DNA and begins with the unwinding of the DNA strands in the double helix.
   a. transcription
   b. translation
   c. replication
   d. cloning

4. The pairing of nucleotides A to G and C to T is known as
   a. mitochondrial DNA.
   b. complementary base pairing.
   c. polymerase chain reaction.
   d. tandem repeats.

5. The inheritable traits that are controlled by DNA arise out of its ability to direct the production of complex molecules called _____ from smaller units called _____.
   a. chromosomes; genes
   b. lipids; triglycerides
   c. proteins; nucleic acids
   d. proteins; amino acids

6. True or False: As the fundamental unit of heredity, genes instruct the body cells to make proteins that determine everything from hair color to susceptibility of disease.

7. True or False: DNA replication is accomplished using a technique known as polymerase chain reaction.

8. True or False: DNA technology is useful in identification because no two humans, except for identical twins, have the same type of tandem repeats in a strand of DNA.

9. True or False: The latest, most successful and widely used DNA profiling procedure is the short tandem repeats.

10. True or False: Mitochondrial DNA is found outside the nucleus of the cell and is inherited solely from the mother.

11. What is DNA and why is it important to forensic scientists?

12. What are genes and what is their function? Of what are genes composed and where are they located?

13. With what discovery are James Watson and Francis Crick credited?

14. Describe the basic structure of the DNA molecule. What is the name given to this type of structure?

15. Name the four bases associated with DNA. How are these bases paired on the DNA molecule?

16. How are proteins made? What determines the shape and function of a protein molecule?

17. What is the human genome? Name two medical applications of information about the human genome.

18. What is PCR? Why is it useful to forensic scientists?

19. What is recombinant DNA? How is recombinant DNA technology used to treat diabetes?

20. What are tandem repeats? How are they useful to forensic scientists?

21. What is a short tandem repeat (STR)? Why are STRs so attractive to forensic scientists?

22. Name two processes by which a forensic scientist can separate STRs for characterization. Which process is preferred and why?

23. What gene is often used to determine the sex of a DNA contributor? What characteristic of the gene allows forensic scientists to make this determination?

24. Name one advantage and three disadvantages of mtDNA analysis compared to nuclear DNA profiling.

25. What is CODIS? How is CODIS useful to forensic scientists?

26. What type of gloves should an evidence collector wear when handling biological evidence? Name two reasons for wearing this type of glove.

27. What type of packaging should not be used for biological evidence? Why? What type of packaging should be used instead for articles containing biological evidence?

28. How should packages containing biological evidence be stored until they are delivered to a laboratory? Name one common exception and describe how it should be stored.

29. List four ways to minimize contamination of biological evidence.

Quick Lab: Buccal Swab

Materials:

Swab/Q-tip
Slide
Light microscope
Methylene blue stain

Procedure:

An important part of obtaining standard/reference samples is determining the DNA profile of any individuals involved in the investigation. One way to do this is by a buccal swab. Take the swab provided by your teacher and rub the inside of your check with it. Next, rub the swab on the slide and add a drop of methylene blue stain; be careful not to get the stain on you or anything but the slide. Place a cover slip on the slide and view it under the microscope.

Follow-Up Questions:

1. How many cells were you able to see on your slide? Were you surprised by the number you saw?

2. What on the slide was stained by the methylene blue?

3. Where in the cell is the DNA located?

Application and Critical Thinking

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

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

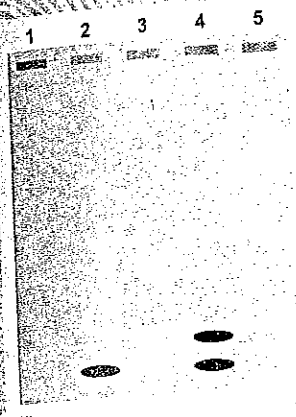
   List the sequence of bases that will form complementary pairs on the other strand of the DNA molecule.

2. Police discover a badly decomposed body buried in an area where a man disappeared some years before. The case was never solved, nor was the victim's body ever recovered. As the lead investigator, you suspect that the newly discovered body is that of the victim. What is your main challenge in using DNA typing to determine whether your suspicion is correct? How would you go about using DNA technology to test your theory?

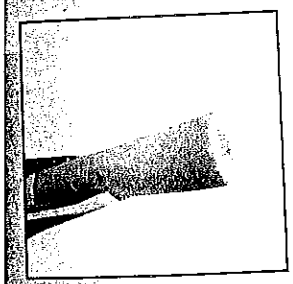
3. You are a forensic scientist performing DNA typing on a blood sample sent to your laboratory. While performing an STR analysis on the sample, you notice a four-band pattern. What conclusion should you draw? Why?

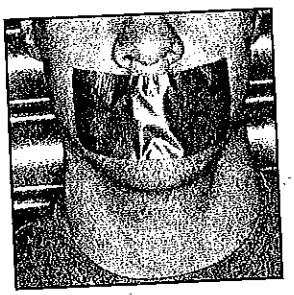
4. A woman reports being mugged by a masked assailant, whom she scratched on the arm during a brief struggle. The victim is not sure whether the attacker was male or female. DNA analysts extract and amplify the amelogenin gene

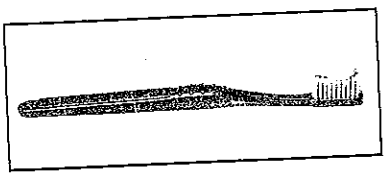
from the epithelial cells under the victim's fingernails (allegedly belonging to the attacker) and from a buccal swab of the victim. The sample is separated by gel electrophoresis with the result shown here. The victim's amelogenin DNA is in lane 2, and the amelogenin DNA from the fingernail scraping is in lane 4. What conclusion can you draw about the attacker from this result? How did you reach this conclusion?

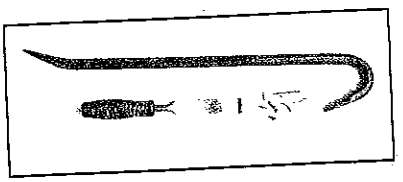


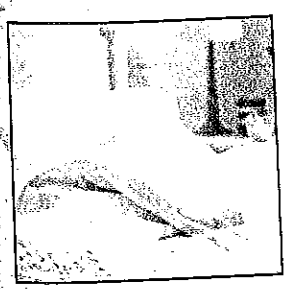
5. At a crime scene you encounter each of the following items. For each item, indicate the potential sources of DNA. The five possible choices are saliva, skin cells, sweat, blood, and semen.

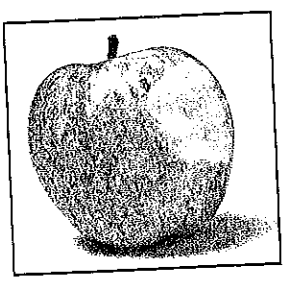


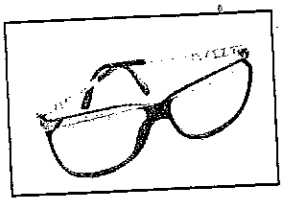












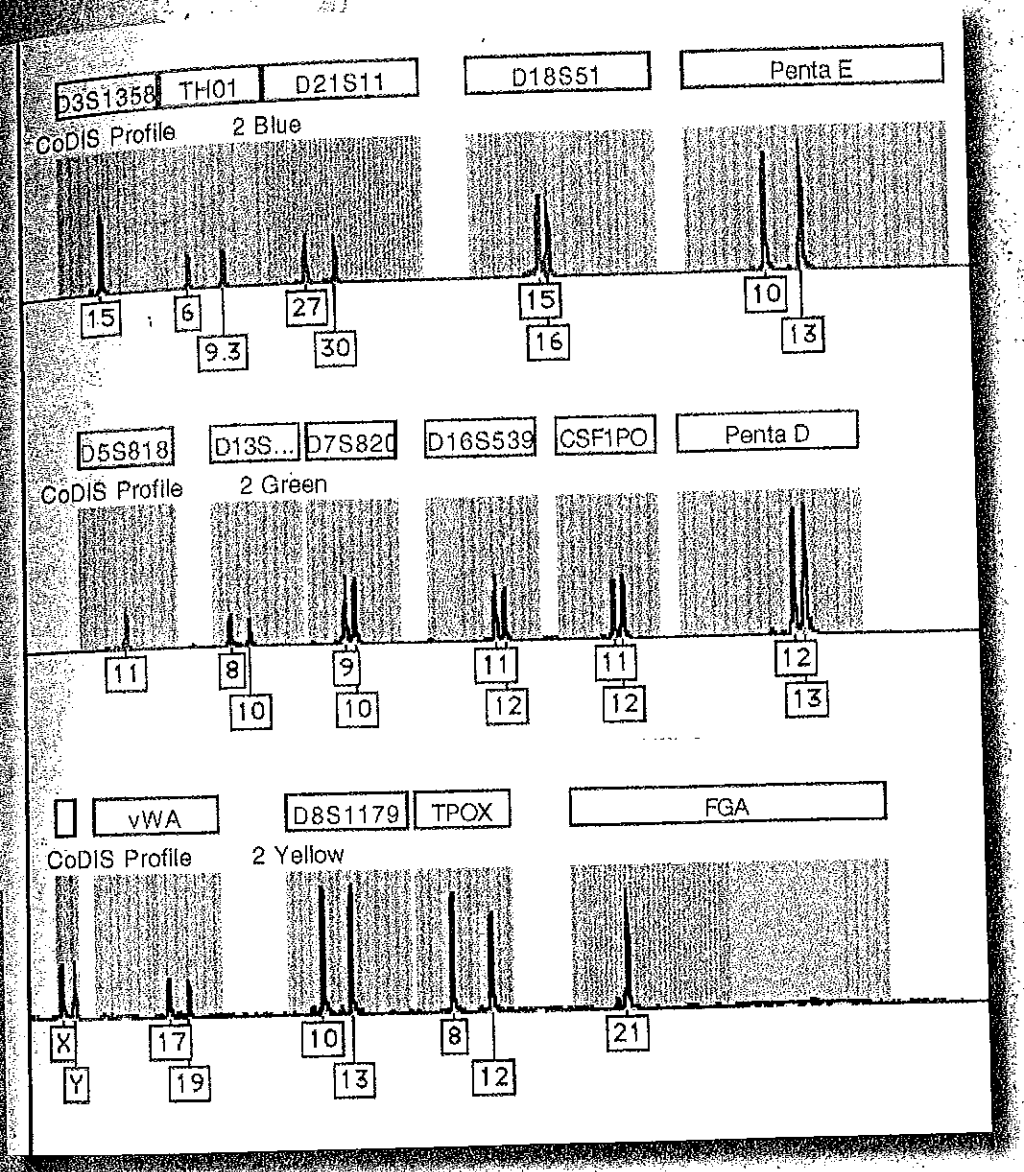
6. The 15-STR locus DNA profile of a missing person, James Dittman, is given in the following table.

STR Loci	Allele
D3S1358	15
THO1	6, 9.3
D21S11	27
D18S51	15,16
PENTA E	10
D13S807	11
D3S1358	10,13
D7S820	9,10
D16S539	11,12
CSF1PO	13
PENTA D	12,13
AMELOGENIN	XY
VWA	17, 19
D8S1179	10, 13
TPOX	8, 12
FGA	21

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

Virtual Lab: Conduct a DNA Analysis

To perform a virtual DNA lab analysis, go to www.pearsoncustom.com/us/vlm/



Endnotes

1. Instructions for assembling proteins are carried from DNA to another region of the cell by ribonucleic acid (RNA). RNA is directly involved in the assembly of the protein, using the genetic code it received from DNA.
2. Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 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.