Chapter 2

The Technologies and Their Applications

"Scientifically speaking, it's like discovering a new star. It's a breakthrough in our lifetime."

John Williams, Police Chief
Woodlawn, Ohio
Associated Press
Mar. **7, 1988**

''Many times, today's science is tomorrow's fiction."

Hal Uhrig, Defense Attorney
Orlando, Florida
Apr. 17, 1989

"You can prove almost anything with the evidence of a small enough segment of time. How often in the search for truth the answer of the minute is positive, the answer of the hour qualified, the answers of the ***year*** contradictory. "

Edwin Way Teale
1899-1980

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The Technologies and Their Applications

Genetics is the study of factors that influence the inheritance of specific traits. People have recognized since time immemorial that like begat like, that individuals share familial features yet have distinct characteristics. In 1865, Austrian monk Gregor Mendel postulated that discrete biological units-genes—were responsible for both the maintenance and variation of certain characteristics from one generation to the next. Genetic uniqueness is a fact of life.

Historically, the thrust of research and applications in human genetics focused on medical uses, especially determination and diagnosis of genetic diseases. Since the turn of the century, however, forensic scientists have exploited human genetic variability in the analysis of evidence from crime scenes. Blood tests have been used as evidence in paternity disputes since the 1920s (18). Yet while these traditional genetic landmarks (often referred to as markers) have been useful for excluding suspects (17,19,20,21), positive identification using these markers has been elusive because they occur in limited combinations. In contrast, much greater variation exists and can be detected with deoxyribonucleic acid (DNA) markers-hence the potential for individualization (box 2-A).

Box 2-A—Terminology

Forensic science involves the application of many scientific expertise (e.g., biology, chemistry, toxicology, medicine) to situations concerned with courts of justice or public debate. This report uses the term "forensic applications" to refer to potential uses of recombinant DNA technologies to identify individuals.

The increased acceptance and popularization of recombinant DNA techniques for forensic uses, especially criminal investigations, have led to some confusing terminology. In particular, some commentators have adopted the terms "genetic fingerprinting," "DNA fingerprinting," or "DNA prints" as generic phrases to describe all techniques, while others use the terms to describe specific techniques by specific companies. This report uses the terms "DNA testing," "DNA identification," "DNA analysis," "DNA typing," and "DNA profiling" to describe the two current and any future technologies-the practical goal of which is unique association or exclusion determined by DNA-based tests.

SOURCE: OfiIce of Technology Assessment 1990.

This chapter briefly outlines the biological basis for using DNA to differentiate individuals, summarizes concepts of recombinant DNA technology, and discusses the two principle techniques currently employed in forensic applications. It also explores future recombinant DNA technologies that could be applied to forensic science. Finally, the chapter describes the wide range of nonmedical applications for which the techniques have or could be used. Other Office of Technology Assessment (OTA) reports address issues of related topics in human genetics and biotechnology (57,58,59,60).

WHAT IS DNA?

Faithful transmission of genes is common to the entire spectrum of living organisms. It is the result of the remarkable capacity of a living cell to reproduce, encode, and translate a chemical into its ultimate biological fate. Except in rare instances, the chemical responsible for the maintenance and expression of inherited characteristics is DNA.

As the chemical dispatcher of genetic information, DNA's structure resembles a twisted ladder, referred to as a double helix (figure 2-l). DNA in all organisms consists, in part, of four chemical subunits commonly called bases. These four bases—guanine (G), adenine (A), thymine (T), and cytosine (C)-are the genetic alphabet. The bases normally pair predictably—A with T, and G with C—to form the rungs of the double-stranded DNA helix, and these combinations are termed base pairs. Their unique order, or sequence, in the helix determines the structure of proteins and the regulation of cell activities. Regions of DNA not involved in such capacities, as far as can be determined, are called noncoding DNA.

In humans, DNA is associated with protein in organized microscopic bundles called chromosomes. Humans have 46 chromosomes-1 pair of sex chromosomes (two X chromosomes for females; an X and a Y for males) and 22 pairs of autosomes. Individuals receive 22 autosomes plus one X chromosome from their mothers, and 22 autosomes plus either an X or a Y from their fathers. Figure 2-2

illustrates the chromosome profile of a normal female.

DNA in humans is found in all body cells except red blood cells. (Blood contains many cell types in addition to red blood cells, such as white blood cells, and it is from these cells that DNA can be obtained.) With few exceptions (that can only be detected through specific, sophisticated laboratory methods), the composition of a person's DNA does not vary from cell to cell, except in egg and sperm cells. These cells have half the complement of DNA present in other body cells.

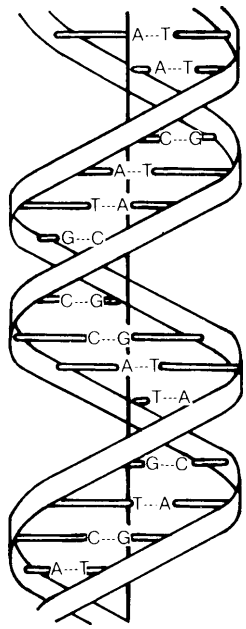
Thus, a forensic scientist can examine DNA from blood (35) or tissue from hair roots and, if the specimens are form the same person, find the same patterns. Similarly, DNA patterns can be matched between DNA isolated from sperm on a vaginal swab or semen stain and DNA from a rape suspect's sample. With semen, the DNA composition differs from sperm-to-sperm, but the DNA profile is a composite of thousands of DNA molecules from thousands of sperm and therefore reveals a man's overall profile (24).

HOW DOES DNA DIFFER FROM PERSON TO PERSON?

Virtually the entire complement of genetic material resides in an individual's chromosomes. This complement, or genome, consists of about 3.3 billion base pairs. Only a fraction of these 3.3 billion base pairs in each person differ between any two individuals (approximately 3 million on average). The challenge to forensic DNA analysis is to detect some of these differences.

To find these differences, scientists rely on the fact that "addresses" can be assigned for genes or DNA sequences. An address, or physical location, of a gene or sequence on a chromosome is called its locus (from Latin for place). Chromosomes contain many loci occupied by different genes or DNA sequences. For example, the locus for the gene responsible for sickle cell anemia is on chromosome 11, and the locus for cystic fibrosis is on chromosome 7. Except for the sex chromosomes, normal individuals have two copies of any given gene or sequence at a particular locus because human chromosomes come in pairs-one copy inherited from the mother and one from the father.

Figure 2-I—The Structure of DNA



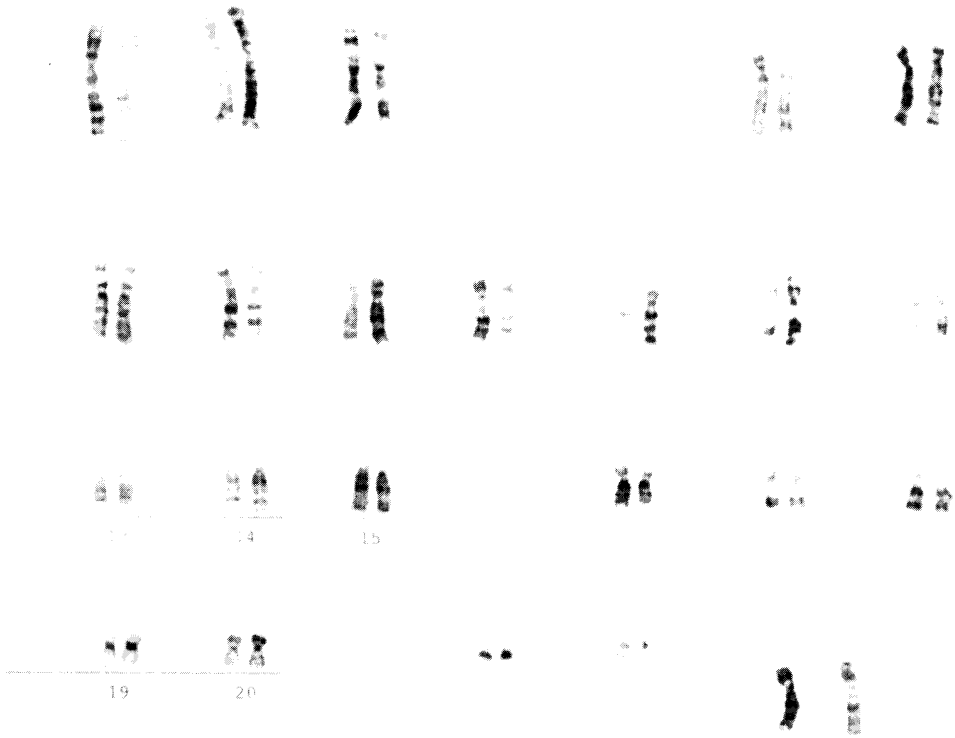
SOURCE: Office of Technology Assessment, 1990.

Genetic variants at a particular locus are called alleles. At each locus along pairs of autosomes, an individual can have two identical, or two different, alleles. If the alleles are the same, the person is said to be homozygous for that particular locus. If the versions differ, the person is said to be heterozygous.

Even though one individual has at most two alleles at a given locus—again, one copy inherited from the mother and one from the father—additional forms can exist in other individuals. That is, many different alleles can exist for that same locus within the population. When multiple alleles exist at a particular locus—as in the case of the common ABO red blood cell typing system—the genetic variant is referred to as a polymorphism. Polymorphisms (i.e., genetic differences among people) are at the heart of forensic applications of DNA typing.

Some addresses (loci) in humans have as many as 50 to 100 different forms (alleles). DNA tests are designed to detect these highly polymorphic loci and to distinguish among the alleles that exist there. DNA analysis does not examine an individual's entire genome, but rather a snapshot of a specific area. And because DNA from any two individuals is more alike than different, relatives or unrelated persons can share the same allele or alleles at any

Figure 2-2—Chromosome Profile of a Normal Human Female



Chromosomes from humans can be matched into 22 pars (the autosomes) plus one pair of sex chromosomes. In this figure, both sex chromosomes are X. A normal male chromosome complement would include the 22 pairs plus an X and a Y. One of each type of autosome (1-22) is inherited from an individual's mother, and one from the father. Chromosome profiles such as this one do not reveal which particular chromosome within the pair derived from which parent.

SOURCE: The Genetics & IVF Institute, Fairfax, VA, 1990.

given locus-even highly polymorphic loci. Thus, forensic uses of DNA tests depend on examining several loci to determine whether DNA types from two different samples match.

WHAT TECHNOLOGIES ARE USED FOR DNA TESTING?

DNA testing technologies encompass an array of molecular techniques developed since the early 1970s, and designed to examine the detailed structure, function, and inheritance patterns of DNA. Applications of two of these technologies-restriction liagment length polymorphism (RFLP) analysis and amplification of DNA by polymerase chain reaction (PCR)-have found their way into courtrooms in the United States and abroad. RFLP analysis is most widely used, although PCR, which is combined with other genetic techniques in forensic analysis, has been used in some casework (see app. A).

Restriction Fragment Length Polymorphism Analysis

Examination of DNA from individuals, other than identical twins, reveals that variations in DNA sequence occur, on average, about once every thousand base pairs. These variations exist in both coding and noncoding regions of DNA, and although most do not lead to functional changes in the protein products of genes, a few can cause disease. In 1978, two scientists demonstrated this phenomenon by showing that a DNA sequence was altered in certain individuals, and that this change in the DNA correlated with the inheritance of sickle cell disease (34).

This important discovery led researchers to propose that these natural differences in DNA sequence @polymorphisms) could be used as markers to track genetic traits through families (5,32). Further research revealed that a specific DNA pattern could be associated with a specific individual-to the extent of positive association (23,29,30,31,66). Although initial research looked at changes associated with disease genes, the majority of the DNA variations now identified, including the majority of markers used in forensic identification, are not linked to disease.

How were these markers discovered and subsequently exploited? At the core of detecting the types of DNA polymorphisms just mentioned are enzymes called restriction endonucleases, or restriction enzymes. Sometimes characterized as molecular scissors, each type of restriction enzyme recognizes a short DNA sequence specific for that enzyme and cuts DNA only at that site. RFLP analysis uses these enzymes to uncover genetic variants. In forensic applications, RFLP analysis detects *size* differences of DNA fragments at specific loci (box 2-B) (29,30,46,64,65,66). It does not directly reveal sequence differences.

Scientists call the basic procedure used in RFLP analysis 'Southern blotting' or 'Southern hybridization,' after the person who developed the technology (54). Generally speaking, it includes the following major steps (figure 2-3):

- isolating DNA from the specimen to be examined;
- . cutting the DNA into discrete pieces with a restriction enzyme;
- . separating the different sized DNA pieces using a process called gel electrophoresis;
- . transferring the DNA from a gel to a nylon membrane (producing the ''Southern blot' ');
- applying, or hybridizing, a DNA probe to the membrane; and

Box 2-B—DNA Differences in Humans: Variable Number of Tandem Repeats

Comparison of two randomly chosen individuals' DNA yields many more similarities than differences, but scientists have identified some of the loci where genetic variation exists. Some variants, or polymorphisms, are single-base pair alterations. Other loci involve more base pairs and take on many forms. In forensic uses of DNA tests, this latter type of polymorphism is most important.

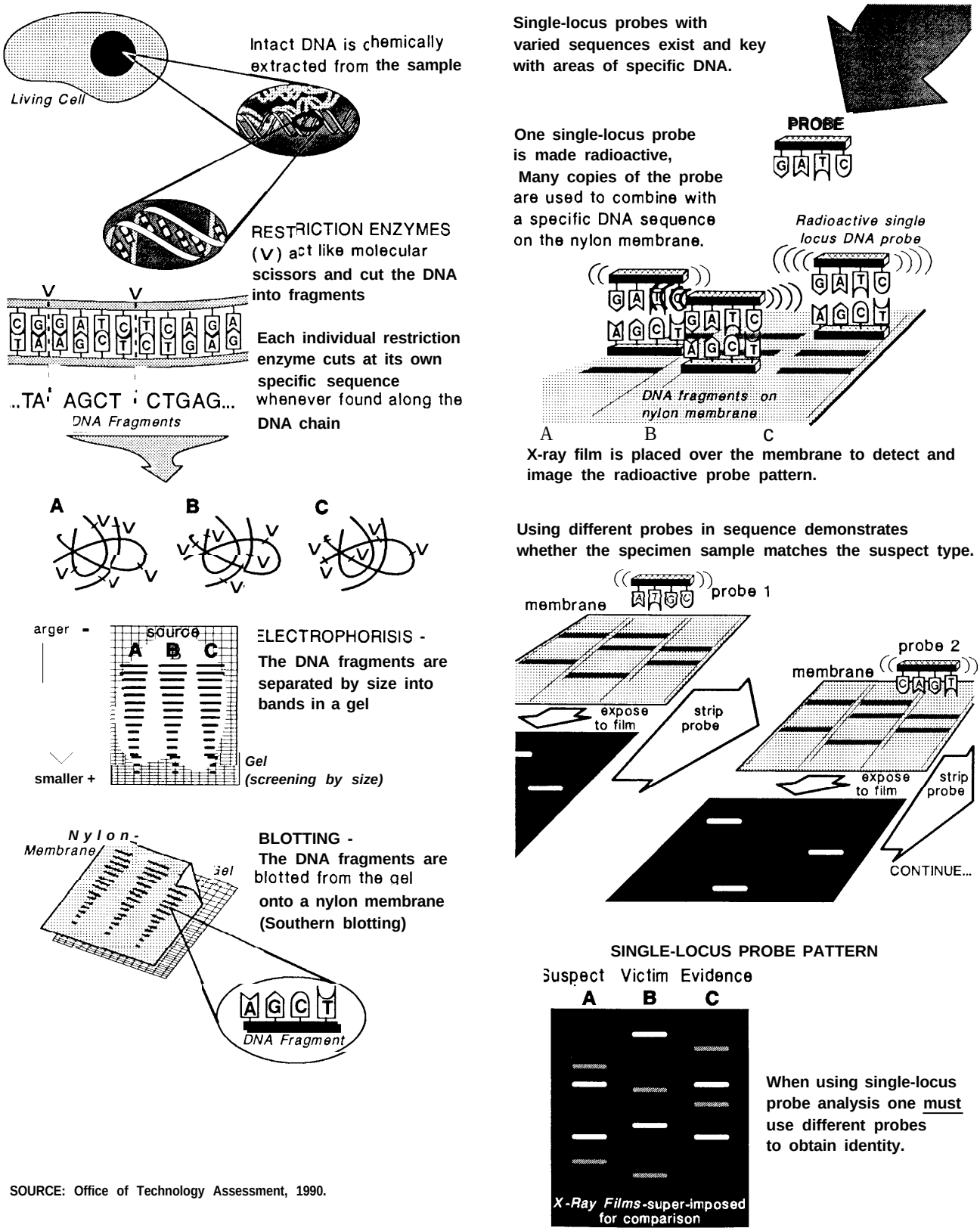
In 1980, scientists discovered that short, identical segments of DNA lined head to tail in a repeating fashion are interspersed throughout the genome. Both the number of base pairs and the actual DNA sequence that comprise the repeated unit can vary from locus-to-locus and chromosome-to-chromosome. Some variants are found at multiple loci, others at only one site--a single-locus-in the human genome, Most importantly, the number of repeated units varies between individuals. Such sequences, or regions of repeated units, are called variable number of tandem repeats, or VNTRs. VNTRs account for the size differences that can be measured by RFLP analysis, and the wide variation in the number of repeats within the human population at VNTR loci is the critical element for forensic DNA analysis. (Single-locus v. multilocus RFLP analysis is described in a following section.)

For example, one VNTR in humans consists of a 17-base pair unit repeated from 70 to 450 times at one locus in the genome. Thus, the total number of base pairs would vary from 1,190 (17 x 70) to 7,650 (17 x 450), Because chromosomes come in pairs (one inherited from the mother and one from the father), individual A could have 74 repeated units at one locus and 300 repeats at the other, yielding two measurable bands in a RFLP analysis. Individual B, a sibling, might also have 74 repeats at one locus, but the other chromosome might have 145 repeats, which would yield a RFLP pattern with one similar and one divergent band. An unrelated individual C might have repeats of 83 and 216. A forensic scientist measures size differences at this locus, not direct sequence variation, to identify the distinctions in the DNA patterns of these individuals.

However, the number of forms (alleles) of a VNTR at a particular locus is limited. Siblings D and E, for example, could be expected to have the same pattern at this particular locus 25 percent of the time if both parents are heterozygotes. Similarly, unrelated individual F could, by chance, share either or both fragments in their pattern. Thus, a DNA fragment pattern derived from examining one locus does not enable the individualization of a DNA specimen. Rather, the power of DNA testing results from analyzing several VNTR loci throughout the human genome. A number of people will have the same fragment at one VNTR locus, but the chances of more than one person having the same *combination* of DNA fragments when 4,5, or 6 different VNTR loci are analyzed becomes vanishingly small. Combining the data from each of these snapshots is one of the keys to statistically positive association.

SOURCE: OffIce of Technology Assessment 1990.

Figure 2-3—Detailed Schematic of Single-locus Probe RFLP Analysis



SOURCE: Office of Technology Assessment, 1990.

- visualizng the location of the probe's hybridization, and hence the DNA pattern—for radioactive probes, usually by exposing the membrane to x-ray film, a process called autoradiography.

DNA Isolation and Restriction Enzyme Digestion

Proper sample collection and storage of evidentiary materials are critical for successful forensic RFLP analysis. Various environmental factors can affect the quantity and quality of DNA isolated from evidentiary stains, sometimes leading to degradation, or breakdown, of the DNA in the sample (43,44). Successful RFLP analysis in forensic casework hinges on obtaining large, intact (high molecular weight) DNA from case specimens.

Once isolated, a small amount of the sample is generally tested to ascertain the general quality and quantity of the DNA. If both are sufficient, a portion of the DNA is then cut, or digested, into discrete flagments with a specific restriction enzyme. This step, digestion with a particular restriction enzyme, is the first element in establishing a DNA profile based on size polymorphisms. Using different enzymes leads to different DNA patterns for the same individual. At this time, RFLP systems used by the two major commercial laboratories and the Federal Bureau of Investigation (FBI) each rely on different restriction enzymes (table 2-l). (See chs. 3 and 5 for discussion of standardization.)

Gel Electrophoresis and Southern Transfer

Because DNA digested with a restriction enzyme creates thousands of DNA fragments ranging in size from a few to tens of thousands of base pairs, the pieces must be separated by size through a process called gel electrophoresis. In this process, the mixture of DNA fragments is placed in a semisolid matrix, called a gel, and exposed to an electric field. Because the chemical makeup of DNA gives it a net negative charge, the DNA fragments travel through the pores in a gel toward a positive electrode. The gel acts as a sieve, with large DNA fragments moving more slowly than small ones. Thus, the mixture is separated, or resolved, according to size. In RFLP analysis of human DNA, the numerous fragments are laddered continuously along the entire length of the gel, and any single DNA fragment cannot be visualized without further effort.

Table 2-l—Restriction Enzymes Currently Used in Forensic Tests

Laboratory	Enzyme	Basic DNAsequenoe recognized
Cellmark	*HinfI*	---GANTC---[a] ---CTNAG---
FBI	HaeIII	---GGCC-- ---CCGG--
Lifecodes	*Pst I*	---CTGCAG--- ---GACGTC---

[a]N/N represents any base pair.

SOURCE: Office of Technology Assessment, 1990.

After a period of time, the electrophoresis is stopped and the DNA transferred out of the gel onto a nylon membrane in a process called Southern transfer. The nylon membrane, or Southern blot, retains the DNA in the orientation obtained in the gel after electrophoresis.

Forensic applications with RFLP analysis currently employ a substance called agarose to form the gel foundation. Small DNA fragments can be separated through another substance, acrylamide, and advances in the method could increase use of this material in forensic casework (2).

Probe Hybridization and Fragment Visualization

After the nylon membrane has been prepared, DNA patterns are revealed by using molecular tools called probes. Probes are short, single strands of a known DNA sequence. Scientists adjust conditions so that probes will seek out and bind, or hybridize, to their complementary sequence among the thousands of sequences that exist on a nylon membrane. For example, a probe with the sequence -G-A-T-C-C-T-A-C-G-T-C-C-A-A- will find pieces of DNA on the membrane with its complementary sequence -C-T-A-G-G-A-T-G-C-A-G-G-T-T-.

Thus, DNA probes can be thought of as reporter molecules that seek out and bind to their complementary sequence on the Southern blot. DNA probes in forensic RFLP analysis are typically tagged with a radioactive label. This radioactive label facilitates the visualization of the particular fragment of DNA that the probe bound to on the Southern blot. By exposing the nylon membrane, or blot, to apiece of x-ray film (or other detection system) after hybridization of the probe, scientists can determine which specific fragment the probe identified from among the thousands in a sample of human DNA. The fragment detected by the probe is actually visualized

as a dark band on the transparent x-ray film. Its size can be approximated by comparing it to pieces of DNA of known length that were electrophoresed on the gel along with the sample.

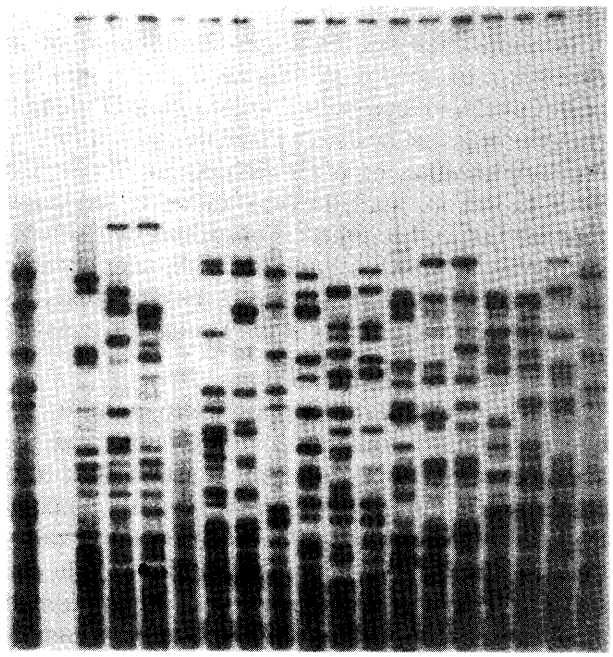
In addition to the restriction enzyme used, probes are the second key element in determining what an individual's DNA profile will look like-i. e., polymorphisms are detected by particular restriction enzyme-probe combinations. For example, if restriction enzyme A and probe A are used on a DNA sample, two restriction fragments might be revealed, one 250 base pairs in length, the other 600. Restriction enzyme A and probe B might detect a different polymorphism, yielding fragments of 2,300 and 900 base pairs for the same individual. Similarly, a third pattern—fragments of 370 and 450 base pairs—might be revealed by restriction enzyme B in combination with probe A. Choice of restriction enzyme and probes affects the DNA banding pattern of each individual's DNA sample. Hundreds of probes exist; several are used in forensic casework. Chapter 3 discusses issues surrounding the number and types of probes that are most appropriate for use in DNA profiling, as well as protocols for determining whether two DNA fragments are of equivalent size.

Two classes of probes are used in forensic applications of RFLP analysis. Multilocus probes recognize and bind to several locations throughout the human genome and reveal a complex DNA banding pattern (figure 2-4). In 1985, a case in the United Kingdom used multilocus probe analysis and became the first use of a DNA technology in a forensic case (29). Multilocus probe analysis in the United States is used by one company, which confines it to paternity examinations (although it has great potential for use in veterinary and agricultural applications). The second class of probes used in forensic RFLP analysis identities a unique genetic address, or single locus. Under defined conditions, single-locus probes generally identify one, two, or a few bands out of the thousands bound to the Southern blot (figure 2-5). Single-locus RFLP analysis is currently the most widely applied DNA technology in forensic casework in the United States.

Polymerase Chain Reaction

The FBI estimates that DNA profiles using single-1ocus RFLP analysis can be obtained from a fresh, dried blood stain approximately the size of a dime and a semen stain about the size of a pencil eraser

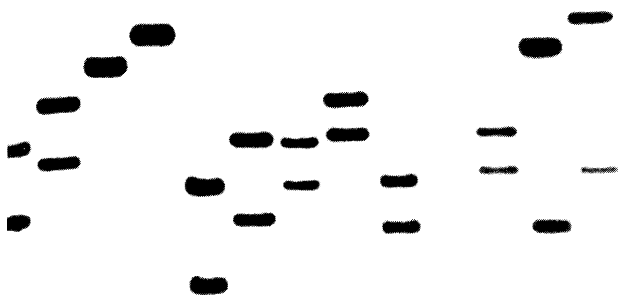
Figure 2-4—DNA Patterns Using a Multilocus Probe



Autoradiogram of restriction fragment length patterns using a multilocus DNA probe (33.15).

SOURCE: Cellmark Diagnostics, Germantown, MD, 1990.

Figure 2-5—DNA Patterns Using a Single-locus Probe



Autoradiogram of restriction fragment length patterns using a single-locus DNA probe (YNH24).

SOURCE: Federal Bureau of Investigation, 1988.

(36); others report success with smaller stains (22). Many forensic specimens, however, are smaller, aged, or have been exposed to extreme environmental conditions that damage DNA and make standard RFLP analysis uninformative. For these types of samples-when the quality and quantity of DNA is unsuitable for RFLP analysis—a different DNA technology, the polymerase chain reaction (PCR), (45,50,51,52) can sometimes surmount these

difficulties and permit the examination of biological evidence (12,15,62).

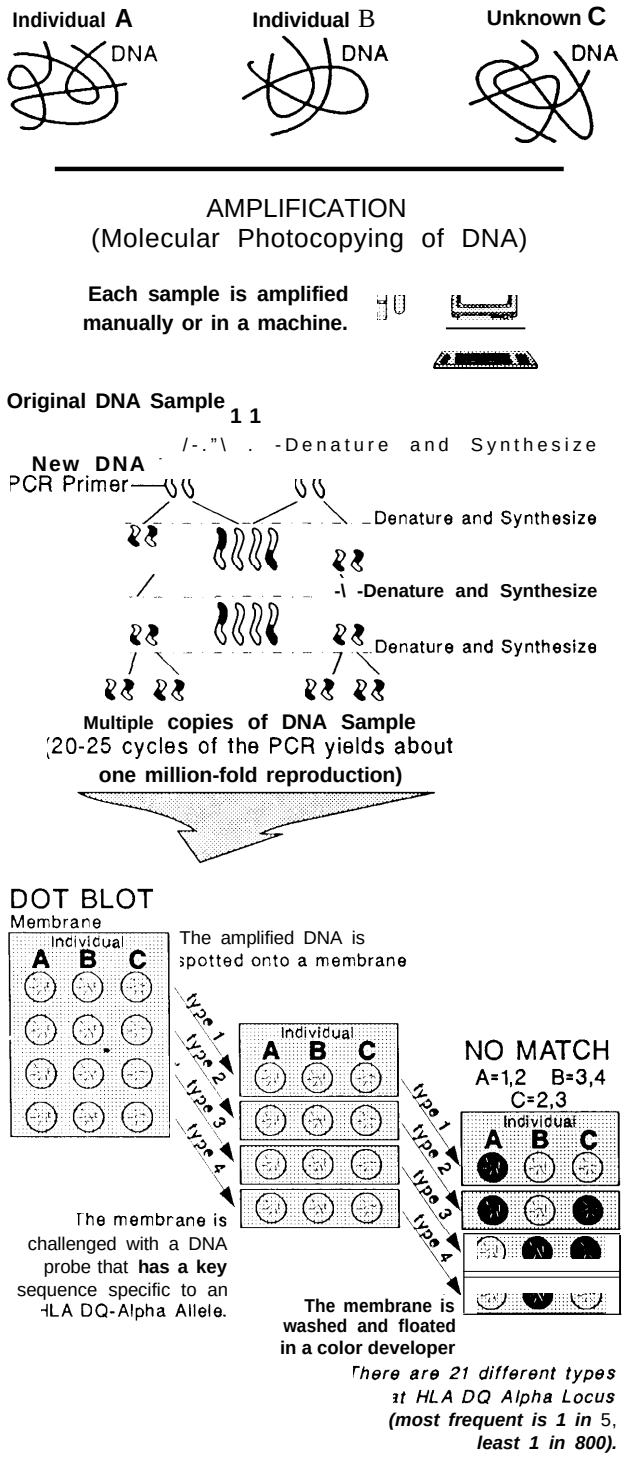
In some respects, PCR can be thought of as molecular photocopying (figure 2-6). It uses repeated cycles to reproduce a target area of DNA until enough copies are available for analysis using Southern hybridization or other techniques. Thus PCR itself is not an analytic tool, rather it facilitates forensic (as well as other) applications by allowing a scientist to take a sample of DNA, which would generally be insufficient to detect the characteristics of the DNA, and amplify it until enough copies are available for further analysis.

Briefly, PCR involves using two specific sequences, called primers, that flank the area the scientist wants to copy. The scientist then sets conditions in the reaction that allow new copies of the DNA of interest to be produced from the primers. Because the products generated in one sample can serve as templates in the next cycle, the number of amplified copies doubles with each cycle. Thus, 20 to 25 cycles of PCR potentially yield about a millionfold reproduction (box 2-C).

Currently, one DNA region is being used in PCR applied to forensic specimens, the human leukocyte antigen (HLA) *DQxct-1* gene, although several others are being examined for their potential utility in forensic casework (12,15). The HLA DQ *x-1 locus,* like its related genes responsible for tissue transplantation rejection, is polymorphic, with 21 different typing possibilities detectable. Overall, the probability of distinguishing between two people chosen at random using this system is 93 percent (62). In contrast, the probability using the conventional red blood cell typing system is 60 percent (1,53).

The HLA DQ *x-1* system distinguishes people by detecting which genetic bases---G,A,T,arere present at certain sites in the allele, rather than measuring the size of DNA fragments, as in RFLP analysis. Instead of gel electrophoresis, the process in forensic casework employs what is called a‘ ‘reverse dot-blot hybridization” (figure 2-7). Some argue that this method is more straightforward because the tests do not define alleles on the basis of size (i.e., mobility through a gel), but are theoretically designed to give “yes’ ’/’’no’ answers. In practice, however, the results can require interpretation for some case samples (36). Finally, because PCR generates large amounts of DNA, analysis need not involve radioactive materials.

Figure 2-6—The Polymerase Chain Reaction



SOURCE: Office of Technology Assessment, 1990.

Box 2-C—The Polymerase Chain Reaction: Step-by-Step

As mentioned earlier, for the most part, all body cells contain the same DNA. Thus, DNA molecules in cells must regenerate copies of themselves each time a cell divides. DNA in all living cells continuously reproduces through a process called replication. During this process, the original strands in the DNA double helix unwind and serve as templates for the building of new, complementary strands, resulting in two identical copies of the original DNA molecule.

The polymerase chain reaction (PCR) is an in vitro technology based on the principles of replication. First described in 1985, PCR is now widely performed in research and clinical laboratories, and many see it as a critical DNA technology for forensic identification.

PCR involves the repeated duplication of a specific area of DNA. For example, consider the following sequence of DNA to be amplified using PCR:

```
-T-T-C-G-A-T-G-G-A-T-A-A-A-C-C-G-A-
-A-A-G-C-T-A-C-C-T-A-T-T-T-G-G-C-T-
```

In order to perform PCR, the sequence of the DNA at both ends of the region of interest must be known, and their complimentary sequences available as short pieces of purified DNA called primers. One primer must be complementary to the end of one strand the second to the opposite end of the other strand. In this case, the primers would be *-A-A-G-C* for the top strand of DNA and *-C-C-G-A* for the bottom strand. These two specific sequences flank the area the scientist wants to copy, and serve as the foundation to which bases can be added and the DNA strand copied. In PCR, the temperature of the sample containing the DNA to be amplified is raised to about 95° C, which results in the separation, or melting, of the double helix to yield single-stranded pieces:

```
-T-T-C-   G-A-T-G-G-A-T-A-A-A-C-C-G-A-

-A-A-G-C-T-A-C-C-T-A-T-T-T-G-G-C-T-
```

Copies of the primers are then allowed to hybridize to the DNA of interest, by lowering the temperature:

```
-T-T-C-G-A-T-G-G-A-T-A-A-A-C-C-G-A-
-A-A-G-C+

-A-A-G-C-T-A-C-C-T-A-T-T-T-G-G-C-T-
                                +C-C-G–A -
```

The scientist then sets conditions in the reaction that allow new copies of the DNA of interest to be produced from the primers (referred to as primer elongation). That is, DNA polymerase (a heat-stable version of the enzyme from *Thermus acquaticus,* a thermophilic micro-organism isolated from a hot spring in Yellowstone National Park) starts at the end of the primers and using bases (G,A,T,C) that are part of the reaction mixture, synthesizes complementary strands of each of the two single strands to yield two strands from the original one:

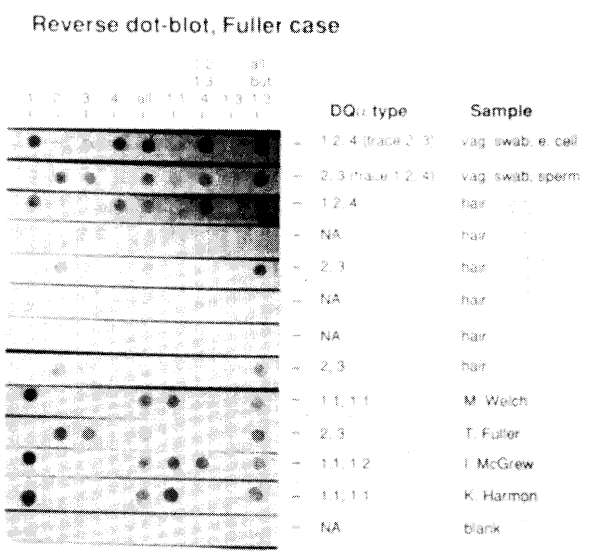
```
-T-T-C-G-A-T-G-G-A-T-A-A-A-C-C-G-A
-A-A-G-C-T-A-C-C-T-A-T-T-T-G-G-C-T----

-A-A-G-C-T-A-C-C-T-A-T-T-T-G-G-C-T-
----T-T-C-G-A-T-G-G-A-T-A-A-A-C-C-G-A-
```

Thus, one cycle of PCR has occurred, doubling the number of DNA copies from the original area of interest. After this first round of synthesis, and for each subsequent cycle, the temperature of the reaction is raised to approximately 95° C to separate the DNA strands. Primers are again allowed to hybridize to the strands, and DNA synthesis allowed to occur. After a second cycle of PCR, the two strands become four, and after 20 to 25 cycles of PCR, the original DNA sequence theoretically has been amplified about a rnillionfold. Generally, 20 to 30 cycles yield enough DNA to perform forensic analysis with one particular genetic locus, the HLA DQ *x-1* locus.

SOURCE: OffIce of Technology Assessment 1990.

Figure 2-7—DNA Typing Using PCR



PCR at the HLA DQa-1 locus using a type of dot-blot hybridization performed by Forensic Science Associates in a rape-murder case.

SOURCE: Cetus Corp., Emeryville, CA, 1990.

In research laboratories, PCR has been used to examine DNA for a range of novel applications involving minute or ancient samples. DNA from a single hair root, cell, or sperm has been amplified and analyzed (27,40), as have DNA from the remains of a 7,000-year-old body found preserved in a Florida peat bog (48) and a 17 million-year-old magnolia tree fossil (25).

Emerging Technologies

RFLP analysis and PCR are only two of a battery of DNA-based tests that can be used in forensic identification. For example, DNA typing techniques that combine principles of PCR and RFLP analysis are being developed in several laboratories. These methods apply PCR amplification to regions of DNA where known variants exist, and would allow RFLP-type analysis to be performed on increasingly smaller quantities of forensic samples (8,9,33,63). Another technique that could prove useful in forensic casework is a form of PCR called multiplex amplification, which simultaneously examines at least nine different polymorphic loci (11). Other research efforts are aimed at enhancing standard RFLP analysis for criminal casework, e.g., eliminating the use of radioactive isotopes by refining nonradioactive methods used in other applications (4,13,14,38,41,56).

Although not in the immediate future, one DNA technology widely used in research laboratories could find its way into forensic applications: DNA sequencing. DNA sequencing would be the ultimate genetic identification because it directly elucidates differences in the arrangement of the genetic alphabet (G's, A's, T's, and C's) among individuals' DNA. Success in adopting DNA sequencing as a forensic tool probably depends on geneticists identifying one or more highly polymorphic addresses (loci) for which DNA sequence data would yield positive identification among individuals. Although such loci remain, as yet, unidentified, many believe that with efforts under way to map and sequence the entire human genome (59), identifying such loci is only a matter of time. Existing automation for DNA sequencing could provide a boost for this technology, although the cost of such instrumentation could place sequencing beyond the reach of some public forensic laboratories.

DNA TYPING: PRESENT AND FUTURE USES

One advantage of DNA typing techniques lies in the array of ways they can be used. Many of the applications provide solutions to practical areas, yielding information previously unattainable. Compared to both subjective evidence like eyewitness testimony and objective evidence like traditional genetic markers, forensic DNA analysis can also provide more definitive and objective evidence to assist in the determination of both the innocence and guilt of persons.

Although traditional genetic tests provide the potential for a high degree of discrimination among different individuals, the upper limit is attained infrequently because of the instability of some of these markers in aged stains. Moreover, of the markers that retain their structure and activity in the dried evidence state, the number of observed or expressed forms is limited. In practice, the individualization of many evidentiary stains cannot be carried out to any great extent given the present array of traditional genetic landmarks (6). In general, conventional genetic tests used in forensic casework at best can associate a suspect to the evidence at about 90 to 95 percent certainty (42). DNA, on the other hand, is more stable, and the range of genetic variability revealed through batteries of DNA-based tests are much broader. Finally, in some cases, an

advantage of DNA v. conventional testing is that a stain from multiple contributors can be more readily detected and, perhaps, deciphered (22).

Thus today, many argue that DNA technologies stand to change the judicial landscape. With recombinant DNA methods, forensic practitioners isolate and examine DNA from traces of human biological material such as blood, semen, or hair roots. As noted, such analyses potentially provide forensic examiners with the ultimate in discrimination power—the ability to statistically connect samples to the exclusion of all other individuals except identical twins. On the other hand, questions about the reliability of forensic DNA analysis have been raised (see ch. 3) (37,47,55), as have privacy concerns (see ch. 5).

Nevertheless, the advent of DNA typing techniques has provided opportunities to apply DNA analysis to a number of situations, including:

- criminal investigations, particularly violent crimes, such as homicides and sexual assaults, and serial crimes;
- identification of unknown remains;
- paternity determination and child support enforcement;
- human rights abuses, such as people who have disappeared in Argentina (see box 2-D);
- immigration;
- missing children;
- incidents with multiple casualties (e.g., plane accidents, war);
- settlement of contested wills and estates; and
- baby swapping.

Other nonhuman applications include identifying purloined endangered species, wildlife management and protection, tracing illegal export of agricultural products, tracking genetic markers in breeding programs, and documenting pedigree, e.g., in thoroughbred horses. While each of these applications is likely to have significant impact, the current policy debates in the United States center on using DNA typing in criminal and paternity investigations.

Of the thousands of murders and nonnegligent manslaughter cases in this country (20,675 reported in 1988) (26), most remain unsolved. Although it is too early to gauge the impact of DNA typing on homicide investigations, estimates comparing casework where DNA typing is used v. non-DNA analysis indicate the effect will be significant (10). In fact, DNA tests might not appreciably affect solution rates, but could increase conviction rates (39). One analysis, however, suggests that DNA

Box 2-D—Mitochondrial Genes and Forensic Identification

Mitochondria are microscopic watermelon-shaped organelles found by the hundreds in every cell in the body. Often referred to as the cell's power supply, mitochondria produce the energy necessary for cellular functions. They carry their own genetic material-multiple copies of tiny circular pieces of DNA, about 16,500 base pairs total (compared with the 3.3 billion in a person's chromosomes).

An unusual aspect of rnitochondrial DNA (mtDNA) is its pattern of inheritance. Whereas individuals inherit half their chromosomes from their mother and half from their father, mtDNA is passed through the maternal lineage. That is, an individual inherits mtDNA only from his or her mother.

One particular region of mtDNA, known as the D-loop, has been found to be highly variable between unrelated individuals. It is generally stable across generations, however, so a child's version of this region almost never varies from his or her mother, sisters, brothers, or even grandmother, maternal aunts and uncles, and other genetic relatives on the *maternal* side.

Recently, scientists have taken advantage of PCR and mtDNA inheritance patterns to identify children kidnapped in Argentina between 1975 and 1983. In all, more than 9,000 people 'disappeared" in Argentina during this 8-year period, including more than 200 infants+ More than 120 pregnant women also were kidnapped, with most forced to bear their children in captivity before being killed. The children, now between 10 and 12 years old, were often sold on the black market. Today, using PCR to amplify the D-loop region of mtDNA, kidnapped children are being matched to surviving biological relatives because the mtDNA pattern of a surviving child is the same as any maternal relative, including any first cousins who are children of a maternal aunt.

SOURCE: OffIce of Technology Assessment, 1990, based on M.-C. King, "Genetics and the Disappeared: The Search for Two Generations," presentation at the 155th Annual Meeting of the American Association for the Advancement of Science, San Francisco, CA, January 1989; and M. Specter, "Microbiology Reunites Families: Long-Lost Children's Genes Match Parents," *The Washington Post,* p. All, Jan. 17, 1989.

Photo credit: Lawrence Livermore National Laboratory, Livermore, CA

Direct image of a chemically unaltered strand of DNA obtained using a scanning tunneling microscope. (Light microscopes magnify objects up to about 1,000 times; electron microscopes about 300,000 times. Scanning tunneling microscopes can magnify images up to 1 million times.)

typing might not significantly improve conviction rates for murders (49).

On the other hand, DNA typing has had, and will likely continue to have, a marked impact on rape cases (49). A woman is forcibly raped every 6 minutes in the United States. In 1988, 92,486 rapes were reported, but because generally less than half the victims report a rape, the actual number is estimated to be much higher. DNA typing of semen stains stands to significantly enhance positive association, because useful protein genetic markers in semen are limited (6). It can have a dramatic impact by exonerating suspects identified through eyewitness testimony who were not excluded by conventional genetic tests. DNA tests are also likely to play an important role in serial crime investigations.

Using blood tests in paternity disputes has been an ongoing practice for over six decades (18). Approximately 150 conventional parentage testing facilities exist in the United States (61). Presently, a limited number of these facilities provide DNA testing services for parentage testing. Indications are, however, that many laboratories performing traditional genetic typing for paternity have begun or plan to include DNA testing, with an eye toward replacing traditional methods.

For paternity disputes, the capability provided by DNA typing appears to be resulting in fewer cases being brought to trial (28), with attendant potential savings to both the clients and the public. Since paternity determination is often a prerequisite to child support actions, DNA typing will have an impact on child support enforcement efforts (61). In the State of Virginia, for example, social service agencies are actively pursuing DNA testing to assist them in efforts to enforce child support laws (16).

The technological advances that allow an individual's genetic blueprint to be examined give rise to concerns that forensic uses of DNA testing will extend beyond the type of tests generally employed today to DNA tests presently confined to clinical and medical uses. Genetic information has been used for political purposes in the past (59). The availability of numerous DNA tests that can reveal medical or diagnostic details about an individual (3,7,57) raises concerns about ensuring the privacy of a person's genetic history, especially when DNA or tissue samples are stored in addition to DNA profile data (see ch. 5).

FINDINGS AND SUMMARY

At present, forensic uses of technologies grouped under the umbrella terms "DNA testing, DNA typing, DNA profiling, or DNA identification" involve two basic techniques: restriction fragment length polymorphism analysis or polymerase chain reaction. RFLP protocols can be subdivided further into single-locus probe analysis and multilocus probe analysis. RFLP analysis has been used by research and clinical laboratories for over a decade; PCR for a few years. In the forensic setting, both techniques can be used to detect differences among individuals at the DNA level.

The molecular tools of DNA analysis offer forensic scientists a greater degree of specificity than traditional genetic methods. DNA technologies provide the best avenue for unequivocal exclusion of innocent suspects. DNA tests can also yield a statistical result that is effectively positive identification.

DNA typing technologies have a broad range of practical applications, including criminal investigations, paternity determination, identification of unknown remains, human rights abuses, and immigration. As more information is gained through genetic research, including efforts to map and sequence the human genome, the range of applications, information gained, and technologies involved is likely to expand.

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