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DNA FINGERPRINTING

An Interactive Qualifying Project Report

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ABSTRACT

The purpose of this IQP was to examine the fundamental principles of DNA fingerprinting technology, analyzing its growth within both the legal and scientific communities, to provide an effective documentation of the impact of this new technology on society. Various methods used to obtain DNA fingerprints were analyzed, including the proper techniques for handling, collecting and storing DNA samples prior to their use in forensic applications. Several landmark court cases using DNA forensics were explored to document the technical proceedings involved in the admittance of DNA evidence into U.S. courts. A few DNA court cases of popular notoriety were covered to give readers a frame of reference for DNA fingerprinting and forensics within the media and current events. Ethical issues of privacy stemming from the advent of DNA forensics and DNA databases were examined.

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PROJECT OBJECTIVE

The purpose of this IQP was to research the new technology and applications of DNA fingerprinting, and document the impact of this technique on society. A main goal of the project was to help eliminate doubt about this controversial technique, directing most of the information toward potential jurors and the general public. This goal was completed by describing, in-depth, the various methods used to obtain DNA fingerprints, as well as protocols for handling, collecting, and storing DNA evidence. Landmark court cases were researched that dictated precedence for admitting DNA evidence into the courtroom. A few sensational court cases were explored that brought DNA fingerprinting and forensics to the public's attention with the use of media. DNA databases were discussed relative to the legal and ethical issues surrounding this new technology.

CHAPTER 1: DNA FINGERPRINTING

DESCRIPTIONS AND TYPES

Introduction

A key component to any credible science is the ability to find unique differences between subjects of interest. The field of biology is a perfect example of this occurrence. For as long as humans have been studying the life around them, there has been a need to distinguish between species of organisms, and even identify individuals within a species. At times this can be challenging since the genetic makeup of members within most species is 99% identical (The Economist, 2004). While members of a species seem to have a great deal in common, many techniques have been developed to identify individuals based on distinct features and specific traits.

Over the past century, humans have made several advances in the field of distinction and identification of individuals, mostly of other humans. While fingerprints, dental record, blood typing and retinal scans were once key tools in this process, a new technique has emerged over the past few decades: DNA fingerprinting. DNA fingerprinting, or DNA profiling to many, is probably most famous for its use in criminal justice, specifically in the case of O.J. Simpson and the investigation of the murder of Jonbenet Ramsey (Meeker-O'Connell, 2004). However, there is still a great deal of ambiguity within the public understanding of the nature of DNA fingerprinting and its multiple applications. So this project investigates the new technology of DNA fingerprinting, and documents its effects on society with respect to landmark court cases involving DNA evidence, and the ethics of DNA databases.

Background on DNA and Genetics for DNA Fingerprinting

To truly have an appreciation for a technique that is rapidly becoming more common in our society, there are several aspects of DNA fingerprinting that require clarification. At the most basic level, the name "DNA *finger*printing" may be a bit misleading. Unlike its inkpad precursor, DNA fingerprinting actually has very little, if anything, to do with fingers. The material used in DNA analysis can come from hair, fingernails, bone samples and bodily fluids such as saliva, blood (white blood cells specifically since red blood cells have no nuclei and therefore no accessible nuclear DNA) or semen (Meeker-O'Connell 2004). Almost any sample containing cells, can be used for DNA fingerprinting.

Cells, the "fundamental units of life," are the building blocks of all organisms (DNA From The Beginning, 2002). Deoxyribonucleic Acid (abbreviated DNA), the genetic blueprint of life and the material used in DNA fingerprinting, is found within the nucleus (as well as the mitochondria) of cells (DNA From The Beginning, 2002). Stored tightly within the nucleus, the components and structures of DNA are categorized by increasing levels of organization.

On a molecular level, DNA consists of complex molecules, known as nucleotides, composed of a sugar, a phosphate group and one of four nitrogen bases (DNA From The Beginning, 2002) (see Figure 1A). The nucleotides are bonded together in a certain pattern in a strand of DNA. The four bases, Adenine, Cytosine, Guanine and Thymine (abbreviated A, C, G and T) are the letters of this code (Bruzel, 1998). Two strands of these sequences form DNA's twisted "double helix" structure, where complementary bases from opposite strands bond to each other (See Figure 2). This bonding is very specific, forming specific "base pairs." In DNA base pairing, "A" always bonds with "T", and "C" always bonds with "G" (Genetic Science Learning Center, 2007).

In addition to the complementary base pairing seen in DNA, there is another interesting characteristic of its double helix. A DNA molecule displays an "anti-parallel" orientation of its strands (Freeman, 2005). The strands in DNA align parallel to each other, but run in opposite directions.

The direction of DNA runs according to the orientation of carbon atoms within the deoxyribose sugar molecule (Freeman, 2005). One end of the strand, called the 3' (pronounced "three prime"), is the end that ends with a hydroxyl group (-OH) bonded to the 3' carbon in the sugar molecule (Freeman, 2005). The other end of a DNA strand, deemed 5' (pronounced "five prime"), is the end of the DNA molecule with a chemical group attached to the 5' carbon in the sugar molecule.

When a phosphate group is the chemical group attached to the 5' carbon, the phosphate group can bond to the hydroxyl group on the 3' end of another molecule of deoxyribose, resulting in the chain of sugar molecules that become strands of DNA (Freeman, 2005). While this may seem a bit complex, the most critical point to understand is that the 3' end of one DNA strands lines up next to the 5' end of the other strand in a DNA molecule (see figure 1B). When a particular sequence of DNA is in the direction towards the 5' end, it is deemed "upstream," and a sequence towards the 3' end is deemed "downstream" (Freeman, 2005). The impact of this orientation on replication and DNA fingerprinting will be explained further in the "PCR" section.

A B

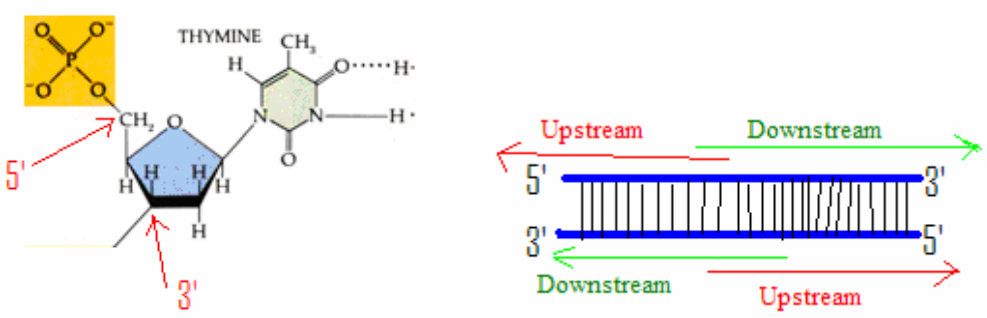


Figure 1: Panel A. Atomic Model of a Nucleotide with Thymine as its Nitrogen Base ©2004 Ohio University (Ohio University, 2004). Panel B. Simplified Model of Antiparallel orientation of DNA.

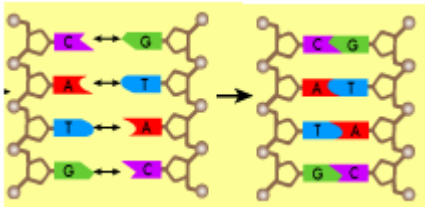


Figure 2: Complementary DNA Strands (Not Twisted for Clarity) (Genetic Science Learning Center, 2007).

Moving up in organization, base pairs within DNA form a code based on specific order of these "letters." These codes are commonly known as "genes": base sequences that often direct protein synthesis (Bruzel, 1998). There are approximately 40,000 genes in human DNA ("Genes," 1999). These different genes are located at specific locations on different sections and segments of DNA, separated by areas of sequence that do not code for proteins.

Moving up from individual helices, multiple segments of DNA are coiled tightly around histone proteins to form structures known as chromosomes (Betsch, 1994) (see Figures 3 & 4). Within the nucleus, chromosomes come in pairs, one inherited from the mother and one from the father (Freeman, 2005). Humans have twenty-three pairs of chromosomes, each containing thousands of genes at a specific location, or loci, on a certain chromosome (Meeker-O'Connell, 2004). Each gene comes in multiple forms, known as alleles (University of Arizona, 2006). Since chromosomes in chromosome pairs contain the same genes, humans contain two alleles of a certain gene. If both alleles are the same, an organism is "homozygous" for that gene. If the alleles are different, the organism is "heterozygous" (University of Arizona, 2006). From the following sections, it will become quite clear that alleles, genes and nucleotides are key components of DNA involved in the theory behind DNA fingerprinting.

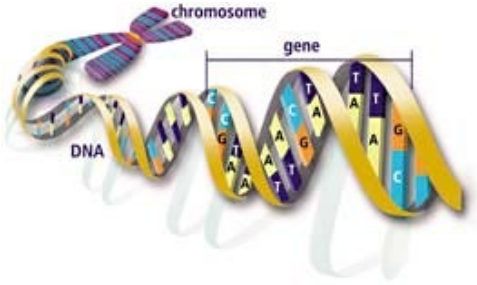


Figure 3: Organization of DNA Into Chromosomes (Meeker-O'Connell, 2004).

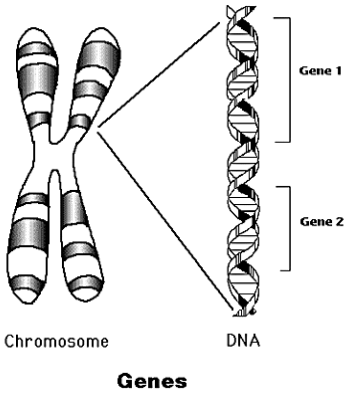


Figure 4: Chromosome and Gene Loci ("Genes," 1999).

Mitochondrial DNA

While the nucleus is the most commonly known site of DNA storage in the cell, there is also another cellular source of DNA used in DNA fingerprinting: the mitochondria. Mitochondria (singular mitochondrion), commonly referred to as the "powerhouses of the cell," are small organelles within the cell that produce the energy needed for cellular functions (Freeman, 2005). Interestingly, each mitochondrion contains its own chromosome, with a different DNA sequence than any of the nuclear DNA (Freeman, 2005). Unlike nuclear DNA, mitochondrial DNA, or mtDNA, comes in the form of one circular chromosome, instead of 23 linear chromosome pairs (Budowle et al., 2000) (see Figure 5).

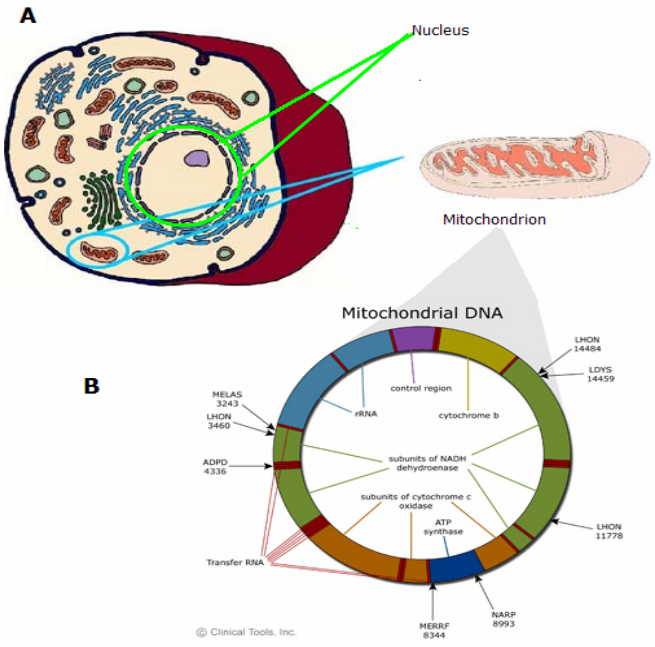


Figure 5: A. Location of Mitochondria Within the Cell. Only a few are shown for clarity. ("Mitochondria," 2007). B. Mitochondrial DNA. Note its circular structure unlike nuclear DNA ("Mitochondrial DNA," 2007).

The DNA found in mitochondria is analyzed, for the most part, using the same basic procedures as nuclear DNA, with a slight increase in the level of detail used during DNA sequencing (Inman and Rudin., 1997). However, there are several advantages of using mitochondrial DNA for DNA profiling applications, as well as a few slight downsides to its use. While the advantages present in mtDNA make it a necessity in certain circumstances, its downsides are a major factor in the favor shown towards nuclear DNA as the major source for most DNA profiling.

As an advantage in some cases, mitochondrial DNA tends to be a much simpler source of information. Only about 16,000 base pairs in length, mtDNA only has one non-coding region, with two hypervariable regions, called HV1 and HV2 (Butler, 2005). As with most of the features of mtDNA, this tends to be both an advantage in some cases and a disadvantage in others. While having a small sequence is advantageous for its ease in characterization, it is bittersweet due to the fact that it proves less decisive as a means of differentiating individuals (Inman and Rudin, 1997).

Despite this drawback, two other features of simplicity in mitochondrial DNA allow for its utility in many DNA profiling applications. One of these bonuses lies in the fact that mtDNA is only maternally inherited, meaning mitochondria only come from the mother, and offspring only have one allele for each locus (Budowle et al., 2000). Similarly, mtDNA does not undergo recombination, meaning that its sequence is highly conserved from generation to generation (Budowle et al., 2000). While this means that siblings and all maternal relatives in a family would have the same sequence, which is a disadvantage for individual DNA identification, it also means that mtDNA can be used in many genealogical studies, tracing the origins of human

history and even tracing the origin of human beings as a species, a hot-topic in the field of biology (Budowle et al, 2000).

Even though it is highly favored for applications in molecular archeology and genetic family trees, mtDNA can still be somewhat advantageous for identification as well. The benefit of mtDNA for discernment of individuals comes from one final characteristic of mitochondria themselves: their high population within the cell. Amazingly, some cells can contain hundreds of mitochondria (Butler, 2005). With each mitochondrion containing 4-5 copies of DNA, this allows for a large chance of finding a source of DNA, even in old and degraded sources with simply not enough nuclear DNA available (Butler, 2005).

Although there is less information stored within mitochondrial DNA, the large number of DNA copies associated with mitochondria provides an alternative to nuclear DNA in extreme cases of tissue and cell degradation. After all, some DNA sample is better than no sample. John Butler, author of Forensic DNA Typing, puts it best, stating, "though nuclear DNA contains much more information, there are only two copies of it in each cell (one maternal and one paternal) while mtDNA has a bit of useful genetic information times hundreds of copies per cell" (Butler, 2005). While this is a brief summary of the differences between the two DNA sources, Table 1 provides an in-depth comparison of mitochondrial DNA to nuclear DNA.

Characteristics	Nuclear DNA	Mitochondrial DNA
Size of Genome	~3.2 billion bp	~16569 bp
Copiers per Cell	2 (1 allele from each parent)	Can be > 100
% of total DNA per cell	99.75%	0.25%
Structure	Linear; packed in chromosomes	Circular
Inherited from	Father & Mother	Mother
Chromosomal Pairing	Diploid (2)	Haploid
Generational Recombination	Yes	No
Replication Repair	Yes	No
Unique	Unique to individual (except identical twins)	Not unique to individual (same as maternal relatives)

Table 1: Comparison of Mitochondrial and Nuclear DNA (Butler, 2005)

Accordingly, to get the best of both worlds under certain conditions, investigators often use both mtDNA and nuclear DNA profiles together in an attempt to get the most information possible. The most notable case of this comes from 9/11, when investigators made use of mtDNA to supplement partially degraded DNA found in less than ideal victim remains (World Trade Center, 2001; Butler, 2005). Additionally, mtDNA is used in conjunction with nuclear DNA in many other applications involving degraded sample sources. Many of the analyses discussed in the "Applications" section involve mitochondrial DNA as a major component as well as applications in numerous other fields of study in genetic research.

Theory and Advantages of DNA Fingerprints

With this background information on DNA and genetics in mind, it's a bit easier to understand what a DNA fingerprint actually is. By separating DNA samples or DNA fragments based on certain characteristics, such as size and sequence, investigators create a pattern of DNA that can be used to identify and distinguish individuals (Betsch, 1994). The pattern usually

resembles a barcode (see Figure 6) and is a very accurate tool for measuring similarities in DNA sequences (Budowle et al., 2000). With this ability to match unknown samples of DNA to suspected sources, DNA fingerprinting has become a highly advantageous procedure in many applications.

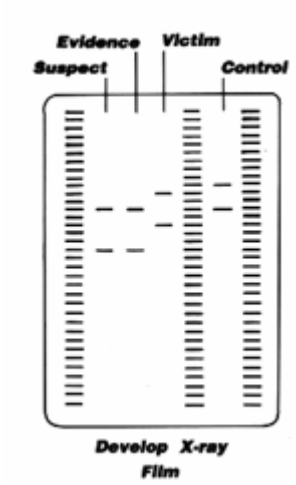


Figure 6: Stylized Depiction of a DNA fingerprint Created via an RFLP. Example of one of several types of DNA fingerprints (Student Web Projects, 2006).

There are several advantages of DNA fingerprinting over older identification techniques, such as conventional fingerprinting. Recently, there has been some doubt as to whether conventional fingerprints are truly unique. However, except for the case of identical twins, DNA sequences are completely unique to individuals (Meeker-O'Connell, 2004). Accordingly, current DNA fingerprinting methods boast accuracy probabilities of approximately trillions to one (The Economist, 2004).

For criminal justice specifically, another advantage to DNA fingerprinting lies in the fact that DNA sequences cannot be altered. Conventional fingerprints, on the other hand, can be altered by surgery or injury (Betsch, 1994). Also, conventional fingerprints can be concealed using gloves. DNA however, is found in so many types of cells that it is almost impossible not leave a sample of DNA at a crime scene (Butler, 2005). For these reasons, DNA fingerprinting can be deemed quite reliable, accurate and versatile.

Types of DNA Fingerprints and Methods of Production

To actually trust the accuracy of a particular technique and understand its applications, the mechanism by which the technique is performed must be completely understood. As with any technology, DNA fingerprinting has evolved since its inception as new scientific advances arose. The original procedure, developed by Dr. Alec Jeffreys in 1985, has been modified and improved considerably in order to make the procedure more efficient and accurate (Butler, 2005).

The basis for most DNA fingerprinting procedures does not lie in the "coding" regions of DNA, known as "exons," where genes encode proteins. This fraction of DNA, which only constitutes 3-10% of the entire human genome, is generally conserved in humans, and would therefore differ very slightly between individuals (Bruzel, 1998). For this reason, investigators tend to use non-coding regions, known as "introns," to spot differences in individuals' genetic profiles, since these regions are more likely to vary between different people (The Economist, 2004).

The most crucial breakthrough in developing a means to distinguish between certain introns came in the discovery of stutter regions (The Economist, 2004). These sequences consist of sections of DNA that have a specific pattern of bases repeated multiple times across a stretch of DNA (The Economist, 2004). The repeated pattern, "…CATG-CATG…" for example, is called the "target sequence" and is flanked on each end by a known sequence of bases (Davidson College, 2006). Using many progressive experimental techniques, Dr. Jeffreys found that many stutter regions are found predictably at the same loci on chromosomes for every individual (The Economist, 2004).

The key to identifying certain areas of repetition was to develop a probe that could bind to the repeated sequence in a single strand of DNA by complementary base pairing (Davidson College, 2006). For example, the probe of a "CATG" would be "GTAC." The probe, a radioactive segment of DNA, could be detected easily and act as a highlighter for repeated sequences of complementary repeats (The Economist, 2004) (see Figure 7). It could therefore be inferred that stutter sequences occurred at loci where multiple probes lined up next to each other. With this in mind, Dr. Jeffreys and his team set out to find a number of specialized "single-locus" probes, or SLPs, for certain stutter regions (The Economist, 2004).

Figure-7: Probing a Specific Locus by Complementary Base Pairing with One Strand of a Double Helix. The probe is shown in yellow, and binds to its complement sequence on the DNA strand (Urovysion, 2006).

Beyond finding specific loci with repeated sequences, Jeffreys found that at each locus the stutter unit for all humans is the same. However, the number of times it's repeated, and therefore the length of a target sequence, is different for many individuals and can result in hundreds of unique alleles for a specific gene (Budowle et al., 2000). The separated bands that make up the "barcode" appearance of DNA fingerprints result from two different allele lengths for each SLP (The Economist, 2004). By analyzing these alleles, Jeffreys hoped to compare the genetic makeup of individuals based on the combinations of alleles they possessed for each locus.

On the other hand, since children inherit one allele from their father and one from their mother, it's possible that closely related individuals could have the same two alleles for one

given locus; however, the possibility of having the same alleles at several different loci decreases exponentially with each additional section analyzed (The Economist, 2004; Freeman, 2005). Therefore, it follows that the differing lengths of these sections at several different loci could be used to distinguish between individuals.

RFLP and VNTRs

There are several types of repeated segments used in DNA fingerprinting and, accordingly, different procedures used to analyze them. The regions first discovered by Dr. Jeffreys were deemed "minisatellites" (The Economist, 2004). These types of larger repeated sequences are also called VNTRs: Variable Number Tandem Repeats. "Variable Number" describes the highly diverse nature of the number of repeats in an allele, while "Tandem Repeats" explains that repeated units are located directly adjacent to each other (Micro 7 UCLA, 2004). The length of the repeated sequence in a VNTR ranges from 6 to 500 base pairs (Freeman, 2005). For example, the "ALU family" of VNTRs has a repeat unit 300 base pairs long repeated over 500,000 times throughout different sections of our DNA (Micro 7 UCLA, 2004).

After discovering the existence of VNTRs and their respective loci, the next logical step was to create a procedure for separating and distinguishing the different sequences at a given locus. The first technique developed to accomplish this was known as Restriction Fragment Length Polymorphism, or RFLP (Hill, 2004). In this process, VNTRs are cut at specific regions to produce segments of varying lengths which could be quantified and used to create a DNA fingerprint (Budowle et al., 2000). "Restriction Fragment Length" refers to the method in which the DNA is cut. "Polymorphism" refers to the trend in these sequences to have many different forms or lengths (The Biology Project, 2000).

The process begins by extraction of the DNA, usually by breaking open cells from tissue samples with an organic or salt-based solvent (Budowle et al., 2000). However, the extraction process must be mild enough not to damage the DNA sample or expose it to the cell's own enzymes (Budowle et al., 2000). These side-effects can lead to degradation of the sample, resulting in an insufficient profile.

Once the DNA has been properly extracted and isolated from the rest of the cell, RFLP proceeds with the actual process of restriction. Restriction is the process whereby DNA is cut at specific sequences using restriction enzymes (Freeman, 2005). Restriction enzymes, or restriction endonucleases, cut at very specific regions known as recognition sites (Hill, 2004). These sites are usually 4 to 6 base pairs long and occur at several different locations in our DNA (Hill, 2004). To use RFLP, the known sequences that flank the target segments must be a recognition site for an available restriction enzyme (Davidson College, 2006). Since restriction enzymes are isolated from a wide variety of bacteria, it's possible to find an enzyme for many restriction sites corresponding to numerous locations in human DNA (Hill, 2004).

For example, EcoRI is an enzyme isolated from the bacteria *E. coli* that cuts the sequence "GAATTC" and is often used in various restriction techniques (Davidson College, 2006) (See Figure 8). However, FBI laboratories generally use an enzyme known as HaeIII, which cuts the sequence GGCC which is found more frequently in our genome (Budowle et al., 2000).

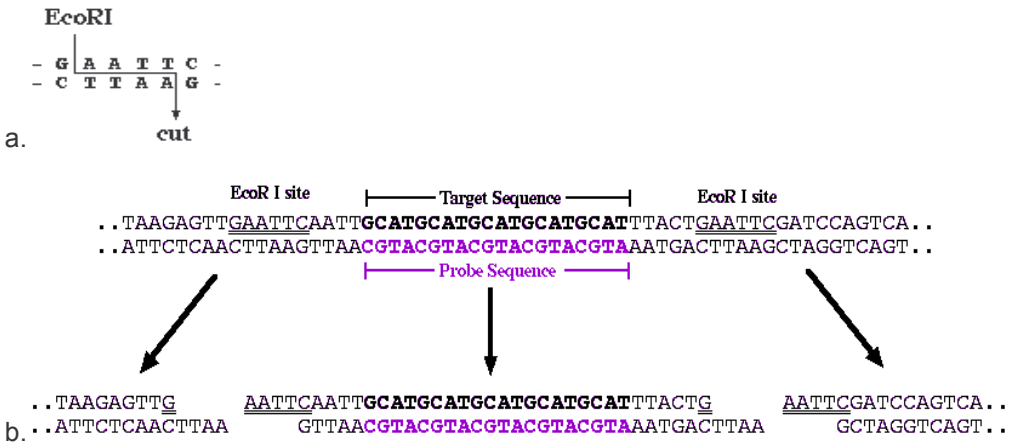


Figure 8: a. Recognition Site of EcoRI. b. Cleavage by EcoRI in RFLP. (Davidson College, 2006).

Since the distance between restriction sites differs based on the length of the polymorphism, segments of differing lengths can be produced for two different individuals. To identify the varying lengths of the segments, a process known as electrophoresis is used (Budowle et al., 2000). In electrophoresis, the DNA segments are transferred to an agarose medium, a porous gel made from a key component of seaweed (Betsch, 1994). Beforehand, the agarose gel is submerged in a buffer solution to maintain its gelatinous consistency (Budowle et al., 2000).

When a charge distribution is applied to the gel, DNA, which has an overall negative charge due to its phosphate residues, travels towards the positively charged end of the plate (since opposites attract). Since smaller fragments can travel through the gel's pores faster than larger ones (picture an 18 wheeler racing a motorcycle through a thickly wooded forest), smaller fragments move closer to the positive charge in a given length of time (The Economist, 2004) (Figure 9). To create a fingerprint, the cut fragments of two or more samples to be compared are usually placed side by side in lanes of the gel to be separated based on size. Additionally, lanes with fragments of known length are often run along side the unknowns as molecular weight markers (Budowle et al., 2000).

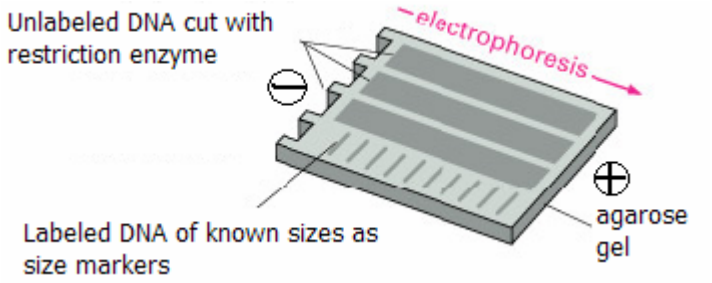


Figure 9: Process of Electrophoresis. ("Southern Blotting," 2004)

Once the fragments have been separated, they must be visualized to determine a match. However, unmarked DNA in agarose gel can not be visualized (Inman and Rudin, 1997). Before transferring the fragments to a membrane for visualization, they must also be denatured, a process where the double-stranded DNA helix is separated out into two separate strands (Budowle et al., 2000). This is often accomplished by exposing the DNA to a basic solution such as Sodium Hydroxide (Khalsa, 2004). This step is essential for two reasons. The first reason is that the nylon or nitrocellulose membrane that the DNA is transferred to after being denatured is only able to bind the DNA firmly if the DNA is single-stranded (Budowle et al., 2000). Secondly, once the DNA is transferred to the membrane, a labeled probe is needed to visualize the DNA, and the probe can only base-pair with single-stranded DNA (Khalsa, 2004).

Once the DNA is denatured, a technique known as Southern Blotting, named after its inventor Edward Southern, is used (Khalsa, 2004). Southern Blotting most often makes use of the phenomenon of capillary action (Budowle et al., 2000). In capillary action, a liquid (in this case an alkali transfer solution) is drawn into another substance by attractive forces between the molecules of the liquid and interaction with the other substance (the membrane in our case) (Budowle et al., 2000). Most often, the membrane is laid onto the agarose gel and then overlaid by dry paper towels, which draw the transfer solution and DNA fragments onto the membrane (Budowle et al., 2000) (Figure 10).

Once the DNA is blotted onto the membrane, it is fixed in its position and remains in its single stranded form ("Restriction Enzyme Digest Analysis", 2002). With the DNA transferred to it, the membrane is pulled off of the agarose and exposed to UV light in order to form stronger (covalent) bonds between DNA fragments and the membrane (Khalsa, 2004).

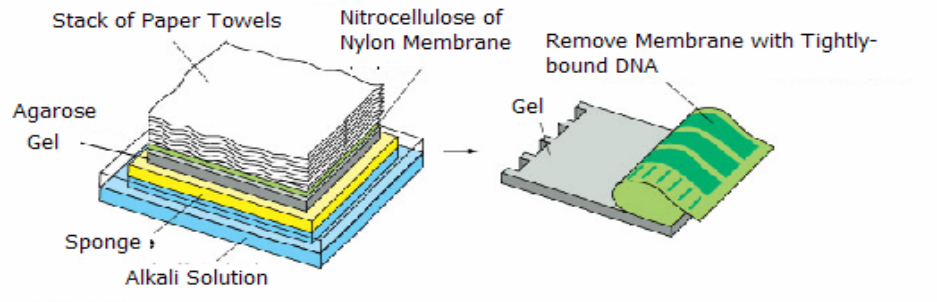


Figure 10: Southern Blotting and Removal of Membrane. ("Southern Blotting," 2004)

Bound to the membrane, the DNA can then be labeled by treating it with probe segments of single-stranded DNA containing radioactive phosphorous isotopes (^{32}P) (Budowle et al., 2000). Like the probes used by Jeffreys to initially find loci of repetition, the probes bind to the complementary bases in the stutter sequences of the DNA fragments in a process known as "hybridization" (Khalsa, 2004). Prior to hybridization, a preliminary step known as "prehybridization," that blocks non-specific binding sites on the membrane, must be done to prevent probes from binding to sequences other than the DNA fragments (Budowle et al., 2000). Also numerous complex and sensitive conditions, ranging from temperature to probe concentration, must be optimized in order to allow an efficient rate of hybridization (Budowle et al., 2000).

Once the probes are hybridized to the fragments, excess probe segments are washed away to leave only the probes that are bound to target DNA fragments (Betsch, 1994). Once this is accomplished, the membrane is exposed to X-ray film, which shows the banding pattern through its sensitivity to the radioactive probes (The Economist, 2004). Alternative methods to radioactive procedures include the use of chemiluminescence and bioluminescence which utilize light as a method for visualizing the DNA fragments' patterns (Budowle et al., 2000).

Once this process is completed, the results can either be analyzed to find a match, or repeated with another locus and probe to increase accuracy (Betsch, 1994). The final products of

RFLP analysis often show several distinct lines of fragments and serve for many practical applications. The RFLP fingerprints in Figure 11, while appearing somewhat complex, serve as examples of the characteristic fingerprints analyzed in various genetic and forensic applications.

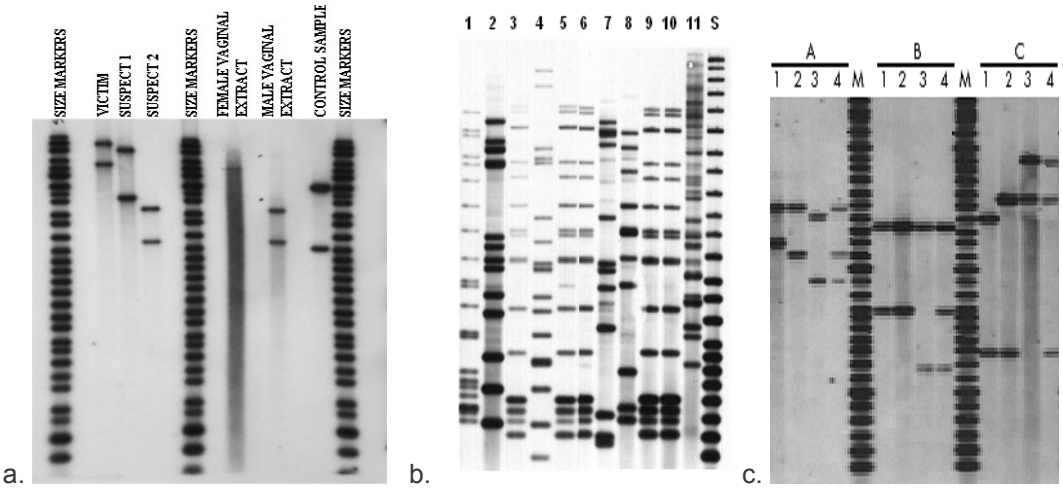


Figure 11: a. Single-Locus RFLP Analysis Used for Rape Investigation ("Thompson Autorads," 1999). b. Multi-Locus RFLP Used in Tuberculosis Study (Center for Disease Control and Prevention, 2007) c. Paternity Tests of 3 individuals (A, B, C) using RFLP (QIAamp System, 2007)

Examining the Final Product of RFLPs

Before going on to another form of DNA fingerprinting, an example of how DNA fingerprints are analyzed to match identities may be useful. While the examples in Figure 11 are analyzed by experts and are therefore a bit complicated to explain, s simplified model of a DNA fingerprint (Figure 12) can be used to explain the overall theory behind using a seemingly chaotic series of bands to identify an individual. As a simplification, only one locus was analyzed. Each locus can either have one or two alleles for each individual which appear as one or two bands in each lane. The bands show the positions where numerous fragments of the same length stop at the same spot and, once probed and exposed to a sheet of X-ray paper, form the characteristic barcode appearance on the paper.

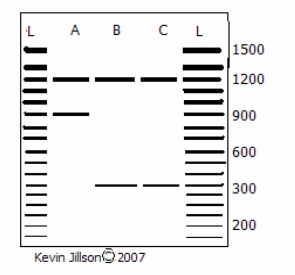


Figure 12: Stylized Representation of an RFLP DNA fingerprint. Lane L, DNA ladder or marker. Lane A, suspect A. Lane B, suspect B. Lane C, crime scene sample. Note that suspect B matches the crime scene DNA, showing he was present at the crime scene.

In Figure 12, the two vertical lanes marked "L" represent molecular weight markers. The markers represent an assortment of DNA bands of known sizes. Currently, the DNA used is most often synthetic. The markers are run in electrophoresis alongside the DNA samples to be analyzed. Although, these are more commonly used in other forms of DNA fingerprinting, they provide a useful means for determining the relative masses of the bands from each DNA sample. The numbers to the right of the figure are the number of base pairs in several of the marker bands and act as a scale for the relative masses of each band.

Let's say that this RFLP was used in a murder case. Supposing lane A comes from a blood sample from one suspect, and lane B comes from a blood sample from another suspect, the two band patterns could be compared to the DNA in lane C, perhaps a sample of hair found at the crime scene, in hopes of ruling out one suspect and identifying the other. Looking at lane A, it can be interpreted that suspect A has two different alleles for this given locus. The bands in lane A line up horizontally with the bands in "L" lanes corresponding to 1200 and 900 base pairs in length. The sample found at the crime scene however, has bands at 1200 and 300 base pairs. Therefore, while both lanes have a band at 1200, the DNA sample in lane A does not match with both bands of the DNA sample found at the crime scene and it can therefore be inferred that the two came from different sources. Lane B, however, has both a band at 1200 base pairs and 300 base pairs. With this information, it is clear that DNA from suspect B could possibly have come from the same source as the DNA found at the crime scene. However, the banding patterns for one locus are estimated to be common to 10,000 people in the population, so multiple loci must be analyzed for a positive match (Inman and Rudin, 1997).

PCR and STRs

PCR

While RFLP analysis of VNTRs seemed to be an exceptional method for creating DNA fingerprints, like most technologies, it had several disadvantages that scientists aimed to remedy. The greatest drawback to RFLP type DNA fingerprinting is the relatively high amount of undamaged DNA sample needed to run the analysis (Butler, 2005). With criminal forensics and molecular archeology, there is often only a trace of blood or one small hair available to analyze, often limiting DNA profiling capabilities. Also, RFLP analysis tends to be quite time-consuming, taking anywhere from six to eight weeks to complete (Butler, 2005).

However, in 1983, Kary Mullis, then a scientist at a small biotech company, developed a process that would eventually revolutionize DNA fingerprinting (The Economist, 2004). His procedure, known as the Polymerase Chain Reaction, or PCR, was a form of "molecular Xeroxing" which could rapidly replicate millions of copies of a small DNA sample with high fidelity and sensitivity to DNA sequence (Inman and Rudin, 1997). This new technique provided a way to gain genetic information from samples that might have been limited by their lack of abundance or degraded starting material (Inman and Rudin, 1997).

The process of PCR makes use of DNA's double stranded nature and a modified version of DNA replication. In general terms, the process starts off with a single molecule of double-stranded DNA and turns each of the strands into an individual DNA molecule, essentially yielding two molecules from one. By carrying out the replication multiple times, an adequate number of DNA strands can be produced with numbers equal to 2 raised the n^{th} power, where n equals the number of times PCR is performed (Brown, 2006).

The specifics of PCR are a bit more difficult to grasp, but can be easily clarified by analyzing the process step by step. Once the DNA sample is extracted (usually by the same

methods used in RFLP), the first step is to separate the two strands of the DNA helix (Brown, 2006). This denaturing step is accomplished by heating the DNA sample to around 95 degrees Celsius (Budowle et al., 2000). At this temperature, each DNA molecule separates into two individual strands.

Next, an interesting method is used to replicate a specific target sequence in the DNA, the locus used in the eventual DNA fingerprint (Inman and Rudin, 1997). To analyze specific sequences in DNA, primers are synthesized with a specific sequence of approximately 20 nucleotide bases (Brown, 2006). These short sequences, otherwise known as oligonucleotides, are single stranded, and base-pair to DNA at specific locations, much like the probes used in RFLP (Budowle et al., 2000). However, the sequences that primers hybridize with are not within the target sequence, but directly flank the sequence, with one primer attached to each strand on opposite sides of the target sequence (Inman and Rudin, 1997) (see Figure 13).

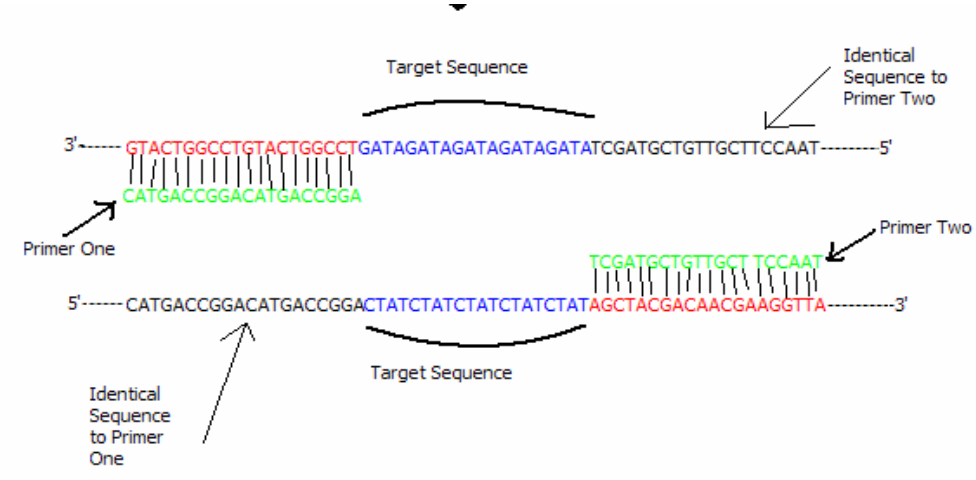


Figure 13: Simplified Version of Primer Hybridization to Separated DNA Strands During PCR.

By having two primers, each on opposite strands, specific sequences within the DNA are replicated in the subsequent steps, without replicating unwanted DNA sequences. Since these known sequences only occur at a particular locus of DNA, target sequences are easily identified

and standardized (Brown, 2006). In essence, the primers serve as markers for where to start DNA replication on each strand and by combining both start regions, only the sequence in between is synthesized (Inman and Rudin, 1997). An example to follow below will elucidate this point.

To allow the primers to hybridize with the flanking sequences, in a process known as annealing, the DNA sample is then cooled down to 45-65 degrees Celsius (Budowle et al., 2000). The exact temperature is determined by the length and sequence of the primers, but almost always lies within this temperature range (Budowle et al., 2000). To ensure that only primers bind to the separated DNA strands and none of the original DNA strands re-pair with each other, a large excess of primer is added to the solution containing the DNA samples (Brown, 2006).

Once the primers successfully pair with their complementary sequences in the DNA, the target sequence is then replicated starting at the primer, a process known as "primer extension" (Inman and Rudin, 1997). To accomplish this, two things are needed: a surplus of each of the four types of nucleotides (those containing A, T, G, or C) and an enzyme to link the molecules together and allow for complementary base pairing (Brown, 2006). However, the enzyme used in PCR must be able to survive and function at the hot temperatures needed to separate strands of DNA (Inman and Rudin, 1997). To find the appropriate enzyme, scientists looked to bacteria that live in hot springs, where temperatures can reach well above 60 degrees Celsius (Butler, 2005). One strain of bacteria they found, called *Thermus aquaticus* (Taq), had the perfect enzyme needed for PCR: Taq Polymerase (Budowle et al., 2000). Taq polymerase is where the "Polymerase" part of the "Polymerase Chain Reaction" comes from.

While primers attach to DNA sequences at 45-65 degrees, Taq polymerase works best at around 72 degrees, so the process is again heated back up to 72 degrees (Budowle et al., 2000).

At this temperature, the primers stay attached to their complementary sequences, and Taq polymerase works at its highest efficiency (Brown, 2006).

The ability of Taq polymerase and PCR to amplify the discrete target section of DNA comes from the antiparallel orientation of the DNA molecule itself. Taq polymerase can only replicate DNA sequences in the 5' to 3' direction (Butler, 2005). Therefore, the two primers serve as the 5' ends of their respective DNA strands and synthesis of new DNA travels only in the 3' direction, leaving any unwanted DNA upstream from the primer not replicated (see Figure 14).

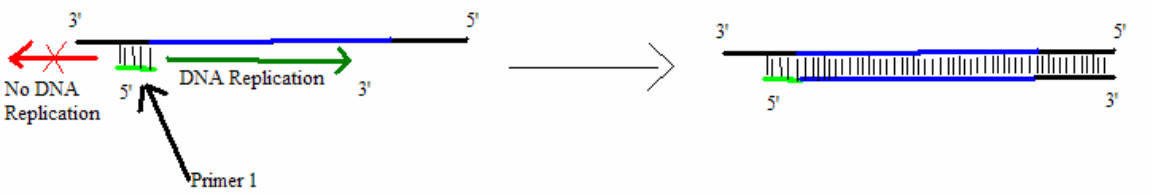


Figure 14: Schematic of PCR Specificity.

However, the unwanted DNA downstream from the target sequence still remains. The key to the elimination of this unwanted DNA within the final product lies within the cyclic nature of PCR. Once one cycle of denaturation, annealing and extension has been accomplished, the cycle is repeated several more times, typically 25 to 36 times (Butler, 2005). After these cycles are completed, an "exponential accumulation," of the discrete target sequence occurs (Budowle et al., 2000). This results from the fact that newly synthesized fragments of DNA from previous cycles serve as templates for amplification in later cycles, with one primer already incorporated in the DNA, and the other serving as the actual primer in the resultant extension (Budowle et al., 2005). The result is at least millions of fragments containing just flanking sequences and the target sequence (Budowle et al., 2000). The numbers of discrete sequences produced by PCR greatly outnumber those containing unwanted DNA sequences, and therefore

allow for high purity of target sequence in the sample to be used in subsequent DNA fingerprinting (Inman and Rudin, 1997).

To clarify the entire process and how it can be used to synthesize a particular target sequence, it's a bit easier to use a simplified example of DNA. For example, take the double stranded DNA sequence below, where "…." Is the target sequence:

STRAND A 5' ---GTAG……CGTC--- 3'
STRAND B 3' ---CATC…….GCAG--- 5'

The first primer for this sequence would be "**GCAG**" (much shorter than a normal primer) and would hybridize with the sequence "CGTC." The second primer, "**GTAG**" would pair with "CATC." Keep in mind that primers are identical to the sequence they replace on opposite strands (i.e. the primer "**GCAG**" replaces a GCAG encoded in Strand B of this example), and when replication occurs, the primer becomes incorporated into the newly-synthesized DNA strand.

A 5' ---GTAG……CGTC--- 3'
GCAG

GTAG
B 3' ---CATC…….GCAG--- 5'

Once the DNA sequence is extended from each primer, only DNA from the primer towards the 5' direction is synthesized, so the next round of extension results in a shorter sequence.

A 5' ---GTAG……CGTC--- 3'
---CATC…… **GCAG** (NO DNA REPLICATION HERE)

(NO DNA REPLICATION HERE)**GTAG**.....CGTC---
B 3' ---CATC……GCAG--- 5'

With several rounds of replication, only the target sequence is duplicated exponentially, and only a few remnants of the unwanted sequence remain in solution, in such little abundance that they would not be visible in DNA fingerprinting. The following schematic only shows one

strand which results in the target, however, keep in mind that with each cycle, more and more target sequence precursors result.

GTAG.....CGTC--- [Another Cycle] → **GTAG**…...CGTC---
CATC……**GCAG** (No Replication Here) [Another Cycle]→CATC……**GCAG}** Target
GTAG……CGTC}Sequence

STRs

With the process of PCR in hand, a change in the target sequence of DNA fingerprinting must be understood. Unfortunately for researchers, PCR analysis is not suitable for large DNA sections like the mini-satellites used in RFLP (Budowle et al., 2000). With this limit, a new form of variation and repetition had to be utilized. What researchers came up with was essentially a smaller version of mini-satellites: Short Tandem Repeats or STRs (The Economist, 2004).

As opposed to the large 6 to 500 base pair repeating units of previous VNTRs, STRs, otherwise known as micro-satellites, are composed of small repeated units only two to five base pairs long (Freeman, 2005). For example, the STR $[CTA]_3$ would represent a small region with the sequence CTACTACTA. These smaller repeats result in much smaller DNA sequences that are compatible with PCR and have many other applicable advantages (Schumm, 1996).

As a side-note, while the forensic community can easily agree on what type of repeats to use, it has had a greater difficulty coming to a consensus on naming each STR locus (Butler, 2005). For example, Genbank, one of several public DNA databases, has its own nomenclature for STRs ("The Biology Project," 2000). Genbanks's naming system differs greatly from another popular system used by several companies, such as Promega, which follows a "D#S####" format (Butler, 2005).

Additionally, there are several other procedures for naming STRs, resulting in a great deal of confusion during the development of the 13 core loci used in forensic analysis (Butler and Reeder, 2004). For this reason, nomenclature of STRs will not be discussed here. However,

the International Society of Forensic Genetics (ISFG) has made several advances in standardizing STR nomenclature (Butler, 2005). Their recommendations can be seen on their website www.isfg.org.

Despite difficulties in naming STR loci, it has still been the general consensus that STRs provide a highly advantageous source for DNA profiling, with the many advances made over the past few decades.

Before delving into the advantages presented by using shorter repeat sequences, there are a few characteristics of STRs that must be understood along with the criteria used in creating a standardized system of STR loci for forensic analysis. For starters, the human genome contains hundreds of thousands of STRs, even some found within protein-coding regions of our DNA (Schumm, 1996). Therefore, to narrow down a key set of STR loci to use, DNA fingerprinting researchers had to make use of several criteria in selecting the standardized STRs.

The ideal traits involved in identifying key loci are based on both characteristics of the repeats themselves and on the flanking regions around them (Bruzel, 1998). In order to allow for ideal DNA fingerprinting, a standardized set of loci and primers specific to individual loci were developed based on these standards for optimal accuracy and differentiation.

In 1997, the FBI announced the selection of 13 STR loci to be used in all United States genetic databases (University of Arizona, 2006). The system, known as CODIS (COmbined DNA Index System) (to be discussed in detail in Chapter 5), has been widely accepted by forensic analysts due to the simplicity of the system's data and the numerous advantages of the 13 loci selected (University of Arizona, 2006) (see Figure 15). In addition, the alleles in CODIS are readily determined using commercially available kits, and the repeat units themselves represent the greatest accuracy, discreteness and precision for nucleotide repeats (University of Arizona, 2006).

Locus	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818
Genotype	15, 18	16, 16	19, 24	12, 13	29, 31	12, 13	11, 13
Frequency	8.2%	4.4%	1.7%	9.9%	2.3%	4.3%	13%

Locus	D13S317	D7S820	D16S539	THO1	TPOX	CSF1PO	AMEL
Genotype	11, 11	10, 10	11, 11	9, 9.3	8, 8	11, 11	X Y
Frequency	1.2%	6.3%	9.5%	9.6%	3.52%	7.2%	(Male)

Figure 15: A typical CODIS DNA profile consisting of the 13 core loci and the "AMEL" locus which establishes the gender of an individual. The genotype the individual has for each locus and the frequency of that particular genotype within the population are also displayed. (University of Arizona, 2006).

In selecting which repeats to use, there are several types of STRs to choose from. The major types are dinucleotide (2 bp), trinucleotide (3 bp), tetranucleotide (4 bp) and pentanucleotide (5 bp) repeats (Butler and Reeder, 2004). However, tetranucleotides have been used the most within the DNA forensics community due to greater fidelity within PCR analysis (Butler and Reeder, 2004). As a result, all 13 loci in CODIS are tetranucleotide repeats (University of Arizona, 2006).

In addition to the length of repeated sections, the traits of the repeats themselves play a key role in deciding which to use. First and foremost, the predicted length of alleles for a certain locus should be from 90-500 bp since smaller sized alleles are better suited for studying degraded and minute DNA samples with PCR (Butler, 2005). This is one of the reasons shorter repeat units are currently favored over VNTRs.

STRs selected for PCR analysis also contain another added benefit due to the narrow size range provided by a smaller repeat. PCR analysis tends to favor a shorter DNA segment when two drastically different-sized sequences are being amplified simultaneously, a phenomenon known as "allelic dropout" resulting from preferential amplification (Butler, 2005). Since the largest and smallest alleles of a certain STR locus usually only differ by 5-10 repeats, it is highly unlikely that one allele will be favored over another during PCR (Inman and Rudin, 1997). Therefore, the size-range of STRs played an important part in the selection process for CODIS.

On the other hand, Promega, a leading company in genetic profiling, states that one of the greatest features used in choosing the best loci is that the STR must be highly variable within the population (Schumm, 1996). Similarly, the alleles for a certain loci should be highly heterozygous within the population to allow for even great discerning power within DNA fingerprints (Butler, 2005). In essence, efficient STR DNA fingerprinting relies on a careful balance between hypervariability (having many different alleles) and a need for narrow allele size-range. For this reason, investigators have to be very cautious and thorough when analyzing the features of a particular locus candidate for STR analysis.

Additionally, STRs must be on separate chromosomal locations to make sure closely-linked loci are not chosen, thereby preventing difficulty in discerning nearby alleles during DNA fingerprint analysis (Butler, 2005). The repeat units should also have an extremely low mutation rate (meaning allele sequence doesn't change much from generation to generation) to avoid false exclusion, especially for use in paternity cases (Schumm, 1996).

With these standards in place, and 13 core loci selected, the process of DNA identification and analysis was largely simplified. Additionally, the selected STR loci have several other advantages over VNTRs which will be discussed in the "-RFLP/VNTRs vs. PCR/STRs-" section. However, these advantages are better understood once the process of DNA profiling with PCR and STRs is explained.

Analysis of PCR Products

While sequencing an entire DNA sequence with PCR is a highly complex process involving different markers for each of the four nucleotides, and simultaneous electrophoresis of the PCR product, using PCR for DNA fingerprinting and identification is quite simple and relies on many of the same basic principles as RFLP (Inman and Rudin, 1997). However, while RFLP

uses restriction enzymes to cut DNA sequences, there is no need for restriction in PCR analysis since the only DNA sequences present are the amplified target sequences of each loci, one maternal and one paternal (Inman and Rudin, 1997). Because of this, products of PCR can be directly analyzed by electrophoresis or even capillary electrophoresis or HPLC chromatography. Accordingly, alleles which differ by four bases in amplification products that are 100-350 base pairs long can be easily distinguished through gel electrophoresis (Schumm, 1996).

Unlike the agarose gel used in RFLP, electrophoresis of PCR products takes place in a different denaturing gel, known as polyacrylamide, which is better suited for the small sequences produced by PCR (Inman and Rudin, 1997). Similarly to RFLP though, a charge is used to separate the DNA molecules by size (Budowle et al., 2000).

Once separate bands of alleles are formed, there are two methods used to analyze the bands after electrophoresis. One method commonly used is to stain the bands directly, usually with a silver stain (Inman and Rudin, 1997). Since no other DNA sequences are present in the gel, there is no need to probe for the sequence, as in the procedure for RFLP, because the stained bands will represent only the target sequence (Inman and Rudin, 1997). The stained gel can then be manually detected in a similar fashion to that of a RFLP fingerprint.

Another visualization technique is becoming more popular within the forensic community: fluorescence detection. PCR primers in many commercial kits used for STR analysis have fluorescent molecules already bonded to the primers (University of Arizona, 2006). Since each synthesized strand has a primer incorporated into it during PCR, all of the products run through the polyacrylamide gel can be easily detected through fluorescence detection. Despite a slight financial burden, computer analysis of the fluorescent gels has become quite prevalent in the field of forensics (Inman and Rudin, 1997). There are numerous types of readers

and computer software that can be used in detection, often involving a laser sensor to analyze the band patterns (Schumm, 1996).

There are two added bonuses to using STRs that are readily apparent during the process of creating a DNA fingerprint. The first comes from the fact that the narrow range of allele lengths for a given locus allows several loci to be run on the same gel without overlapping, in a procedure known as a multiplex (Butler, 2005). In a multiplex, several loci are run in the same lane of the gel and provide a more efficient method for determining a match of two DNA samples (Inman and Rudin, 1997). Instead of running each single-locus analysis separately, as is the case for RFLP, several STR loci can be analyzed simultaneously, saving time on analysis and allowing for a more accurate method of matching DNA fingerprints (Schumm, 1996).

To make sure that none of the loci in the multiplex overlap, the distance between primers and the repeated section is carefully adjusted to create larger size differences between the alleles found at different loci, therefore spreading the bands farther apart (University of Arizona, 2006). In addition to the 13 CODIS loci, a gender allele "AMEL" is often run within a multiplex, with only two possible allelic combinations: male (XY) or female (XX) (Inman and Rudin, 1997).

Additionally, allelic ladders are often run in a lane next to that of the loci being analyzed (Butler, 2005). An allelic ladder is an "artificial mixture of the common alleles present in the human population for a particular STR marker," serving as size markers for analysis of a STR fingerprinting (Butler, 2005). To create these ladders, samples from multiple individuals within a population are amplified together by PCR and then run through electrophoresis alongside unknown DNA samples (Butler, 2005). As long as the same fluorescent primers are used to amplify the ladders and the unknown DNA, the two will migrate through the gel at exactly the same rate for segments of corresponding length (Schumm, 1996). From this, the length of each band can be determined and individuals can be typed as having certain alleles for a given locus,

by comparison with an allelic ladder. In figure 15, for example, the individual has one allele of 15 repeats and one allele of 18 repeats for the locus D3S1358.

Figure 16 shows three different multiplexes, each with four different loci run together. While this is somewhat complicated to analyze, it provides a good reference for what an actual STR fingerprint looks like. The lanes labeled "L" are allelic ladders to be compared to six different DNA samples for each multiplex. Each allelic ladder is labeled on the right side of the column based on the number of repeats in its largest and smallest alleles.

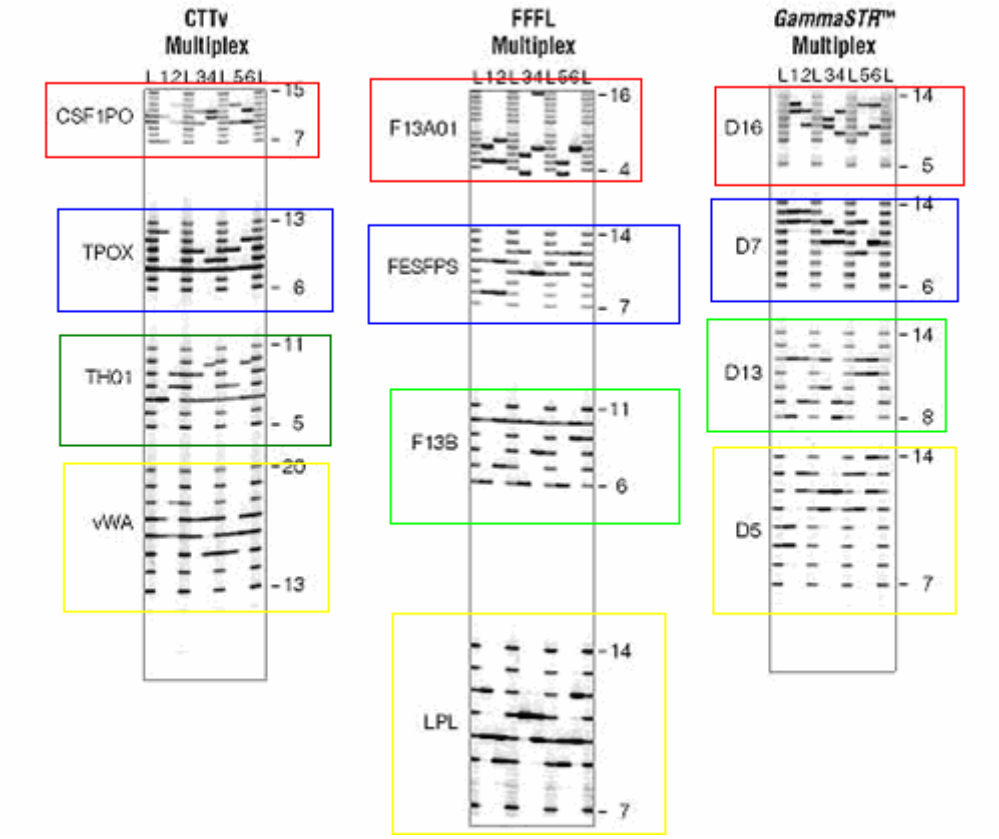


Figure 16: Example of Three STR DNA Fingerprint Multiplexes. On the left of each locus's section (Boxed for clarity) is the name of the locus and the on the right is the number of repeats in its largest and smallest alleles. (Schumm, 1996).

Examining the Final Product of PCR/STR

While the example in figure 16 is a complex view of the analysis needed to match DNA profiles using STR analysis, an explanation of a simplified example might be quite beneficial. Figure 17 is a somewhat simpler view of the matching ability produced by a multiplex of three

PCR products. While the bands from different alleles actually overlap in this example, the different colors could be achieved by the use of several different probes, which some of the more advanced STR reading systems have the ability to distinguish (Schumm, 1996).

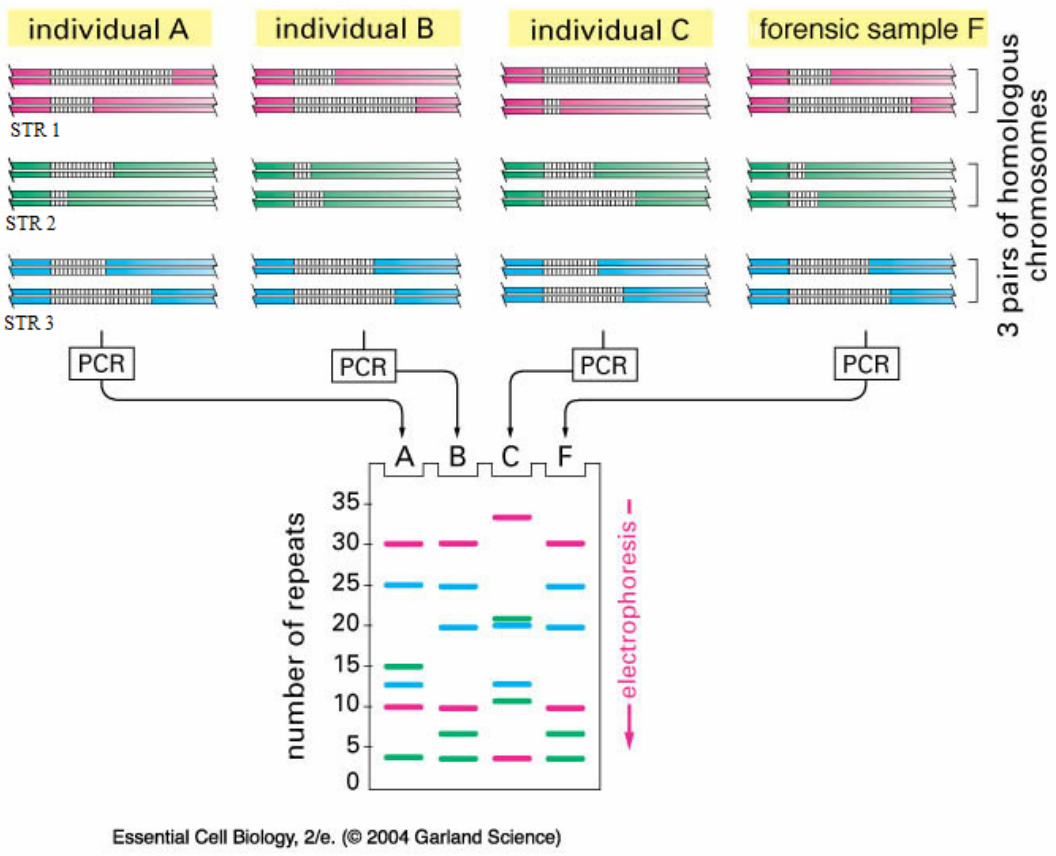


Figure 17: Example of a multiplex of PCR Products. ("Use of PCR in Forensic Science," The National Health Museum, 2004).

In this example, target sequences from the same three loci were taken for three individuals (A, B and C). The two alleles of each loci for each suspect were then run through electrophoresis and compared to the corresponding sequence of a DNA sample found at a crime scene. While no actual allelic ladders are included, the scale on the left side of the fingerprint labeled "number of repeats" is equivalent to the information an allelic ladder would produce.

First, the genotype of the crime scene sample must be determined. It can easily be seen that it has the genotype (10, 30), (4, 6) and (20, 25) for STRs 1, 2 and 3, respectively. In

contrast, individual A's pattern shows a genotype of (10, 30), (4, 15) and (13, 25) for STRs 1, 2 and 3, and therefore, individual A is proven not to be the source of this DNA sample.

Likewise, individual C has a genotype of (4, 33), (11, 21) and (13, 20) for STRs 1, 2 and 3. These also do not match up with those of the crime scene DNA sample, and individual C is ruled out as well.

However, individual B has the genotype (10, 30), (4, 6) and (20, 25) for STRs 1, 2 and 3, a perfect match to those three loci in the crime scene sample. While this makes individual B more likely to be the source, an absolute positive match cannot be made. The random match probability, "the probability that a randomly selected individual from a population will have an identical…combination of genotypes," for an STR analysis of three STR markers is not small enough to say with absolute certainty that individual C is the source when compared to the total human population, unless the STR analysis is being used simply to select one individual from an already assembled short list of suspects (Butler, 2005). For three STRs, the probability is only 2000 to 1 (Bruzel, 1998). To be absolutely certain, thirteen loci should be analyzed.

However, despite a need for analysis of several more loci, this simple example shows the power of using STRs to compare the DNA of two or more individuals with great accuracy. Accordingly, STR and VNTR analyses have both proven to be essential tools in identifying individual organisms based on their genetic makeup. Before exploring these many applications, it's quite useful to weigh out the advantages and disadvantages of these two very different procedures.

RFLP/VNTRs vs. PCR/STRs

As is the case with most technologies that build off of older ones, STR analysis, which built off of VNTR analysis, has become the current favorite of most genetic and forensic

researchers today because of the many advantages it provides over older RFLP methods. The numerous benefits of STR procedures tend to overshadow the few small drawbacks involved in the processes of their amplification and analysis.

The most obvious advantage to the use of STRs is their compatibility with PCR, which allows the amplification of a trace amount of DNA to an adequate quantity for DNA fingerprinting (Butler, 2005). While RFLP requires 50 – 500 nanograms (ng) of relatively intact DNA, PCR methods only need 0.1 – 1 ng of DNA sample to amplify and create a DNA fingerprint, roughly the amount of DNA found in a fleck of dandruff or a small drop of saliva (Butler, 2005). The relative amounts of DNA found in many other sample sources will be discussed further in Chapter two.

A similar advantage to PCR lies in its broad range of acceptable DNA samples. RFLP analysis requires intact, double stranded DNA in order for the restriction enzymes to work (Butler, 2005). However, PCR analysis can be undertaken on highly degraded DNA samples. Additionally, these samples can be single-stranded or double-stranded, since PCR only requires one strand for replication (Budowle et al., 2000). With this added versatility, the compatibility of STRs with PCR once again tends to favor their use in DNA fingerprinting.

However, beyond the quality and quantity of acceptable DNA samples, PCR also offers several other advantages in terms of convenience and efficiency. On a timescale, the actual polymerase chain reaction takes only a few hours, and provides a complete and succinct DNA fingerprint within one or two days (Budowle et al., 2000). RFLP analysis, on the other hand, can take anywhere from one to eight weeks, depending on the type of probe used and other lab conditions (Butler, 2005).

An added convenience of PCR comes from its ability to work in conjunction with several automated technological advances. Not only can PCR be read quite easily by laser and

fluorescence techniques, it is also well-suited with several types of computer software that allow for immediate and accurate analysis of the eventual DNA fingerprint (Inman and Rudin, 1997).

In addition to the benefit of quick and simple analysis, the digital capabilities of PCR analysis make it perfect for use in our current national database (University of Arizona, 2006). Since all STR analyses entered into CODIS are converted to a digital format, investigators in one city have access to DNA profiles from all over the country (University of Arizona, 2006). RFLP however, is currently incompatible with most computerized and automated systems, due to the indiscrete nature of its loci, and, for the most part, the electrophoretic banding patterns must be analyzed by humans on a case by case basis (Butler, 2005).

Beyond compatibility with PCR, another advantageous aspect of the smaller allele size involved in STR analysis comes from recent studies that show higher variability in smaller repeated sequences (Freeman, 2005). However, a drawback to increased genetic variety is that higher variability implies a higher mutation rate, a trait that must be minimized in most DNA fingerprint processes, especially if the PCR primers are to hybridize correctly (Lewin, 1989). For this reason, researchers must carefully balance variability and mutation rate when selecting loci. Despite all of this, hypervariability has always been a highly sought after trait in DNA profiling systems and the smaller nature of STRs makes them much more well-suited for this aspect of identification.

The smaller size of short tandem repeats themselves and their representative alleles provides several more advantages to their use over traditional VNTR methods. The small size range of STRs not only allows for accurate amplification during PCR (See “STRs” section), but also creates bands that can be spread out easily, and precisely analyzed (Butler, 2005).

Restriction techniques however, tend to yield fragments with a very broad size range. The drawback to this outcome is that in the process of separation, smaller DNA fragments can be

run completely off of the gel leaving an incomplete picture of the DNA fingerprint (Lewin, 1989). Therefore, the narrow size range provided by STR markers is again quite crucial to obtain accurate depictions of an individual's genetic makeup.

Also, the discrete alleles that can be found by using a smaller repeated sequence can be differentiated based on a difference in a single base-pair length (Butler et al., 2004). However, the size of RFLP products cannot be precisely determined, and therefore, bands of similar size tend to be classified together in a process known as "binning" (Butler, 2005). For example, since VNTR sequences are so large, a fragment that is 1.2 kilobases (1200 bases) long could be easily confused with a 1.1 kilobase fragment, even though these sequences could be quite different. However, in the discrete alleles of STRs, a person who has the genotype (9.1, 5.2) for a certain locus can be easily distinguished from another person who is (9, 5.1) for the same locus (where the numbers in parentheses represent the number of repeats of each allele of a certain STR).

Along the same lines, the discrete nature of STRs allows for the use of two previously mentioned procedural benefits: allelic ladders and multiplexes. As described before, allelic ladders allow for the precise assignment of the number of repeats in a given STR allele. The allelic ladders used as molecular weight markers for STR analysis are much more accurate than those used in RFLP profiling. This is due to the fact that the exact same probe (fluorescent primers) and starting materials are used to make both the ladder components and the PCR products (Butler, 2005). Because of this, the allelic ladder components will migrate through the polyacrylamide gel in the exact same fashion as their corresponding PCR products and will line up perfectly, providing a reliable measure of repeat length (Schumm, 1996). Since, an allele of a certain size will always line up with a corresponding ladder marker run under the same conditions, even though different laboratories may use different probes, the accurate allele

determination provided by allelic ladders allows different labs to compare their results with reliability and confidence (Schumm, 1996)

RFLP molecular weight markers, however, are ambiguous in terms of precise allele determination. Not only does the large size of VNTRs cause binning, but the molecular weight markers are also affected non-uniformly by differing laboratory conditions and probes (Butler, 2005). For this reason, laboratories have a difficult time comparing results and defining an allele as having an exact repeat length. Individual RFLP bands can usually only be used in conjunction with another pattern in order to provide useful genetic identification. In other words, RFLP fingerprinting cannot categorize a person's individual alleles but only makes comparisons based on the overall fingerprint produced.

However, to obtain low enough random match probabilities, both techniques require analysis of more than one locus. STR analysis though, makes the process of multi-locus probing much simpler, through its ability to make use of multiplexes. Multiplexes, (like those shown in the "PCR Analysis" section) provide a way to analyze several different loci simultaneously. Since PCR primers can be easily adjusted to change the distance between flanking regions, and thus increase the size differences of two different loci, multiplexes have been developed for STR loci that have no overlap and therefore provide a clear-cut DNA fingerprint for several different loci (University of Arizona, 2006). These "high-throughput" systems are suited much better for PCR analysis than RFLP analysis (Schumm, 1996).

Since restriction enzymes are specific for a certain sequence, it's difficult to adjust the size differences of RFLPs for two different VNTR loci (Butler, 2005). Therefore, cutting a DNA strand for one locus may produce two bands of 4.2 and 3.4 kilobases, and cutting a different VNTR loci may produce 3.4 and 2.9 kilobase bands. By themselves, the bands from these two loci are easily distinguished and identified. However, this would involve running two different

RFLP analyses, which would be time-consuming and inefficient. Additionally, if these two loci were run together, it would cause quite a bit of confusion. It would very difficult to distinguish a person who is (4.2, 3.4) for locus A and (3.4, 2.9) for locus B, from someone who is (3.4, 2.9) for locus A and (4.2, 3.4) for locus B.

This discrepancy also ties in somewhat with previously mentioned size-range issues, but the overall STR advantage comes from the fact that the PCR products from different loci can be run in the same gel, but kept isolated, forming the final product: a multiplex (Schumm, 1996). Additionally, returning to the automated nature of PCR analysis, two different colored probes can be developed for multiplexes, allowing for even simpler analysis of STRs (Schumm, 1996). These distinguishable fluorescent probes are developed by incorporation of different fluorescent elements into the PCR primers for two different loci (Budowle et al., 2000). Specially-made scanners can then distinguish these two probes from one another for increased clarification in STR analyses (Schumm, 1996) (see Figure 18).

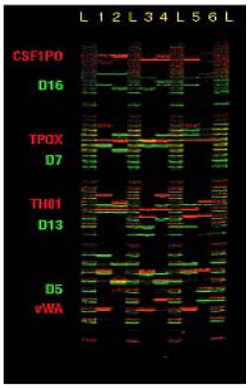


Figure 18: STR multiplex using two different colored primers to distinguish between different loci (Schumm, 1996).

On the other hand, since most RFLP analysis depends on X-ray film, which produces a black and white fingerprint, there would be no way to distinguish between the radioactive probes used for VNTRs (Budowle et al., 2000). The only way to accomplish this would be by using the newer chemiluminescent probes for VNTRs (Budowle et al., 2000). However, chemiluminescent probes are not easily distinguished like the two-tone probes used in PCR primers (Schumm, 1996). Unfortunately, there is little documentation regarding use of two-tone probes in Southern blotting similar to those used as PCR primers. Furthermore, since allelic ladders are made using the same primers, the scanners made to analyze the fluorescent tags on PCR products can compare them with the allelic ladder "rung" closest to them, allowing for instant and precise allele determination (Schumm, 1996).

Coming back to the precise allele determination possible by STR analysis, there are several interesting capabilities that come from being able to exactly classify a person's specific genotype for a given locus. Using population genetics principles, investigators can determine the percentage of a certain population that contains a given allele or given genotype for each locus analyzed (University of Arizona, 2006). In doing so, researchers can gain useful insight into certain traits, such as sex, race or even disease status in certain cases (The Economist, 2004). To estimate physical traits, race for example, investigators analyze which group within a population tends to have the highest percentage of a given genotype in order to discern possible traits found in the DNA source (University of Arizona, 2006). For example, if certain genotype for given locus was the most prevalent in Caucasians, investigators could make a preemptive assumption regarding a DNA source's race.

In some cases, since a few STRs are actually found within coding regions, information can be ascertained that provides even greater detail about the individual a DNA sample comes from (Butler, 2005). For gender, the AMEL locus, found in the gene coding for the protein

Amelogen is analyzed to determine whether an individual's genotype is XX or XY and determine their gender accordingly (Schumm, 1996). While the AMEL locus is not a true STR, it can still be included in STR multiplexes, giving STRs another advantage over VNTRs (Schumm, 1996). With these small pieces of information about an individual, researchers can begin piecing together details about where the DNA came from, without having an exact DNA fingerprint match. The ethical debate sparked by this "big-brother"-like ability will be discussed in later chapters (The Economist, 2004).

While, STR and PCR methods clearly have a great deal of advantages over VNTR and RFLP analysis, it's important to remember that the use of STRs evolved from VNTR analysis, and that the two still have much in common. Both procedures have numerous highly-specific loci and probes which allow them to distinguish between individuals based on the length of DNA sections; in fact, there are more than two thousand specific STRs available for genetic mapping (Schumm, 1996). Additionally, both techniques can be used in many applications, including the case of a sample containing mixtures of DNA from different sources, provided a single locus probe is used (Butler, 2005). Of course, that's only one of numerous applications that both procedures are capable of handling.

Advantages of VNTR Analysis

While STRs obviously have many defining advantages, explaining their overall popularity and favor in the forensic world, there are still a few advantages involved in RFLP analysis that have not been outsourced by PCR and STR. Consequently, while few in number, these advantages aren't found in PCR and STR analyses, and therefore provide a lifeline for the use of RFLP analysis in the world of molecular biology. One slight advantage to VNTRs is their tendency to be more heterozygous than STRs (Inman and Rudin, 1997). Therefore, VNTRs are

more likely to form two bands for a given locus than STRs, making them slightly more advantageous in terms of creating a unique fingerprint for a single locus (Butler, 2005). However, this becomes less of a problem when more than one locus is analyzed.

In terms of multi-loci analysis to decrease random match probability and thus make an accurate identification, VNTRs are at a slight advantage to STR analysis in terms of the number of loci needed (Butler, 2005). To have an adequate level of certainty for matching DNA fingerprints, one in a billion is usually the minimum random match probability aimed for by investigators (Bruzel, 1998). To accomplish this, only six RFLP loci are needed; STR analysis, on the other hand, requires approximately thirteen loci to achieve this certainty (Butler, 2005). However, since PCR analysis of STR loci is accomplished much more rapidly and efficiently, the need for more loci tends to be somewhat inconsequential.

However, the greatest and most definitive advantage to using RFLP analysis of VNTRs is its ability to overcome the effects of contaminants in a DNA sample (Butler, 2005). DNA at crime scenes and other sites of interest is often contaminated with substances such as leaf and soil residue, certain oils, cleaners, spermicides, dyes etc. (Butler, 2005). Since PCR analysis is so sensitive to trace amounts of DNA (usually its major advantage), it can be easily thrown off by multiple substances in a sample (Inman and Rudin, 1997). Many substances actually co-purify with DNA after extraction and would therefore be present in the sample during the subsequent amplification (Budowle et al., 2000).

Furthermore, since PCR relies heavily on the enzyme Taq polymerase, contaminants can also inhibit this enzyme, which is much more sensitive than the restriction enzymes used in RFLP, inhibiting the PCR amplification (Butler, 2005) (see Table 2 for several common PCR inhibitors and their sources). For this reason, many precautions and protocols are needed to avoid contamination in STR samples that are not necessary with RFLP.

Possible Forensic Source	PCR Inhibitor
Blood	Heme (Hematin)
Tissue and Hair	Melanin
Feces	Polysaccharides
Feces	Bile Salts
Soil	Humic Compounds
Urine	Urea
Blue jeans	Textile dyes (denim)

Table 2: Common Forensic Sources and Associated PCR Inhibitors (Butler, 2005).

Many solutions exist for overcoming Taq polymerase inhibition, including the addition of certain counter-enzymes and cleansers (Butler, 2005). For instance, dyes found in certain types of denim must be cleansed thoroughly with a specific reagent before they can be amplified with PCR (Inman and Rudin, 1997). While both restriction and polymerizing enzymes are inhibited by these dyes, the procedure for overcoming inhibition of RFLP is slightly more facile, provided there is enough sample available (Inman and Rudin, 1997). Therefore, the advantage to RFLP use in cases of contamination is dependent on several factors of the surrounding environment and the DNA sample itself.

With these many advantages and disadvantages in mind, it's quite clear that the type of repeat analyzed really depends on the conditions of the DNA sample available and the specifications set forth by the intended use of the DNA profile. For this reason, both techniques are often seen as options for certain applications.

Uses of DNA Fingerprinting and Profiling

Forensics/Justice System Uses

With the major advances and many choices of techniques for forensic applications, DNA fingerprinting has recently had an increasing influence on many aspects of our society, especially our justice system. Forensic scientists, for instance, have developed and improved several DNA fingerprinting methods to analyze samples found at crime scenes. By comparing DNA samples from a suspect's blood or other tissue (often cheek swabs to avoid suspect objections based on "cruel and unusual punishment") to DNA samples found from virtually any substance at a crime scene containing white blood cells, authorities can determine the identity of individuals involved in a crime. By verifying a match to the sequences of the thirteen core loci of CODIS, investigators can provide highly accurate evidence to be used in murder or rape cases (Meeker-O'Connell, 2004).

For example, soon after developing his initial procedure for paternity testing in 1985, Dr. Alec Jeffreys, used his new technique in a double rape and murder investigation (The Economist, 2004). By comparing samples of semen left on the two victims with samples from a large group of men, Jeffreys not only ruled out one potential suspect, but eventually helped authorities convict the guilty party (Butler, 2005).

Pushing beyond the use of DNA fingerprinting on a case-by-case basis, in 1997, the FBI introduced the Combined DNA Index System, or CODIS, the standardized system of the thirteen specific loci used by investigators nationwide (University of Arizona, 2006). The system is to be used in conjunction with a newly emerging National Database where convicted offenders of violent crimes are required by law to submit a DNA sample to compare to evidence from future crime scenes (University of Arizona, 2006). As discussed previously, this has several

advantages within our criminal justice system, making DNA fingerprinting a very powerful forensic tool for identifying criminals.

Additionally, in terms of criminal forensics, despite its strong use for positive identification, otherwise called "inclusion," DNA fingerprinting has also been an accurate method for *exonerating* suspected criminals. In fact, to quote Dr. Jeffrey's, "the first time DNA was ever used, it didn't prove guilt, it proved innocence" (The Economist, 2004). By showing that the suspect was undoubtedly not the source of crime scene DNA since the two fingerprints didn't match, Jeffreys helped set an innocent man free. In fact, as of 2005, one hundred and thirty-six prisoners in the United States prison system were proven innocent by DNA fingerprinting of previously overlooked crime scene evidence (Freeman, 2005).

Beyond criminal investigations, another added advantage of DNA fingerprinting comes with its immense versatility. The ability to sequence and identify similarities and differences in DNA has been a vital component in many fields of study beyond forensics. For example, in a related field to criminal forensics, DNA profiling has also been used in the justice system to settle paternity cases. In fact, hundreds of thousands of paternity tests are conducted each year in American labs alone (The Economist, 2004).

However, there is a bit of finesse required in determining paternity using DNA samples. To do so, a sample of the child's DNA is taken and is compared to samples from the both the child's mother and the suspected father (Biotechnology Industry Organization, 2003). Paternity testing is commonly done by RFLP, since there is usually no limit based on the amount of sample available, but STRs have also become quite popular due to the discrete nature of their alleles (Butler, 2005).

Examining several loci, sequences that the child and mother share are ignored, since these sequences would be maternally inherited (Biotechnology Industry Organization, 2003). By

comparing sequences in the child's DNA that aren't common to the mother with sequences from the alleged father's DNA, an accurate paternity ruling can be established (Davidson College, 2006). Like most DNA analyses, paternity testing is more effective as a tool for ruling out a suspect rather than indisputably naming a father, since there is always the slim chance that not enough loci are examined (Davidson College, 2006).

Using Figure 19 as an example, the theory behind paternity testing can be easily clarified. In the first situation (shown on the left), Jill is the mother of Payle, and Jack is the suspected father. Using the following logic, it can be deduced that Jack is potentially Payle's father.

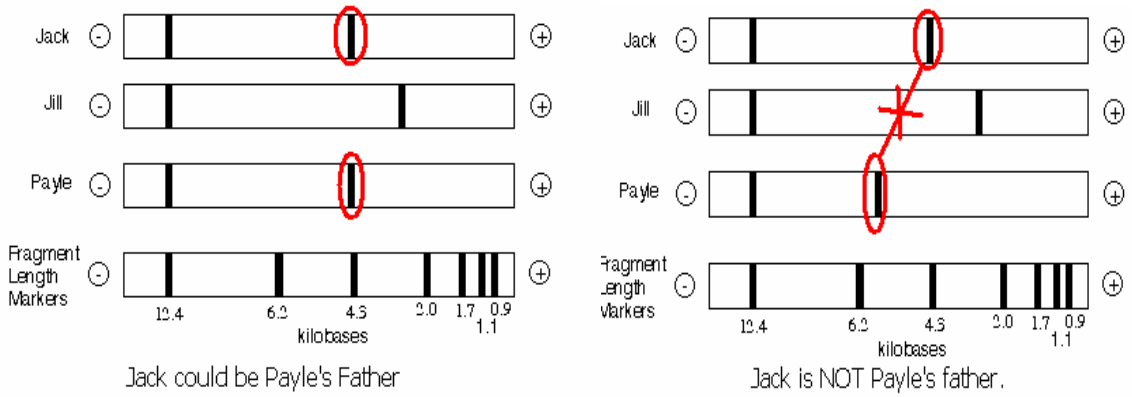


Figure 19: Example of paternity analysis of one genetic locus (Davidson College, 2006).

First, looking at Payle's fingerprint shows that she has an allele that is 12.4 kilobases (kb) long and one that is 4.5 kilobases long for this specific locus. Next, Jill's fingerprint is analyzed to show that she has alleles that are approximately 3 kilobases and 12.4 kilobases long. It can therefore be deduced that Payle inherited her 12.4 kb allele from her mother, since Jill's 3 kb band does not match up with Payle's 4.5 kb band. From this, it's clear that Payle inherited her 4.5 kb allele from her father. By looking at Jack's DNA fingerprint, it's clear that Jack and Payle not only share the 12.4 kb allele (most likely a coincidence), but also that the two share Payle's paternally inherited 4.5 kb allele, narrowing down Jack as a candidate for being Payle's

father. However, since analysis of one loci only yields a random match probability of 1 in 2000, analysis of several more loci must be completed to name Jack as the father with certainty.

In the second situation, Jack is ruled out as being Payle's father. Using the same logic as before, it's found that Payle's maternal allele is 12.4 kb and her paternal allele is again 4.5 kb. However, in this scenario, Jack is found to be 12.4 kb and 6.0 kb for this locus. Although Payle does in fact have a band at 12.4 kb, comparison to Jill's DNA shows that Jack must have a 4.5 kb allele to be Payle's father. Therefore, it can be said with strong certainty that Jack is not Payle's father.

While paternity testing is one of the more well-known applications of DNA fingerprinting to determine lineages and familial relations, there have been several other cases of DNA fingerprinting used to identify or exclude individuals as being related. One of the most famous cases of genetic testing in genealogy involved the Romanov family: the royal family in Russia executed during the Bolshevik revolution (Butler, 2005). A Russian woman had managed to convince the public that she was the Grand Duchess Anastasia and had survived the massacre (Biotechnology Industry Organization, 2003). However, DNA comparison of her blood with bone samples from remains of the Romanovs disproved this woman's claim to nobility (Biotechnology Industry Organization, 2003).

Furthermore, the analysis of DNA found in remains to identify deceased individuals has found its way into many practical aspects of society. For example, the United States military has recently began work on developing its own "DNA databank" of all military personnel as a backup to metal dog-tags currently used in the field (Biotechnology Industry Organization, 2003). By submitting DNA samples, soldiers can be identified much more accurately than by the previous methods of dental records or ambiguous dog-tags (Betsch, 1994).

Additionally, DNA fingerprinting has recently been used to identify victims of disasters other than casualties of warfare. Specifically, identifying victims of the World Trade Center attacks has presented a significant challenge for DNA profiling. Most of the remains at the World Trade Center were so badly damaged that DNA samples taken from them would have less than ideal potential for analysis (Butler, 2005).

With limited DNA samples, investigators would have to use several types of DNA: both nuclear and mitochondrial in hopes of narrowing down possible identities (World Trade Center, 2001). Also, since most victims were not entered into any DNA databases, investigators had to find other sources of DNA to find a match (World Trade Center, 2001). Toothbrushes found in the homes of possible victims, while not completely reliable sources, provided several samples for DNA comparison (World Trade Center, 2001). Also, investigators attempted comparisons between living family members, but since relatives only have similar and not identical sequences, this information remains speculative (World Trade Center, 2001).

However, even if a positive match is unattainable, a DNA profile can provide information about victims such as race, sex and other traits that could have been hidden by the drastically damaging effects of a large disaster (Meeker-O'Connell, 2004). In the case of 9/11 and other disasters such as tsunamis and volcanic eruptions, DNA fingerprinting provides a limited, but useful technique for comparison and identification.

Environmental Research

However, when the identity of an individual is already known, there are several other uses for DNA sequence analysis. In fact, there are many applications of DNA profiling that don't even involve individuals or even human beings for that matter. In environmental biology, DNA fingerprinting can be used to compare different species and identify risks to a certain

population. For example, DNA profiling was used to identify the risk of extinction cheetahs face due to "virtually no genetic variation" in the species (Biotechnology Industry Organization, 2003). By showing that most cheetahs have virtually the same genetic makeup, researchers inferred that this lack of variation could be a threat to the survival of the species in conditions where evolution and natural selection are necessary.

Additionally, wildlife authorities can use DNA testing to identify illegal poaching as well as harvesting and importation of natural resources, including certain types of caviar, ivory and redwood (Biotechnology Industry Organization, 2003; Meeker-O'Connell, 2004). For example, environmental police can match DNA from stumps of illegally harvested timber to that of the wood in a logger's possession, thereby providing evidence for violation of environmental codes (Bruzel, 1998).

In an even more interesting study, environmentalists have used RFLP techniques to simplify the study of certain birds' mating patterns (Lewin, 1989). The two greatest uses of DNA fingerprinting in this field involve "identifying the parentage of offspring" of certain individuals and determining how closely individuals in a group are related to each other (Lewin, 1989). These pieces of information are crucial for environmental biology because they allow researchers to identify which traits and genes within a species' gene-pool produce the greatest reproductive success (Lewin, 1989). To do so, researchers must count the number offspring a certain bird produces and relate this figure to their genetic make up.

However, there are several phenomena within the bird population that present a few problems for accurate determination of reproductive success (Lewin, 1989). The most interesting of these is the behavior displayed by several species of birds where non-breeding individuals care for the young of other members of the population in a form of environmental "altruism" (Lewin, 1989). This behavior makes it difficult to accurately match young birds to

their parents, often skewing environmental data. However, by creating DNA fingerprints of members within the species, researchers can not only match parental birds to their young, but also determine which genes provide the advantages necessary for reproductive success (Lewin, 1989).

Disease Research and Control

In terms of conservation of the human species, DNA profiling has proved quite useful as a technique for identifying disease risks and possible treatments in the human population. For direct measures, DNA fingerprinting can be used to diagnose individuals as having a certain disease. As previously discussed, PCR can be used to rapidly detect the presence of specific segments of DNA (Biotechnology Industry Organization, 2003). Using this principle, PCR can be run to check individuals for infection with certain diseases, such as HIV or *Chlamydia*, by detecting DNA sections unique for those viruses (Biotechnology Industry Organization, 2003).

However, the most practical use for DNA fingerprinting in disease control is its role in identifying risk factors for inherited diseases, therefore providing a method for prevention. Inherited disorders are caused by genetic inheritance from parents, rather than by infection of a virus or bacteria (Betsch, 1994). There are a wide range of inheritable diseases, including cystic fibrosis, hemophilia, Huntington's disease, sickle cell anemia, and Alzheimer's and DNA fingerprinting has provided various methods for analysis and prevention of these diseases (Betsch, 1994).

In one sense, RFLP analysis can be used to test couples with a family history of certain disorders and help them make educated decisions about starting a family (Davidson College, 2006). Inherited disorders usually result from a specific allele which can be passed on from parent to child. Cystic fibrosis, for example, is a recessive disease, meaning both parents have to

be carriers of at least one cystic fibrosis diseased allele (Davidson College, 2006). Since researchers know the locus of the gene responsible for cystic fibrosis, single-locus RFLP analysis can be done for the CF gene to determine the genotype of the parents (i.e. determining whether or not parents are homozygous or heterozygous) (Davidson College, 2006). This information can then be used to determine the probability that a child would have the disease and aid parents in planning their pregnancy or, for certain diseases, help medical staff prepare for proper treatment of the child (Betsch, 1994).

For example, Figure 20 shows a schematic of several representative fingerprints for different genotypes of the CF locus. A WT (Wild type), for example, is homozygous for the healthy form of the CF gene and would therefore only show one band at this location. Similarly, a person with cystic fibrosis (CF) would be homozygous for the disease form of the CF gene. Carriers for the disease have both a healthy allele and a CF allele and would therefore have two bands for the CF locus. By comparing prospective parents' DNA fingerprints to these banding patterns, researchers can determine the probability that parents would have a child with CF and therefore help them explore their parenthood options.

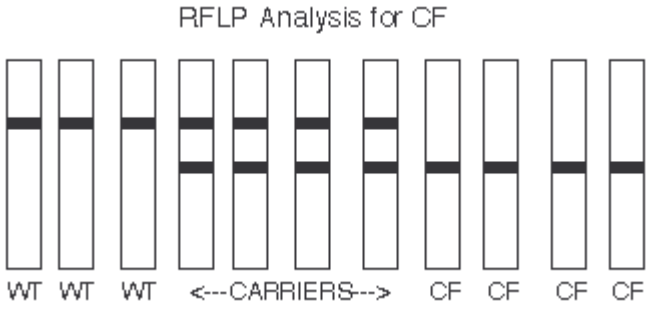


Figure 20: RFLP Analysis for the CF Allele in Prospective Parents. WT indicates a parent with two healthy alleles. Carriers have a healthy allele and a cystic fibrosis allele. CF indicates a person with two copies of the gene responsible for cystic fibrosis and no normal allele, and are therefore affected by CF. (Davidson College, 2006).

Additionally, study of the loci for other inherited disorders can be used in hopes of developing a cure for these diseases. Researchers often run DNA fingerprints on members of families with a history of a certain disease, Alzheimer's for example (Meeker-O'Connell, 2004). By comparing the DNA fingerprints of family members with the disease in question to those without, researchers attempt to identify certain chromosomal patterns in hopes of discerning the loci affected and possible methods of treatment for these diseases (Meeker-O'Connell, 2004; Betsch, 1994).

In the cases of disease research and other genetic studies, scientists often essentially create pedigrees of families. These pedigrees, which are charts resembling family trees that show certain familial lineages and the representative genetic makeup of members, are used in hopes of identifying the inheritance and genetic background to certain inherited traits and alleles (Davidson College, 2006). In the same fashion, researchers in molecular archaeology often use genetic sequences to trace biological ancestry (of the human species especially) in hopes of identifying key pieces of a certain organism's genetic history.

Molecular Archaeology

While the uses of DNA fingerprinting in biological lineage and origins are as vast as the human pedigree itself, there have been several interesting representative cases of its application recently. For instance, in 1991, the remains of a mummified human being were found in the Tyrolean Alps (Handt et al., 1994). Using carbon dating of the mummy's tissues, the remains were found to be well over 5000 years old, making it the oldest mummy known to man (Handt et al., 1994).

Researchers hoped to determine the so-called "ice man's" genetic makeup in hopes of revealing aspects of his "ethnic affiliation," and therefore gain insight into the genetic ancestry of

surrounding areas (Handt et al., 1994). However, since his tissues and resulting DNA samples were highly degraded, mitochondrial DNA was the primary genetic source used due to its higher prevalence relative to nuclear DNA (Ingman et al., 2000). In fact, the iceman's DNA was degraded to fragments with an average length of less than 150 bp (Handt et al., 1994). Fortunately though, since minute samples of nuclear DNA were available, both mitochondrial DNA and nuclear DNA were used in conjunction with each other to achieve the best possible analysis of the iceman's genetic makeup (Handt et al., 1994).

Using somewhat complex methods, researchers were not only able to quantify the amount of available DNA for STR analysis; they also removed contaminating DNA from within the iceman's samples and identified several areas where potentially misleading base pair insertions (essentially extraneous genetic mutations) occurred (Handt et al., 1994). The most likely source of these inconsistencies came in the form of degrading microorganisms contaminating the iceman's DNA with their own (Inman and Rudin, 1997; Handt et al., 1994).

After several lengthy and intricate procedures to rule out potential outliers and isolate authentic genetic information, researchers were able to compare the iceman's mitochondrial sequences with data sets of mitochondrial DNA from various regions in the world (Handt et al., 1994). By calculating the genetic divergence, or the amount by which the iceman's DNA differed from the average sequence of a certain population, researchers were able to narrow down the iceman's ethnic origin to being from the Alpine regions of Europe (Handt et al., 1994). Continuing research on the iceman is currently being undertaken to gain a more precise genetic determination as more information on the genetic diversity among European populations becomes available (Handt et al., 1994).

While the Tyrolean iceman was a particularly specific and sensationalistic study of genetic lineage, one of the largest and most extensive studies in molecular archaeology deals

with the human race as a whole. Stemming from a need to trace mankind's roots to a specific point of ancestry, researchers have recently begun delving into the field of population genomics, using DNA sequencing and locus analysis as their greatest tools (Hedges, 2000).

In hopes of finding a common origin for all mankind, researchers have been attempting to pinpoint the geographical location of our "most recent common ancestor," the first *Homo sapien* population with descendents from every race and region of the world (Ingman et al., 2000). While some scientists believe that modern humans arose simultaneously, but individually, in different regions of the world, DNA evidence and sequencing have provided a great deal of support for the theory of a common ancestor (Hedges, 2000). Using DNA evidence and phylogenic trees, structures similar to family trees that show levels of relation between different human populations, researchers found that modern humans came about in Africa roughly 100,000 to 200,000 years ago (Ingman et al., 2000).

To prove this, researchers analyzed particular loci in mitochondrial DNA mostly (Ingman, et al., 2000). Since mtDNA has very little recombination (a phenomenon where offspring have different alleles from either parents due to combination of both alleles), but high variability in its "control region," an area of the circular mitochondrial chromosome most widely used for evolutionary studies, it can provide useful information for comparing populations of humans, rather than individuals, in hopes of estimating genetic differences between peoples spread throughout the globe (Ingman et al., 2000).

By comparing the mitochondrial DNA from representative members of different populations, researchers began to develop phylogenic trees to be used to approximate the degree of genetic similarity of different populations (Hedges, 2000). Also, since mitochondrial DNA approximately follows the "molecular clock theory" that states that the rate of genetic evolution

in a given lineage is roughly constant over time, differences in sequence can be used to estimate the approximate time of divergence between two genetic lines (Ingman et al., 2000).

Figure 21, for example, shows a phylogenic tree constructed based on sequencing of the mitochondrial genomes of individuals from every major ethnic population on the planet. While this particular figure is somewhat complex, it basically consists of "branches" determined by average mitochondrial differences ending with "twigs" of individual populations on the far right. Interestingly, the main focus of the tree is the bisecting line that separates African lineages from non-African genetic lines, a site of significant difference in global genetics.

From this analysis of the different levels of variation within certain populations and several deep-rooted mitochondrial lineages, it can be proven with strong certainty that the similarities present between different populations lead back to Africa, the apparent starting point for the human race (Ingman et al., 2000). Using mutations in several loci, including mitochondrial sequences, as the "raw data," these evolutionary trees and molecular clocks can be used to determine approximate time periods of evolutionary change and expansion (Hedges, 2000).

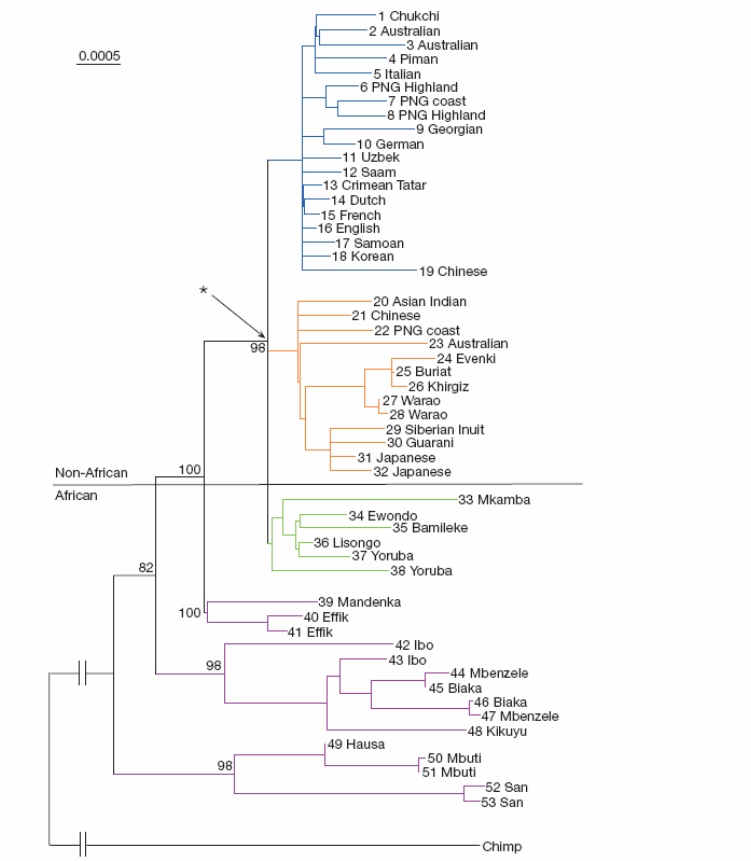


Figure 21: Example of a phylogenic tree constructed using mitochondrial DNA loci. In this particular tree, 53 different populations (the "twigs" at the end of the "branches") were studied to show their relative genetic distance and determine the origin of mankind (Ingman et al., 2000).

However, the major drawback to studying mitochondrial DNA is that it only reflects the genetic history of females, since it is only maternally inherited (Ingman et al., 2000). For this reason, mitochondrial studies for molecular archaeology are often known as the search for "mitochondrial Eve" (Gibbons, 1997). To gain an accurate depiction of human descent though, researchers were also forced to look for mankind's genetic "Adam." Since mitochondria are passed from mother to daughter, researchers clearly had to find a genetic source that was passed exclusively from father to son; the most obvious site being the Y chromosome (Gibbons, 1997). While the Y chromosome is not nearly as variable as mitochondrial DNA, there were still several sites hidden in non-coding regions that could be analyzed (Gibbons, 1997).

In addition to a more in-depth comparison of human DNA to our closest relative, the chimpanzee, studies of Y chromosomal lineages undertaken in a similar fashion to mitochondrial studies (i.e. constructing phylogenic trees) confirmed the conclusions drawn from these maternal lineages: the human descent of man most likely came out of Africa (Gibbons, 1997). The information gained from both mitochondrial analysis and studies of the Y chromosome has recently opened the doors for many more ongoing studies of human evolution and the formation of more and more genetic pedigrees. These studies hope to shed light on the many questions researchers still have regarding where humans came from and where we're going as a species.

General Conclusion on DNA Fingerprinting

While these several preceding examples are only a few in a field of seemingly innumerable DNA fingerprinting and sequencing applications, it's clear to see that DNA fingerprinting has become nearly omnipotent in biological applications, ranging from criminal forensics to tracing the origin of man. With such a need for DNA fingerprinting, it's almost certain that further advances will be made in order to allow increased application of the numerous benefits of genetic-based identification.

There are several definitive characteristics of current DNA fingerprinting techniques and applications that make it so intriguing and expansive. Starting from the original RFLP procedure, DNA fingerprinting has evolved over the past few decades in terms of accuracy and versatility. This extensive ability to build off and improve fingerprinting techniques defines DNA fingerprinting as an extremely resilient analytical technique. The wide variety of available DNA sources and applications only add to its viability within the scientific community.

Additionally, with the ability to standardize the results of DNA fingerprinting, there is a great potential for widespread use of the same experimental protocols and methods worldwide,

increasing the efficiency and coverage of such a substantial and necessary tool in numerous fields of study.

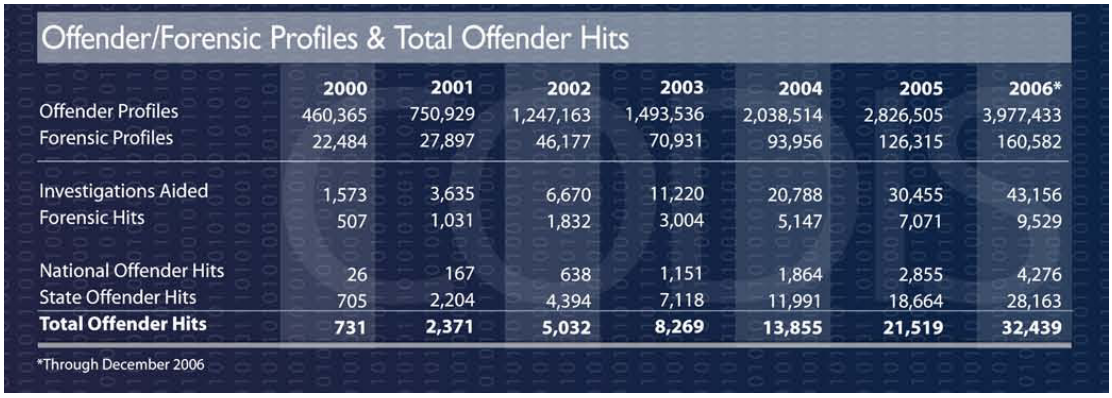
Additionally, while there are still long-standing ethical and legal issues surrounding DNA fingerprinting and its applications (to be covered in the following chapters), a great deal of optimism and enthusiasm remains within the scientific community as more advancements are made to perfect and enhance this fundamental technique. As DNA fingerprinting expands further into the global scientific population, there seems to be virtually no limit to the opportunities for additional growth and application of DNA profiling and sequencing techniques.

CHAPTER 2: DNA FORENSICS

Introduction

DNA fingerprinting has been a vital tool for identifying individuals based on their genetic makeup. Despite having numerous practical applications (discussed in Chapter-1), the most notable use of DNA fingerprinting over the past few decades has been within our nation's judicial system. While there are various techniques used to analyze DNA profiles, the overall theory of DNA fingerprinting in forensics is practically the same for any technique. By taking samples of DNA at crime scenes and comparing them to a suspect's DNA, investigators attempt to match the pattern of allelic bands for several key loci in hopes of narrowing down individuals with a high level of certainty.

With the numerous advances made in the field of DNA forensics over the past several decades, including the 1997 inception of a standardized CODIS system of 13 core loci, and the national DNA database of genetic profiles of violent offenders discussed in Chapters 1 and 5, law enforcement officials have been able to provide increasing levels of substantial DNA evidence used for convicting guilty parties, as well as exonerating innocent individuals. Table 3, which shows the number of positive matches, or "hits," made by the CODIS system in the past seven years, shows a steady increase in the number of criminal cases supported using DNA evidence. However, despite the large-scale progress made in the field of DNA forensics, this vital technology has come under a great deal of scrutiny and attack by defense attorneys and other legal affiliates.



Offender/Forensic Profiles & Total Offender Hits

	2000	2001	2002	2003	2004	2005	2006*
Offender Profiles	460,365	750,929	1,247,163	1,493,536	2,038,514	2,826,505	3,977,433
Forensic Profiles	22,484	27,897	46,177	70,931	93,956	126,315	160,582
Investigations Aided	1,573	3,635	6,670	11,220	20,788	30,455	43,156
Forensic Hits	507	1,031	1,832	3,004	5,147	7,071	9,529
National Offender Hits	26	167	638	1,151	1,864	2,855	4,276
State Offender Hits	705	2,204	4,394	7,118	11,991	18,664	28,163
Total Offender Hits	**731**	**2,371**	**5,032**	**8,269**	**13,855**	**21,519**	**32,439**

*Through December 2006

Table 3: Successful "Hits" of the CODIS System (FBI, 2006)

Skeptics of DNA forensics have made a variety of claims against DNA profiling, ranging from accuracy to contamination. Contrary to their initial intentions, these claims and objections have actually strengthened forensic credibility and reliability. Forensic techniques have withstood the battery of doubts and accusations and, with several procedural improvements, have paved the way for a greater acceptance of DNA evidence within the courtroom.

As is the case with most new technologies, the first major objection to DNA fingerprinting was in regards to its accuracy. After a few years of general acceptance of Alex Jeffreys's original RFLP analysis in the UK, questions of random match probability, especially in newer STR technologies, quickly came about in courtrooms throughout the world (Lynch and Jasanoff, 1998). However, the use of CODIS's 13 core loci, which have calculated random match probabilities above 1 in a billion, quickly put an end to any doubts in the accuracy of DNA profiling (Bickel, 1997).

The most controversial disapproval of DNA forensic evidence actually had little to do with "trust in the technology," but more to do with "trust in the system," (Lazer, 2003). While DNA forensics proved to be more and more reliable in terms of its ability to distinguish or identify individuals based on genetic characteristics, opponents soon began attacking the ways in which forensic analyses were carried out, aiming to blame human error rather than technological flaws. Specific objections dealt with a variety of discrepancies involving the manner in which

DNA evidence was handled: most notably in terms of "collection, transport, and preservation of evidentiary samples, (and) chain-of-custody documentation" (Bieber, 2003).

One of the most famous objections to DNA forensic evidence based on human error was made in the 1994 murder case against OJ Simpson. Interestingly, instead of attacking the science of DNA fingerprinting, defense attorneys put a large emphasis on how DNA evidence in that specific case was handled sloppily, including practices where samples could have been potentially contaminated, yielding an inaccurate DNA profile (Lazer, 2003). Despite having numerous DNA samples with the potential to link Simpson to the scene of the crime, the prosecution's case was ruined by improper and unorganized handling of DNA samples (Jasanoff, 1998). Unfortunately, these mistakes left room for several arguments about contamination, degradation and possible conspiracy (Jasanoff, 1998).

However, the loss of Simpson's conviction was greatly beneficial to the DNA forensic community as a whole. Over the past decade, numerous advances have been made in the field of forensic science to limit the ambiguity surrounding DNA samples that have been collected, transported and analyzed from crime scenes. With these strict protocols for evidence handling and documentation, law enforcement officials have been able to establish DNA forensics as a highly reliable source of evidence, and have maintained its substantial accuracy for identification purposes. From these procedural advances, more and more DNA evidence has been admissible into courtrooms where justice can been served.

Problems Leading to Inaccurate Profiles or Ambiguous Matches

Before discussing the steps taken by investigators to maintain the integrity of DNA evidence, it's important to have a brief discussion of the potential problems there are for DNA evidence in terms of contamination, degradation and documentation. While experts in the field

of DNA forensics encounter numerous sources of potential error they must resolve, the underlying problems in the treatment of potential DNA evidence can be simplified and explained quite easily.

The process of contamination poses an incredibly large challenge to investigators hoping to obtain an accurate DNA profile. Contaminants in DNA samples can come from a wide variety of sources (See Table 2 in Chapter-1 for examples), but always have the potential to threaten the integrity of the DNA sample itself or the process used to analyze it. In general, there are two types of contamination that can occur in DNA forensics: sample-to-sample (dirt contaminating a blood stain, for example) and person-to-sample (i.e. an investigator coughing into a sample of blood) (Reliagene, 2006).

In one sense, contaminants threaten the process of DNA analysis itself. Since RFLP and PCR both rely on enzymes to accomplish either restriction in RFLP or amplification in PCR, contaminants such as extraneous chemicals or enzymes surrounding the DNA sample have the potential to inhibit these enzymes and therefore prevent the possibility of obtaining a sufficient DNA profile (Inman and Rudin, 1997). Additionally, mixing DNA from outside sources, such as microorganisms or even from investigators themselves, will result in an inaccurate DNA fingerprint, often due to co-amplification in the PCR process (President's DNA Initiative, 1999).

The process of degradation, however, occurs when DNA samples are broken down into small, often unusable, segments prior to DNA analysis. As an extreme example, the DNA found within the Tyrolean Iceman mentioned in Chapter-1 was so heavily degraded by time and exposure to the elements that most of his DNA came in small fragments less than 150 base pairs long (Handt et al., 1994). Whether degradation occurs on a 5000 year old mummy or a DNA sample from a more recent crime scene, it is a serious problem for forensic scientists. Exposure to intense sunlight, moisture, degradative bacteria or many other elemental factors can

dramatically reduce the amount of usable DNA found at a crime scene (President's DNA Initiative, 1999). Therefore, investigators must take many precautions to prevent further degradation of potential pieces of evidence.

The final common source of forensic discrepancy comes in the form of ambiguity regarding who handled DNA samples and when they handled them. If evidence is not clearly documented when it changes hands, then theories of tampering and contamination are open to interpretation. In the O.J Simpson case for example, a lab technician failed to write her initials on a sample of DNA she was given by the LAPD, and defense attorneys were able to establish the evidence as inadmissible due to this gap in the sample's history (Wang, 2001). To ensure that there is no doubt that DNA came directly from a crime scene and that no tampering with the evidence occurred, a strict chain of custody must be established to represent the exact timeline of how evidence was handled during its movement from the crime scene to the lab, and how the data ascertained within the lab moved to the courtroom (Schiro, 2001).

However, in spite of the numerous problems that can arise from these sources of error and ambiguity, investigators have developed many techniques to overcome these potential setbacks. By following strict guidelines on how to handle, transport and preserve pieces of evidence, law enforcement officials and scientists alike have a good chance of obtaining admissible DNA evidence.

Procedural Advances: Safe Passage of DNA from the Crime Scene to the Courtroom

At the Crime Scene

Once the location of a crime has been determined, a key step in preventing contamination is to create a barrier around the scene to limit access and therefore limit the amount of potential outside contaminants (Byrd, 2000). Once the area is secure, including an initial inspection

(where nothing is touched), photography, and other non-forensic studies, investigators begin looking for pieces of DNA evidence (Byrd, 2000). As discussed in Chapter-1, DNA can be found in all sorts of biological samples, but a few of the more common sources include saliva, seminal fluid, hair, skin, perspiration and blood (Kramer, 2002). Table 4 provides a summary of the more common DNA sample types, the amount of DNA they contain, and common sources of the sample.

Type of Sample[1]	Amount of DNA [1]	Common Sources [2]
Liquid Blood	20000-40000 ng/mL	Weapon, facial tissue, laundry, bullet, fingernail, Investigators.
Blood Stain	250-500 ng/mL	
Liquid Semen	150000-300000 ng/mL	Facial tissue, laundry, condom, blanket, pillow, sheet.
Post-coital Vaginal Swab	10-3000 ng/swab	Investigators (Victim Sample)
Plucked hair (with root)	1-750 ng/root	Investigators (Suspect Sample)
Shed hair (with root)	1-10 ng/root	Hat, bandanna, mask, laundry.
Liquid Saliva	1000-10000 ng/mL	Toothpick, cigarette, stamp or envelope, bottle, can or glass, bite mark.
Oral Swab/ Buccal Cells	100-1500 ng/swab	Investigators (Suspect), Chewing Gum
Urine	1-20 ng/mL	Investigators (Suspect), carpeting
Bone	3-10 ng/mg	Cadaver
Tissue/ Skin	50-500 ng/mg	Weapon, hat, laundry, eyeglasses, tape/ligature, condom, bullet, fingernail.

Table 4: Common Sources and Amounts of DNA for Forensic Use; "Investigators" signifies samples obtained after the crime for comparison ([1]Butler, 2005; [2]President's DNA Initiative, 1999).

From its high DNA content and common occurrence at crime scenes, blood is a highly favored DNA source for forensic investigators. Even before DNA testing was developed, blood was used to narrow down suspects by their ABO blood type (Schiro, 2001). However, DNA profiling proved to be much more advantageous for identifying suspected criminals. Furthermore, while the methods used to collect, transport and store samples from differing

tissues are all a bit different depending on the sample, blood can serve as a good example for the different techniques used to gather DNA evidence while preventing possible contamination.

The first step to collecting evidence is to identify and locate the source of DNA. Unless present in a large quantity, many types of samples are not easily noticeable without some sort of visualization technique. However, as law enforcement officials have been reminded recently, "just because you cannot see a stain does not mean there aren't enough cells for DNA typing" (President's DNA Initiative, 1999). Accordingly, trace amounts of blood, semen, and saliva are often the first bodily fluids officials look for when investigating a crime scene (Butler, 2005).

Fortunately for investigators, blood is particularly difficult to remove completely, even after criminals use rigorous cleaning techniques (Schiro, 2001). Even when blood has been diluted up to 10 million times its initial concentration, investigators still have several techniques for detecting its presence (Butler, 2005). The first technique investigators often use is a high intensity light which can illuminate minute shading differences in crime scene materials, ultimately showing the presence of stains (Schiro, 2001). This technique is used first because it causes no apparent damage to DNA samples and can discriminate stains quite efficiently.

However, when stains are too dilute to see using a high intensity light, investigators, often quite reluctantly, use a test known as the Luminol reaction: the glow-in-the-dark spray made famous by TV shows like CSI and Law and Order (Schiro, 2001). To test for the presence of hemoglobin, an iron-rich component of blood, investigators spray a suspected area with a specialized water-based chemical, Luminol, which glows
bright blue in the dark (As in Figure 22) when it comes in contact with iron (Harris, 1998).

Figure 22: A simulation of Luminol at work: Before spraying Luminol, there is no sign of blood (left panel). After spraying Luminol (right panel), the latent blood traces emit a blue glow (Harris, 1998).

While TV depicts Luminol as an omnipotent reagent that investigators spray liberally throughout a crime scene, there are several complications that can result from its use. For one thing, Luminol is often used to detect blood stains that are so diluted that they are actually insufficient for DNA analysis and therefore must be used for more qualitative information rather than quantitative measures (Schiro, 2001). For this reason, Luminol is a prime example of a "presumptive test for blood": a test used only to support the existence of blood rather than analyze the blood directly (Della Manna and Montpetit, 2000).

Also, not only does Luminol often react with substances other than blood, such as bleach and some common dyes, it is also suspected to damage certain genetic markers needed for DNA fingerprinting, as well dilute blood samples further due to its water-based consistency (Schiro, 2001). However, research has been done recently to test the affects of Luminol on various stains and develop more efficient collection methods for Luminol treated samples (Della Manna and Montpetit, 2000). Despite these advances, investigators must still be very cautious when using Luminol to find blood stains.

Semen stains, on the other hand, have very specific and accurate presence tests. Using UV light and several different reagents specific for proteins found in semen, large areas that commonly have semen stains, such as bed sheets or mattresses, can be tested without damaging the sperm DNA needed for fingerprinting (Butler, 2005). Once the presence of semen is confirmed, sperm cells are easily identified using staining techniques and a simple light microscope (Butler, 2005). Similarly, saliva stains are also tested by an assay for the salivary-specific enzyme, amylase (Butler, 2005). Hairs, with their roots still intact, can also be seen fairly easily without the use of chemical reagents or assays, and provide a good source of DNA (Koblinsky et al., 2005).

With such a variety of detectable DNA sources, investigators must often decide which pieces of evidence are crucial to the case and will provide the most useful information about a crime scene without overloading law enforcement officials with extraneous pieces of information (Schiro, 2001). However, the ultimate goal of an efficient collection method is the ability to obtain as much sample and as many useful samples as possible (Spear, 2004). For this reason, investigators often follow the mantra that "too much evidence collected is better than not enough evidence collected" (Schiro, 2001).

Collection

Once the presence of explicitly useful DNA-containing samples is confirmed, investigators go about the actual process of collecting the evidence in a manner where contamination and degradation are minimized. While documentation is an integral part of the process, contamination prevention is paramount at this stage. The first barrier to contamination is literally in the investigators hands. To prevent contacting evidence with their own skin, investigators must consistently wear gloves and other clothing to minimize person-to-sample contamination (Reliagene, 2006).

Another interesting procedure often used by non-forensic personnel at the crime scene is to keep their hands occupied by carrying flashlights, notebooks, pens etc., in order to prevent any inadvertent contact with potential evidence (Schiro, 2001). Also, disposable instruments are used whenever possible in order to prevent cross contamination with evidence from previous cases (President's DNA Initiative, 1999).

Investigators are also extremely cautious in making sure not to talk, sneeze, or cough on or around the evidence in order to keep from contaminating samples with other forms of DNA

(President's DNA Initiative, 1999). Following these simple initial guidelines, investigators can begin to confidently undergo more specialized collection techniques without contamination.

While DNA samples come in a wide variety of consistencies and quantities, fluid samples typically come in two forms: those absorbed into material and those adhered to its surface (Handbook of Forensic Services, 2003). For this reason, there are several different techniques used to collect samples based on their condition and liquid or physical state (Handbook of Forensic Services, 2003).

In most conditions, DNA usually remains intact within sample sources as it is being collected and is extracted later in a laboratory (Handbook of Forensic Services, 2003). Therefore, common collection methods generally tend to retain the sample's original integrity until it reaches a secure laboratory (Handbook of Forensic Services, 2003). This usually provides a greater chance for conducting useful examinations. Accordingly, forensic standards encourage investigators not to remove a stain or sample from an object, but instead to collect the entire surface where the stain is located (Spear, 2004).

If a stained item is small enough to be moved to a lab, a ski mask or a handgun for example, the entire item is packaged in an appropriate container (to be discussed in the next section) and transported directly to a forensic lab (Schiro, 2001). While in its packaging, the stain should also be protected from its surrounding environment by covering it with appropriate barriers such as paper or cardboard (Spear, 2004). While this often requires more storage space, it's quite beneficial for preventing contamination because it minimizes pre-analytical contact with the evidence (Schiro, 2001).

Unfortunately, samples are often left on objects that are too large to move from the crime scene, quite typically on carpets or furniture (Spear, 2004). To remove these samples, the material containing the stain is often removed by cutting out the stain and a bit of its surrounding

material with freshly washed scissors (Spear, 2004). However, the implements used to cut the material should never be washed with bleach or other similar cleaning products since these have the potential to damage biological evidence; instead, distilled water is a preferred cleaner (Butler, 2005).

If the sample is on a surface that can't be cut, like a concrete floor or bathroom tiles, the most common procedure to remove the stain is by absorbing it onto a collection medium, often called the "swab method" (Kramer, 2002). The most common materials used for collecting samples are cotton implements such as cotton swabs, cotton balls held using clean forceps, or small pieces of cotton thread (Schiro, 2001). Figure 23 shows some of the typical collecting media used by investigators.

Figure 23: Common collection media used in sample collection. From left: cotton swabs, cotton balls, absorbent squares, forceps (to be used with cotton balls or threads), and lift tape (Evidence Collection and Protection, Inc., 1998).

To absorb the sample stain, the collection medium is often moistened with distilled water and applied to the stain until the greatest amount of sample is absorbed (Handbook of Forensic Services, 2003). During the swabbing process, several considerations must be taken to prevent contamination; otherwise, it will generate an unusable sample. When collecting the sample, investigators aim to concentrate the stain as much as possible by using a minimal amount of water, and swabbing the same area to get the most sample on their swab as possible (Kramer, 2002). Also, investigators swab very small areas with a new swab for each area to avoid sample-to-sample contamination (Kramer, 2002).

In contrast to cutting-out methods, where surrounding material is left alongside the stain, in many cases, the goal of investigators attempting to isolate samples for transport is to get as

little surrounding material as possible to avoid potential contamination (Spear, 2004). For example, when collecting cigarette butts for potential saliva samples, investigators aim to only collect mouthpieces and try to leave as much ash behind as possible since ashes can inhibit the enzymes involved in DNA analysis (Spear, 2004; Handbook of Forensic Services, 2003). The same principle applies to collecting biological samples from soil in order to leave behind enzymes and microorganisms in the soil, both of which can cause insufficient DNA analysis (Spear, 2004).

If samples are not absorbed into a material, but instead remain on the surface as dried stains, clotted blood or dried semen for example, they can be collected using the same moist-swab techniques used for absorbed stains (Schiro, 2001). However, if the stain is too small and using a moist technique has the potential for diluting the sample excessively, investigators often choose to use scraping or tape-lifting which can remove samples without the use of water (Kramer, 2002).

In the scraping method, a clean, sharp instrument is used to scrape the sample stain into a paper packet where it's packaged in the same manner as other DNA samples (Schiro, 2001). While this method prevents dilution, it comes at a price. When sample stains are scraped, they tend to break up into small flakes which can easily disperse, resulting in a loss of sample and potential contamination of other samples (Spear, 2004). Therefore, investigators must be very cautious and aware of any surrounding material when collecting dried samples.

The other method to prevent dilution, the tape lift, conveniently uses the same tape, shown in Figure 2, used to collect conventional fingerprints from surfaces (Kramer, 2002). By gently placing the adhesive side of the tape onto small, dried samples, investigators can easily pick up the stain, without drastically affecting its integrity (Kramer, 2002). The tape is then placed onto a paper or vinyl acetate backing to allow the biological sample to "breathe" (Kramer,

2002; Schiro, 2001). Protected by this backing, the sample can then be packaged normally and sent to the lab.

For samples other than stains, such as hair, chewing gum or other solid objects, investigators often use forceps or occasionally gloved hands to collect and package the material (Handbook of Forensic Services, 2003). This protects the sample from contamination while keeping it in its original environment.

Before delving into the ways collected samples are packaged and documented for transport to the lab, there is one more consideration that investigators must make when collecting samples of suspected DNA evidence. When samples are analyzed by forensic scientists, there must be a sample to compare with evidence in order to demonstrate that testing was done correctly (Koblinsky et al., 2005). These DNA-free samples, known as control samples, usually consist of samples taken from unstained areas close to the stain of interest (Spear, 2004).

To ensure that the control is accurate though, it must be documented, collected and preserved in the exact same manner as the control sample (Kramer, 2002). For example, before an investigator swabs a blood-stained area of carpeting, a control swab should be taken in an unstained area using the same type of swab, packaging and even distilled water from the same batch if the swab is to be moistened (Kramer, 2002). However, a control sample from a blood-stained material or collected under different conditions would obviously be unacceptable (Schiro, 2001). With a proper control sample, laboratory analysis can be done on the sample of evidentiary DNA and compared to a sample containing no DNA in order to maintain the validity of the tests done on the evidence needed for the investigation.

Additionally, investigators must also think ahead to subsequent investigations, laboratory analyses and legal issues and therefore collect what are known as “elimination samples” (U.S. Dept. of Justice, 2000). Elimination samples are used to rule out collected DNA as coming from

extraneous individuals that aren't involved with the crime at hand (President's DNA Initiative, 1999). These DNA samples are often taken from victims in murder cases or from recent consensual partners in rape cases (President's DNA Initiative, 1999).

Packaging

Once proper evidentiary samples, control samples, and elimination samples are taken, they must be packaged correctly in order to prevent degradation and contamination while they are transported to a lab. Before the sample is placed in any type of packaging, it is absolutely critical for evidence to be completely air-dried (Reliagene, 2006). Figure 24 presents several types of drying techniques and their relative rates of drying. No matter what method is used, complete drying is crucial, since any moisture remaining in the sample can support the numerous types of biological processes that lead to DNA degradation (Spear, 2004).

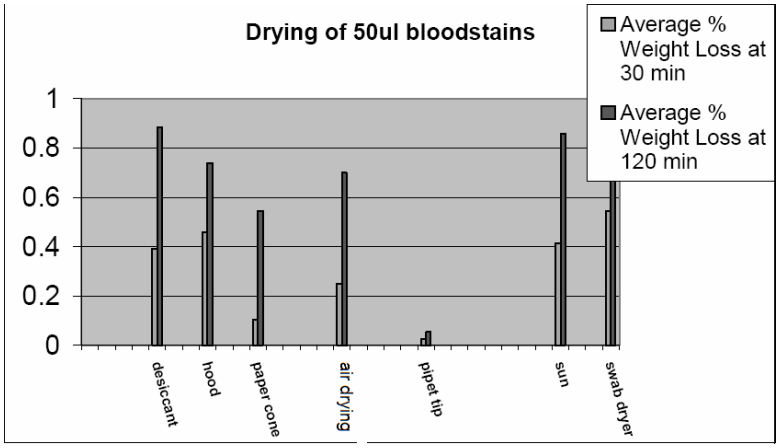


Figure 24: Graph of the relative rates of drying for several techniques (Spear, 2004).

While Figure 3 shows the amount of moisture lost during drying, it is also important to recognize that other factors of the drying process influence an investigator's choice of technique. For instance, high temperatures can also lead to DNA degradation; so, drying techniques that use heating elements, most notably hair dryers, are potentially harmful to the sample (Kramer, 2002). To overcome the effects of both heat and moisture, investigators take great care to package DNA

samples in suitable containers that can prevent exposure to damaging elements, such as direct sunlight or humid atmospheric conditions (President's DNA Initiative, 1999). The packaging used must also provide protection from potential contaminants surrounding the evidence (Kremer, 2002).

The best material used to package DNA samples is, surprisingly, also one of the simplest: paper. For most forms of collected evidence; whether swabs, hair strands or even intact surfaces; paper or manila envelopes are often the container of choice for investigators (Kramer, 2002). Since paper is quite porous, it allows samples to breathe while still maintaining a barrier from potential contaminants (Spear, 2004). Plastic bags, on the other hand, will retain moisture and allow for the growth of bacteria and other harmful organisms (President's DNA Initiative, 1999). Another advantage to paper packaging is that paper is quite rigid and can usually be closed tightly without the use of sealants, such as staples, that can host harmful contaminants and inhibitors (President's DNA Initiative, 1999).

Once samples are adequately prepped for packaging, they are carefully placed in individual, appropriately-sized paper containers to prevent sample-to-sample contamination (Handbook of Forensic Services, 2003). Accordingly, control samples must also be kept separate from their DNA containing counterparts (Kramer, 2002). While packaging dried materials, investigators use extreme caution to keep samples from coming in contact with other biological materials in order to prevent potential contamination (Spear, 2004).

Additionally, the dried samples must also be kept at a cool temperature while being transported to their eventual storage site in order to prevent any degradation (Reliagene, 2006). Ideally, samples are kept refrigerated, but not frozen, while they make their way to their eventual storage destination (Handbook of Forensic Services, 2003).

The same caution is used for liquid samples that can't be dried. In the case of liquid blood, semen, saliva or other bodily fluids, a wide variety of plastic containers are available for storage (Handbook of Forensic Services, 2003). While the use of plastic containers may seem counterintuitive, liquid samples can be kept intact within these specially-made "Vacutainers," provided a proper preservative is available within the container (Schiro, 2001). The containers, as shown in Figure 25, are distinguished by different colored tops, including Yellow, Purple, Red and Gray, based on the type of preservative they contain (Schiro, 2001).

Figure 25: Color-coded "Vacutainers" used for packaging liquid samples. (Evidence Collection and Protection, Inc., 1998).

Gray tops, for example, contain sodium fluoride (NaF), while Purple-topped containers have a preservative known as EDTA (Handbook of Forensic Services, 2003). The type of preservative used is dependent on several factors, including the type of sample, how long it needs to be preserved and the types of analyses that must be conducted (Spear, 2004).

Chain of Custody & Documentation

Once the sample is packaged into the correct container, there is one more issue that investigators must deal with in order to ensure that samples can be used as sufficient pieces of evidence. Before sending samples to the lab, investigators at the crime scene must document the evidence in the appropriate fashion to maintain a strong and continuous "chain of custody" (U.S. Dept. of Justice, 2000). Chain of custody is defined as "the possession, time and date of transfer, and location of physical evidence from the time it is obtained to the time it is presented in court" (Koblinsky et al., 2005).

In order to prevent any inconsistencies within a piece of evidence's history, all samples to be sent to the lab must be clearly marked with their location at the crime scene, the name of the investigator that collected the evidence, and the time and date of its collection (U.S. Dept. of Justice, 2000). Also, in addition to labeling the evidence, investigators must often fill out chain of custody forms in order to provide several levels of documentation (Schiro, 2001). Furthermore, the chain of custody commonly contains several different "checkpoints" as a safety net for the detection of evidence mislabeling or mishandling along the path of evidence transport (Kaye and Sensabaugh, 2000). To establish the evidence's history as accurately as possible, other factors must also be documented, including the initial storage temperature and other handling factors, that provide a detailed account of how the evidence was sustained between its collection and its entrance to the courtroom (Koblinsky et al., 2005).

Once the first link in the chain of custody has been established and the evidence is properly labeled, it can then be transferred to the laboratory for extraction and analysis. In order to rule out theories of third-party interference, every time a sample changes hands, the name of the two parties involved, and the time and date of the transfer must be documented (Koblinsky et al., 2005). Usually, evidence sent from the crime scene stays in the hands of a property clerk that keeps documentation and control of the evidence when it's not being analyzed by laboratory technicians (Koblinsky et al., 2005). The property clerk is a specialized link in the chain of custody designed to keep tabs on who handles evidence and the manner in which it's handled at all times.

In the Laboratory

Once the DNA is securely transferred from the crime scene to the laboratory or storage facility, the first step is to store the evidence properly to prevent any further degradation while

DNA analysis is pending. While cool temperatures are needed, freezing is often avoided, since condensation can arise when a sample is removed from a freezer (Spear, 2004). However, the most common form of storage is usually to keep the sample in its original packaging and under dry and cool conditions, often in an extremely low-moisture refrigerator or by using dry ice (Kramer, 2002; Reliagene, 2006). While samples can survive at room temperature for long periods of time, these extra precautions are necessary to maintain the highest possible integrity of the DNA sample (Spear, 2004; U.S. Dept. of Justice, 2000). As previously mentioned, warm and moist conditions are the major causes of DNA degradation, so any suitable storage technique must minimize exposure to these elements while the sample awaits testing (Handbook of Forensic Services, 2003).

However, before DNA from a sample is analyzed, a forensic scientist, known as a serologist, must test the sample to identify the nature of the material or substance (Koblinsky et al., 2005). While the type of sample is often clear to investigators (i.e. a large, dark blood stain or a tuft of hair), in many cases it's difficult to distinguish between faint samples as to whether they come from blood, semen, or other bodily fluids, even by using presumptive tests in the field. A serologist's job is to identify the exact source, quantity and quality of DNA within a sample so that the correct extraction and analysis procedures are used (Inman and Rudin, 1997; Koblinsky et al., 2005). A variety of laboratory tests and reagents are available that can precisely identify what type of material a biological sample is composed of.

Once the source of DNA is determined, there is still one more precaution to take before extraction can be done. In many cases, when a sample is transported on its original substrate, scientists identify potential inhibitors surrounding the sample that must be removed before extraction can take place (Spear, 2004). When stains are found on fabrics, denim and leather especially, scientists must identify potential dyes that could pose a threat to PCR analysis (Spear,

2004; Inman and Rudin, 1997). In these cases, scientists often choose to transfer the stain completely (by swabbing usually) or to treat the material with several chemicals that can protect the sample from inhibiting agents (Inman and Rudin, 1997). Samples found in soil are another example of substrates with potential inhibiting factors, mostly microorganisms and environmental enzymes, that must be dealt with before proper extraction can be accomplished (Inman and Rudin, 1997). Table 2 in Chapter-1 has several examples of common PCR inhibitors that have pre-extraction treatments available.

With these precautions in place, scientists can finally begin the process of extraction. While minimizing contact with the sample itself through the vigilant use of gloves, autoclaved containers and other sterile techniques, forensic scientists begin by attempting to isolate a small portion of the stain for extraction procedures (Spear, 2004). In general, scientists try to obtain the smallest amount of sample that will produce an observable result in order to leave a large enough remainder for subsequent testing and to avoid objections from defense attorneys regarding irreproducibility (Koblinsky et al., 2005). In terms of chain-of-custody, the exact quantity of sample removed should be documented succinctly so that every portion of the evidence can be accounted for (Handbook of Forensic Services, 2003).

Once the sample is isolated, there are a variety of extraction techniques available to remove DNA from cellular or other biological components. Since biological samples obtained from a crime scene contain many cellular substances other than DNA that can inhibit DNA analysis, DNA must be separated from other cellular material before it can be examined (Butler, 2005). Most of the available extraction procedures are deemed chemical techniques and use specialized detergents to remove cellular components from samples; however, several physical techniques are currently in development that use synthetic membranes to bind and remove DNA molecules from individual samples (Bieber, 2003).

Chemical extraction, the most common method used today, uses a variety of different reagents and detergents, both organic and inorganic (Koblinsky et al., 2005). However, despite the wide variety of substances used in these procedures, all chemical extractions follow the same basic principle. First, cells that do not contain DNA, red blood cells for example, are separated from DNA-containing cells, usually by centrifugation (Butler, 2005). Centrifugation is a process that uses a rapidly spinning system to separate components of a mixture based on their molecular weight (Koblinsky et al., 2005). After cells containing DNA are centrifuged, they are isolated and then lysed, a process that uses chemicals such as sodium docecylsulfate and the enzyme proteinase K to disrupt plasma membranes, eventually releasing DNA, proteins and other cellular components (Butler, 2005).

After the cells are lysed, there are a wide variety of treatments, often involving further centrifugation of the sample, that isolate DNA from the rest of the cellular components, leaving it ready for RFLP or PCR analysis (Butler, 2005). Extraction techniques can be quite complex and involve many complicated steps. These steps are necessary to break open cells properly in a manner that prevents DNA degradation by the cell's own enzymes (Butler, 2005). However, the disadvantage to having so many steps is that each subsequent treatment requires a transfer of material to a new tube, creating many opportunities for contamination of the extracted DNA (Butler, 2005).

While the process of extraction and its numerous steps can be quite complex and cumbersome, the basic principle of chemical extraction is to break open cells and their nuclei using specific detergents and chemicals and then isolate the DNA from potential biological contaminants and degradative substances using centrifugation or similar techniques.

In cases of sexual assault, one of the most interesting types of chemical extraction is often used to isolate sperm cell DNA. In many rape cases, vaginal swabs, which are taken from the

victim by investigators to obtain samples of the attacker's DNA, often contain large amounts of the victim's cells mixed together with the attacker's sperm cells (Gill et al., 1985). However, using a process similar to most chemical extractions, vaginal epithelial cells can be preferentially lysed and centrifuged out by using detergents that sperm nuclei are impervious to, effectively removing vaginal cells without releasing the attacker's DNA (Gill et al., 1985). Once the vaginal cells are removed, sperm DNA can be extracted by normal chemical extraction methods (Butler, 2005).

Another form of extraction, the Chelex method, also relies heavily on cell lysis and centrifugation (Koblinsky et al., 2005). However, Chelex extraction also makes use of a magnetic procedure that removes metal ions from cellular components, which are needed for most enzymatic reactions that destroy DNA (Butler, 2005). This effectively prevents DNA degradation, while significantly reducing the number of steps and material transfers needed in the process of chemical extraction (Butler, 2005).

For physical methods, the most common technique makes use of a specialized material known as FTA Paper (Butler, 2005). The paper essentially breaks apart cells during centrifugation, while separating DNA and storing it within the paper's fibers, protecting it from degradation and contamination in one simple step (Koblinsky et al., 2005). Not only does FTA paper allow for relatively long-term storage of DNA samples at room temperature, it also eliminates several transferring steps, since PCR can be run directly on the paper (Butler, 2005). Figure 26 shows a schematic of FTA paper extraction, as well as generic schematics for organic and Chelex extractions.

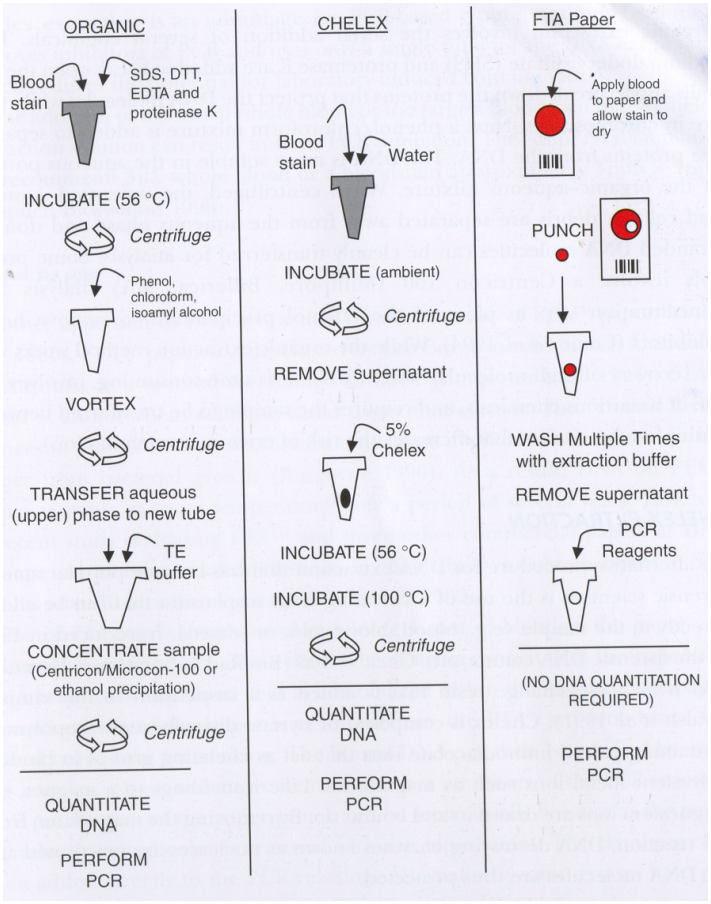


Figure 26: Schematics of Organic, Chelex, and FTA paper extraction methods (Butler, 2005).

Regardless of which extraction procedure scientists choose to use, laboratories must go to great lengths to prevent contamination during the extraction and subsequent analysis process. Since DNA samples are most susceptible to sample-to-sample contamination and introduction of extraneous DNA during extraction, it's very important that scientists take the same precautions used in the field (not talking or coughing around samples, for example) (Butler, 2005). Many labs go as far as using bio-safety hoods and processing reference samples and evidence samples in completely different rooms while using strict lab-space and instrument sterilization protocols (Kaye and Sensabaugh, 2000; Butler, 2005).

After careful extraction has taken place, the procedures described in Chapter-1 are used to analyze the isolated DNA and create a genetic profile of the evidence. Once the process of

DNA analysis is finished, every retainable sample is repackaged and labeled in a manner where it can be directly traced back to its original location at the crime scene, often through the use of "sub" item numbers and other organized documentation protocols (Spear, 2004).

When feasible, laboratories often try to retain as much of the original crime scene samples and extracts as possible in order to allow for sufficient retesting of the evidence, often at the request of defense attorneys (Kaye and Sensabaugh, 2000). Both extracted DNA, often stored in a buffer solution, and other evidentiary samples with appropriate preservatives, are usually packaged in sealing containers for long-term storage in order to prevent samples from reducing to a small residue that is insufficient for secondary testing (Spear, 2004). Once samples are properly sealed under low-moisture conditions, they are typically frozen and stored at -20 to -80 degrees Celsius (Butler, 2005). For samples with intact nuclei, this prevents the activity of DNA-degrading nucleases, and for extracted DNA, helps to significantly slow the process of degradation (Butler, 2005).

To complete the chain of custody, samples are also documented with the name and other pertinent information of the lab worker that handled the evidence (Handbook of Forensic Services, 2003). Not only does this maintain a strict history of the evidence's movement, it also ensures that analysts have the appropriate credentials for DNA analysis, including a laboratory accreditation and yearly proficiency testing required for analysts (Kaye and Sensabaugh, 2000).

Use of DNA Evidence in Court

Once DNA analysis results and their corresponding documentation are completed, they eventually find their way into the courtroom. However, to be useful pieces of evidence, the results of PCR or RFLP analysis must be compared to another DNA sample in order to link elements of crime and hopefully make a definitive conviction (Koblinsky et al., 2005). For this

reason, crime scene investigators must vigilantly think ahead to which evidence might be useful when its handed over to the district attorney's office (Byrd, 2000).

DNA evidence can be used to help prove guilt in several ways. One way DNA is often used as evidence is when a victim's genetic profile is found in a sample on or associated with the suspect: a bloody glove at the suspect's residence for instance (Schiro, 2001). This evidence can show that the suspect has come in contact with the victim, with blood often hinting at violent contact. Alternatively, finding the suspect's DNA at the crime scene or on something in the victim's possession is another way of supporting guilt by association; for example, an envelope containing a threatening letter from a stalker can be analyzed for DNA found within the suspect's saliva (President's DNA Initiative, 1999).

However, in both cases a comparison must be made between the DNA found by investigators and either the DNA of the victim or the suspect: what investigators deem the "known print" (President's DNA Initiative, 1999; Ramsland, 2007). Obtaining DNA from victims is often a bit easier than obtaining a suspect's DNA. In murder cases, medical examiners can often provide blood samples taken from the victim directly to the laboratory (Handbook of Forensic Services, 2003). However, in cases where the victim is still alive, rape or other assault cases for example, victims can choose to give a blood sample, which is often collected in a lab affiliated with the laboratory running the DNA analysis (Handbook of Forensic Services, 2003). Each tube is labeled with the "date, time, subject's name, location, collector's name, case number and evidence number," and is handled using the same precautions used for crime scene evidence. Alternatively, for victims that are a bit squeamish, oftentimes children, victim DNA can be taken using a swab, or toothpaste-free toothbrush for children, in order to collect buccal cells, the cells that line our cheeks (Handbook of Forensic Services, 2003).

Taking DNA samples from suspects can prove to be a bit more difficult. The first

obstacle for investigators is finding a suspect to take a sample from. Provided that law enforcement officials have a suspect, there is usually a bit of red tape they must deal with, often dealing with Fourth Amendment Rights or other civil liberties, before they can take an admissible sample ("DNA Forensics," 2002). Problems with forcing suspects to donate "painful" blood samples have been greatly reduced by the introduction of previously mentioned buccal swabs (Handbook of Forensic Services, 2003).

Once it is collected, suspect DNA must be documented significantly and kept completely isolated from crime scene evidence to prevent allegations of contaminating crime scene DNA with suspect DNA (Kaye and Sensabaugh, 2000; Koblinsky et al., 2005). Once a "known print" can be determined from a suspect under proper documentation and isolation, it can be analyzed and compared using the logic discussed in Chapter-1 with the hope of linking elements of the case together.

Alternatively, if investigators cannot determine a probable suspect, there is another option to obtain DNA profiles for comparison. CODIS, the FBI's electronic DNA database that was introduced in Chapter-1 and will be discussed in greater detail in Chapter-5, can be used by investigators in hopes of matching genetic profiles ascertained from a crime scene with the profile of a known violent offender (FBI, 2006). By entering the DNA profile from evidence of an unknown source into CODIS, law enforcement officials sometimes have the ability to find suspects when "no prior suspect existed" (President's DNA Initiative, 1999).

However, CODIS also works both ways. Beyond searching for potential suspects, investigators can also match suspects of one crime to previously unsolved crimes. According to the DNA Identification Act of 1994, once a suspect of a violent crime has submitted a DNA sample to investigators, their DNA profile must be entered into CODIS ("DNA Forensics," 2002). With the suspect's DNA submitted to CODIS, crime scene DNA samples from preceding

crimes are compared to a suspect's profile in case the suspect could have been involved in prior criminal acts (FBI, 2006). CODIS will be covered in greater detail in Chapter-5.

With suspect and victim comparisons set in place, the last step in the use of DNA evidence is presenting the evidence in court (that is, until defense attorneys begin the process of contesting DNA evidence). DNA evidence usually makes its way into the courtroom in three ways. First, the DNA evidence samples are often presented to the judge in a pre-trial hearing, undoubtedly accompanied by their chain of custody forms, to serve as hard evidence for the condition of the DNA evidence (Schiro, 2001). Secondly, with each DNA analysis, forensic scientists must submit a report of their findings which serves to summarize the scientific outcomes of the analysis (Kaye and Sensabaugh, 2000; Koblinsky et al., 2005). Finally, DNA evidence also enters the court in the form of expert testimony from forensic scientists known as "criminalists" (Koblinsky et al., 2005). Criminalists present the court with an interpretation of the results based on their experience and expert opinion (Koblinsky et al., 2005). While a criminalist cannot testify that a DNA sample came from a specific individual, they can explain the one in a billion probability of someone randomly having a certain genetic profile and can testify whether or not a victim or suspect has that specific profile, thereby presenting the likelihood of a possible match (Schiro, 2001). Their testimony often provides a useful explanation for laymen jurors who might otherwise not completely understand the results of DNA analysis.

Conclusion

Once DNA evidence has safely arrived into the courtroom, its applicability is in the hands of district attorneys, jurors and other members of our legal system. The main objective of forensic scientists and other officials involved in DNA forensics is to maintain the integrity of

the sample and resultant DNA so that it can withstand attacks from defense attorneys and hopefully lead to fair and unbiased convictions. The advancements made in the field of DNA forensics in the past ten years have made wondrous strides in terms of preventing contamination and degradation, and establishing an indisputable chain of custody for DNA samples. With these standards and protocols in place, the amount of admissible DNA evidence involved in various criminal convictions has grown substantially.

Chapter 3: Landmark DNA Cases

Science has played a powerful role in the courtroom, starting around the turn of the 20th century, and in many cases has decided the outcome of a particular trial. The problem with accepting complex scientific information in court is that the jurors often do not have the background to understand all the technical information. So the problem becomes whether the specific scientific technique is accepted by experts, and whether that technique was correctly used in a given court case. DNA evidence is still looking for full acceptance in the courtroom as we get better about including controls, minimizing contamination, and protocol standardization, and it still seeks to be the standard for forensic technical evidence.

In this chapter, several landmark cases will be explored that set the precedence for admitting technical information, sometimes DNA, into the courtroom. Some of these cases created tests for determining technique reliability, while others established the concept of pre-trial hearings, and identified required steps or "prongs" for admitting the evidence.

1923, Frye v. United States

In 1923, the landmark case of Frye v. United States established that "while courts will go a long way in admitting expert testimony deduced from a well-recognized scientific principle or discovery, the thing from which the deduction is made must be *sufficiently established* to have gained general acceptance in the particular field in which it belongs" (Frye v. United States, 1923). Basically, the "Frye Standard" states that scientific evidence or technique must have *general acceptance* in the scientific community to be admitted into court.

In 1923, James Frye was arrested for murder in the second degree. Frye appealed his conviction, and the defense offered a "witness" to testify to Frye's innocence. A "deception test" was used, also known as a crude polygraph or lie detector test. This test was recently invented,

at that time, and found Frye to be "telling the truth," proving his innocence. However, the court ruled against this new technique stating, "we think the systolic blood pressure deception test has not yet gained such standing and scientific recognition among physiological and psychological authorities as would justify the courts in admitting expert testimony deduced from the discovery, development, and experiments thus far made" (Frye v. United State, 1923). This case concluded with a new standard stating how evidence was to be admitted into the court room; the evidence or technique must be accepted by the whole scientific community before it will be allowed in court. Many cases over the years referred back to this so-called "Frye Standard" when new scientific techniques or evidence was to be admitted into a trial, however proving a general acceptance in the scientific community was decidedly harder to actually achieve.

1975, Federal Rules of Evidence 702 (Rule 702)

Congress, in 1975, enacted the Federal Rules of Evidence 702, more commonly referred to as Rule 702, which provided more lenient guidelines than the vague Frye Standard. Rule 702 states:

> If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence of to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise, if (1) the testimony is sufficiently based upon reliable facts or data, (2) the testimony is the product of reliable principles and methods, and (3) the witness has applied the principles and methods reliably to the facts of the case (Rule 702, 2000).

"In other words, if a jury would find the testimony of one with specialized knowledge to be *helpful*, a court may admit it. Following the enactment of Rule 702, many courts rejected the *Frye* standard of "general acceptance" in favor of the more liberal "*helpfulness standard*" of Rule 702" (New Technologies, 2004). Thus, Rule 702 allows the use of qualified expert witnesses, with specialized knowledge, to assist the court in understanding complex technical evidence, and

allows new techniques, if they are shown to be *reliable*, even if such techniques are not generally accepted.

Rule 702, even though it was enacted in 1975, did not really come into play until the mid 1980s when DNA fingerprinting techniques started to rise. Since it was less strict than the Frye Standard, it allowed the use of DNA in the court room as a reliable technique, even prior to its general acceptance. Without this lenient rule, DNA would not have made it into the courtroom until much later when the whole scientific community approved.

1985, U.S. v. Downing

In 1985, John Downing was charged, in the U.S. District Court for the Eastern District of Pennsylvania, with mail fraud, wire fraud, interstate transportation of stolen property, and aiding and abetting (U.S. v. Downing, 1985). Downing, along with a group called the "Universal League of Clergy" or ULC, defrauded vendors by collecting their money but never delivering any merchandise. After Downing was caught, many vendors were willing to testify against him. Although the witnesses provided substantial eyewitness testimony, his lawyers tried to discredit them saying all eyewitness testimony is unreliable and can not be used to determine guilt. The defense asked to use a psychologist as an expert to prove the witnesses unreliable, but the District Court denied the defense request, ruling the psychologist's testimony did not meet the *helpfulness standard* of Rule 702. With the eyewitness testimony accepted in trial, the jury found Downing guilty of all charges, except for the interstate transportation of stolen property.

Downing appealed his conviction to the United States Court of Appeals. The Court of Appeals ruled that the District Court was wrong to exclude the psychologist's testimony and remanded the case back to the District Court with orders to reinstate the expert's testimony. However the District Court threw out Downing's case stating "the court declines to admit the

expert psychological testimony proffered by the defendant under Rule 702," leaving the guilty verdict standing (U.S. v. Downing, 1985). This landmark case allowed for any *misleading evidence* to be dismissed by a pre-trial hearing, and concluded that *relevance* to the case is the main basis to admit evidence, not the strict Frye Standard of general acceptance in the scientific community.

1986, Colin Pitchfork: First Person to be Convicted of Murder Based on DNA

Since Sir Alec Jeffreys discovered DNA fingerprinting in 1984, many cases have been solved, or court decisions overturned, based on DNA evidence. The world's first conviction of murder using DNA occurred in 1987 in Narborough, England. The conviction took place following the rapes and murders of two victims, one in 1983, and the other in 1987. In 1983, "Lynda Mann, just fifteen years old, was discovered along a shady footpath, savagely raped and strangled" (Batt, 1999). The police were not able to gain any leads, however a minute sample of semen was collected and stored. In 1987, another rape and murder occurred in the same area. "Dawn Ashforth, also 15 years old, was brutally raped and strangled. The similarity between the two cases was too evident to ignore, and the police realized they were looking for the same man" (Batt, 1999). A manhunt began but turned up nothing. Police were tipped off and had a possible suspect in custody, John Buckland a seventeen year old dishwasher. He admitted to killing Dawn, the second victim, but proclaimed he did not kill Linda. Police decided to test the new DNA fingerprinting technique and found that Buckland's DNA did not match the semen found at either scene, and Buckland was proved innocent.

The police were now left with no suspects, but felt that if DNA could prove innocence it could also prove guilt. DNA was taken from males between 13 and 30 years old in three nearby villages (Batt, 1999). Unfortunately, there was no match to any of the men and the case was

going nowhere. Finally a break came, "a young woman who managed a local bakery overheard a man bragging to his friend that he had paid someone else to go in his place and be tested in his name. The woman reported the man to police who picked him up and took him into custody" (Batt, 1999). Ian Kelly was brought in for questioning and confessed that he had given a sample of his DNA in Colin Pitchfork's place (see Figure 27). "On January 22, 1988, Pitchfork pleaded guilty in court and was given a life sentence. He thus became the first murderer caught because of DNA evidence" (Autopsy, 2004). This landmark case was a breakthrough for the new DNA fingerprinting technique, because not only did the test prove that Pitchfork was guilty, but it also cleared a suspect, Buckland, who was innocent.

Figure 27: Colin Pitchfork. A picture of the first man to be convicted of murder using DNA fingerprinting (Autopsy, 2004)

1988, Andrews v. Florida: First U.S. Case to Allow DNA Evidence

Following the first conviction based on DNA in England, it was time for DNA evidence to appear in the United States' courts. In 1986, in Orlando, Florida, a serial rapist, Tommie Lee Andrews, was caught, charged and sentenced to twenty two years in prison for only one of his many rapes (Andrews v. Florida, 1988). Following his incarceration for the first rape, investigators collected DNA from Andrews, and sent it for testing at Lifecodes lab, to be compared against the DNA samples collected from the other rapes. Since DNA testing was a new technique in the United States, the court required a pre-trial hearing to determine if the evidence was admissible. The pretrial was "long and complex, but finally the judge allowed the evidence into the case" (Ramsland, 2003). The results proved that the rapes were committed by

one man, and the DNA matched Andrews, so he was eventually charged with all the rapes. His prison sentenced was increased from 22 years to 115 years (Ramsland, 2003).

This was one step in the right direction for DNA evidence in the United States, but it still would take some time before DNA was more widely accepted in U.S. courts.

1989, People (New York) v. Castro: The Three Pronged Test

In the case of People v. Castro (1989), DNA fingerprinting was greatly scrutinized. José Castro, a handyman in a New York City neighborhood, was charged with murdering Vilma Ponce and her two-year old daughter (Patton, 1990). Police noticed a bloodstain on Castro's watch when he was in custody. Blood samples taken from the victims and the watch were sent to Lifecodes lab for analysis. The samples were a match to Castro, and the prosecution attempted to have the results admitted as evidence (Patton, 1990). A lengthy pre-trial hearing was held, and the court established a *three-prong test* that would determine whether DNA evidence should be admitted in a U.S. trial. The test included:

1. Is there a generally accepted scientific theory stating that DNA testing can be reliable?
2. Do techniques exist that can produce reliable DNA results?
3. Did the testing laboratory perform these accepted DNA tests in this trial?

The case ended in late 1989 when Castro confessed to the murders. However, the DNA evidence would not have been admitted in this case due to the fact that Lifecodes did not follow approved scientific techniques while testing the samples (People v. Castro, 1989).

After the trial, the FBI created the Technical Working Group on DNA Analysis Methods, or TWGDAM (Miller, 2004). "TWGDAM is comprised of scientists from industry, forensic laboratories, and the academic community, who meet several times each year in efforts to build consensus, and to define guidelines for DNA laboratories, quality assurance guidelines for

forensic DNA testing, and guidelines for DNA proficiency (Miller, 2004). These guidelines have become a major factor in determining the admissibility of DNA tests into evidence. The landmark Castro case was the first to strongly question the use of DNA tests and to standardize the methods used.

1990, Two Bulls v. US: The Five Pronged Test

In 1990, Matthew Two Bulls was convicted of aggravated sexual abuse and sexual abuse of a fourteen-year old girl on an Indian Reservation in South Dakota. The girl's underwear was recovered and was sent for testing. The FBI used DNA fingerprinting and determined there was a very high probability that the semen on the underwear came from Two Bulls (Two Bulls v. US, 1990). During the pre-trial hearing, the district court allowed the DNA evidence to be admitted, however the defense appealed the case, arguing that the DNA evidence should have followed the stricter Frye Standard, not Rule 702 (Two Bulls v. US, 1990). The defense also argued that the testing procedures should also have been questioned. The appellate court sent the case back to be re-tried using a new standard. While trying to configure this new standard, the Castro three-prong test was considered too strict by the appellate court, and the Frye Standard and Rule 702 were determined to be correct. The new test to be performed at the pre-trial hearing was now a five-prong test to be used whenever DNA evidence was included in a trial. The five prong test asks:

1. Whether DNA evidence is generally accepted by the scientific community?
2. Whether the testing procedures used in this case are generally accepted as reliable if performed properly?
3. Whether the test was performed properly in this case?
4. Whether the evidence is more prejudicial than probative in this case?

5. Whether the statistics used to determine the probability of someone else having the same genetic characteristics is more probative than prejudicial under Rule 403? (Two Bulls v. U.S., 1990)

After a long trial, the appellate court ruled that the DNA evidence was admissible in this case, and the Two Bulls' conviction was upheld. This case, like Castro, cautions future cases to question DNA testing to prove that the test was performed properly, while also asking whether the evidence is more probative or prejudicial.

1991, People (Illinois) v. Miles: Supported the TWGDAM Guidelines Established by Castro

In 1991, Reggie Miles was convicted, in Illinois, on two counts of home invasion, five counts of aggravated criminal sexual assault, one count of criminal sexual assault, one count of aggravated unlawful restraint, one count of armed robbery, and two counts of residential burglary (People v. Miles, 1991). The DNA evidence collected was sent to Cellmark Diagnostics to be tested. Cellmark, on previous occasions, failed to comply with testing standards required by TWGDAM and was under scrutiny. In this trial, the DNA testing lead to a guilty verdict, but Miles appealed the conviction hoping the testing was botched in some way. The appellate court ruled that the techniques used by Cellmark produced reliable results and were performed correctly, which upheld the State's decision. The case's ruling ended with strong support for DNA evidence, proving DNA to be reliable for future cases, and faith for the standards established by TWGDAM.

1989, 1991, 1993, Daubert v. Merrell Dow Pharmaceuticals

Daubert v. Merrell Dow Pharmaceuticals, Inc., a landmark case, questioned whether the Frye Standard for admitting scientific testimony had been superseded by Rule 702 (Daubert v.

Merrell Dow Pharmaceuticals, 1989, 1991, 1993). Both Jason Daubert and Eric Schuller sued Merrell Dow Pharmaceuticals, Inc., arguing that the drug Bendectin, taken by the children's mothers to control nausea during pregnancy, caused the babies to have birth defects. Merrell Dow provided expert testimony stating "that none of the more than thirty published studies, involving more than one hundred and thirty thousand patients, showed any evidence that Bendectin caused birth defects, and that none of the studies had found Bendectin to be capable of causing malformations in fetuses" (Daubert v Merrell Dow, 1993). The plaintiff's responded with expert testimony as well, stating their testimony relied on animal studies; their re-examination of the published studies indicated that Bendectin did cause birth defects. The District Court ruled that "scientific evidence is admissible only if the principle upon which it is based is sufficiently established to have general acceptance in the field to which it belongs" (Daubert v Merrell Dow, 1993). In other words, the District Court argued that the Frye Standard supersedes Rule 702.

Daubert and Schuller appealed to the United States Court of Appeals. The Court affirmed the District Court's ruling, declaring that the expert's opinion was "based on a methodology that diverged significantly from the procedures accepted by recognized authorities in the field… and cannot be shown to be *generally accepted*," i.e. via the Frye standard (Daubert v Merrell Dow, 1991). Thus, the U.S. Court of Appeals argued that the Frye test supersedes Rule 702.

Daubert and Schuller then appealed to the United States Supreme Court, arguing that when Rule 702 was revised, it abandoned the Frye Standard. The Supreme Court agreed, stating that the Federal Rules of Evidence were proposed to expand upon the range of admissible evidence. The decision is now known as the *Daubert Standard of Evidence Admissibility*; this ruling lets the trial judge determine whether an expert's testimony rests on a reliable foundation

and is relevant to the case (Daubert v Merrell Dow, 1993). The *Daubert Standard of Evidence Admissibility* uses five criteria:

1. Whether the theory or technique has been tested?
2. Whether the theory or technique has been subjected to peer review and publication?
3. Whether the theory or technique has a known or potential rate of error.
4. Whether the theory or technique has standards for controlling the technique's operation.
5. The degree to which the theory or technique has been accepted in the relevant scientific community (Daubert v Merrell Dow, 1993).

The Supreme Court reversed the Appellate Court's exclusion of the plaintiff's evidence, and sent the case back to the Appellate Court to be re-tried. The Appellate Court found that the plaintiff's evidence under the new standard should also be excluded (Daubert v Merrell Dow, 1993).

This landmark case established that Rule 702 supersedes the Frye Standard, and the Daubert Standard has been applied to DNA evidence in all cases after 1993.

2003, Paul Eugene Robinson: First Conviction Based Solely on DNA

In 2000, with the six-year statue of limitations looming on a rape case, Paul Eugene Robinson was arrested (see Figure 28). "District Attorney Jan Scully announced that Paul Eugene Robinson was sentenced to the maximum term of 65 years in state prison for five counts of sexual assault occurring in August 1994" (Scully, 2003). The press release, in June of 2003, stating the arrest of a suspect, was a significant step for DNA evidence at that time. This was the first case where the suspect was convicted solely using DNA evidence.

During 1993-1994, several sexual assaults occurred in the Cal Expo area, in California. The six-year statute of limitations deadline was approaching fast, and there were still no suspects in the case, so a "John Doe" warrant was filed for an individual based on his DNA characteristics, as found on the crime scene evidence instead of his physical characteristics (see Figure 29), then the case was temporarily closed.

Later, after the state of California established a DNA database, Paul Eugene Robinson was in jail for violating his parole. While in jail, blood was drawn and saliva samples were taken for testing. His DNA produced a "cold hit" in the state's database; Robinson's DNA matched the samples from his victim. Robinson was convicted on five counts of sexual assault, and sentenced to the maximum term of 65 years in a state prison (Scully, 2003). If the police did not issue the "John Doe" DNA warrant, the case would have closed and Robinson would not have been caught. This landmark case shows the enormous steps forward that DNA fingerprinting has taken in the judicial system and in the scientific community.

Figure-28: Paul Eugene Robinson. Picture of the first man convicted of a crime based solely on DNA evidence. (Delsohn, 2001)

THE PEOPLE OF THE STATE OF CALIFORNIA,

vs.

JOHN DOE ,unknown male with Short Tandem Repeat (STR) Deoxyribonucleic Acid (DNA) Profile at the following Genetic Locations, using the COfiler and Profiler Plus Polymerase Chain Reaction (PCR) amplifications kits: D3S1358 (15,15), D16S539 (9,10), THO1(7,7), TPOX (6,9), CSF1PO (10,11), D7S820 (8,11), vWa (18,19), FGA (22,24), D8S1179 (12,15), D21S11 (28,28), D18S51 (20,20), D5S818 (8,13), D13S317 (10,11), with said Genetic Profile being unique, occurring in approximately 1 in 21 sextillion of the Caucasian population, 1 in 650 quadrillion of the African American population, 1in 420 sextillion of the Hispanic population

Figure-29: Photo of the John Doe Warrant in the Eugene Robinson Case. This warrant was issued when the DNA profile was known, but the person it belonged to was not yet identified. (Delsohn, 2001)

Chapter 4: Sensational DNA Cases

Introduction

The introduction of DNA evidence into the courtroom has been a long and tough road. Criminal trials have shown light on the new technique of DNA Fingerprinting, but not all criminal trials have set legal precedents like the cases discussed in the previous chapter. The cases discussed in this chapter will go in-depth on a couple of very famous cases that brought DNA fingerprinting to the public's attention.

The Boston Strangler

Boston, and the surrounding area, was terrorized by a serial killer, or possibly several killers, from June 14, 1962 to January 4, 1964. Eleven of the thirteen murders were officially known as the victims of the Boston Strangler. The police believed the murders were committed by several people, however, the public felt only one person was responsible for all the murders. All of the women were murdered, sexually molested, and strangled with an article of clothing, most of the time the victim was wearing the article. None of the homes showed any signs of forced entry, meaning that the women did not know they let the killer, or killer, into their homes. (Bardsley and Bell, 2003; Kelly 1995)

Anna Slesers, fifty-five yeas old, was the first victim of the Boston Strangler on the evening of June 14, 1962. Later in the evening, Slesers' son, Juris, came by the apartment to pick up his mother for church services, and became annoyed when she did not answer the door right away. Juris forced his way into the apartment and found his mother strangled to death on the bathroom floor with the tie of her bathrobe around her neck. The apartment was ransacked but nothing appeared to be taken. The crime appeared to be a burglary gone wrong and the

intruder was overcome by an urge to sexually assault her in the end. (Bardsley and Bell, 2003; Kelly 1995)

On June 30, less than three weeks after Slesers death, sixty-eight year old Nina Nichols was found strangled with two of her own stockings tied in a bow around her neck. There were also signs that she had been sexually molested. Her apartment, at 1940 Commonwealth Avenue, had been ripped apart but nothing was taken. The same day, Helen Blake was killed, in the same fashion, over fifteen miles north of the Nichols' scene. The sixty-five year old woman's apartment was ransacked, but this time two rings that Blake wore were taken. The killer also tried to pry open a metal strongbox and footlocker, but was unsuccessful. (Bardsley and Bell, 2003; Kelly 1995)

The police became worried and sent out a warning to all women in and around Boston to be alert of all strangers and to lock all of their doors. The Boston police turned all of their attention to the case, hoping to end the crimes quickly. Sex offenders and violent mental patients were thoroughly investigated as potential suspects. Also, seminars were set up by the FBI, to educate detectives on how to deal with these crimes. (Bardsley and Bell, 2003; Kelly 1995)

On August 19, a few weeks after Nichols and Blake were found murdered, seventy-five year old Ida Irga was found strangled with a knotted pillowcase around her neck, sexually molested, and left in the doorway of her living room. Across town, sixty-seven year old Jane Sullivan was also found dead in her bathroom. She was strangled with her nylons, sexually assaulted, and left in her bathtub; no one found her until ten days after. In both crimes, neither apartment was ransacked or forced open, however, Sullivan's purse was found open. (Bardsley and Bell, 2003; Kelly 1995)

Boston's citizens lived in fear that they would be the next potential victim, but the city was given a three-month breather from the attacks. On December 5, the Strangler struck again,

but this time with a young victim. Sophie Clark, twenty-one years old, was killed in her apartment, a couple of blocks away from the first victim, Anna Slesers. Clark was strangled with her own stocking, molested, and left in the living room. There were some differences with this murder that had not surfaced with the other attacks. Clark was young, African-American, and did not live alone like the older women. Also, for the first time, there was semen present at the scene. Clark was the first victim of a new series of attacks on young women by the Strangler. (Bardsley and Bell, 2003; Kelly 1995)

Three weeks went by before Patricia Bissette, twenty-three years old, was discovered on December 31. Her boss was worried when she did not answer the door for a ride into work and failed to show up later. Her boss went to back to her apartment and climbed into a window, with the help of a custodian, and found Bissette strangled, molested, and left dead in her bed. Her apartment was searched but nothing was taken. (Bardsley and Bell, 2003; Kelly 1995)

A few months went by when things were quiet but in March of 1963, sixty-eight year old Mary Brown was found dead in her apartment. She had been beaten, strangled, and raped. Two months later, on May 8, 1963, Beverly Samans, twenty-three years old, was found dead by her friend. Unlike the other women, Samans was stabbed to death, and oddly enough, the stockings were tied too loose around her neck to actually cause strangulation. She also had not been sexually assaulted. Samans was stabbed twenty-two times, eighteen in the chest and the fatal four in her throat. She was studying to be an opera singer and police speculated that her throat muscles were too strong and made strangulation too difficult, which resulted in the stabbing. (Bardsley and Bell, 2003; Kelly 1995)

The police were frustrated with no progression in the case. They called Paul Gordon, who claimed to have ESP qualities. Gordon, after talking to the police, gave accurate details of the cases and possible killers. Gordon named Arnold Wallace as the alleged Strangler. Wallace

was investigated and it was discovered that he was a mental patient and had escaped from the hospital multiple times, which happened to coincide with the days of the murders. An investigation of Gordon's life was looked at before police further investigated Wallace. The police found out that Gordon had been to the hospital and could have talked to Wallace. This caused doubt in Gordon, and suspicion arose that his ESP's abilities could be a hoax. Wallace was given a lie detector test to prove if he was the Boston Strangler, but the results proved to be inconclusive and he was taken back to the hospital. (Bardsley and Bell, 2003; Kelly 1995)

Another quiet period occurred until the summer of 1963. On September 8, 1963, Evelyn Corbin, fifty-eight years old, was found murdered. She was strangled with two of her stockings, gagged with her underwear, and left on her bed. Her jewelry was found of the floor and her purse had been emptied onto the sofa, but nothing appeared to be taken. (Bardsley and Bell, 2003; Kelly 1995)

On November 25, while Boston and the rest of America was still grieving the death of JFK three days before, Joann Graff, twenty three, was raped and murdered in her apartment. Graff was strangled, with her stockings tied around her neck, and she was sexually assaulted, just like the other women had been. There were also teeth marks found on her breast. However, this case differed slightly from the others. Around 3:25 P.M., a student that lived upstairs from Graff heard knocking on the door across the hall. He opened the door to find a man around thirty and asked the student, "Does Joan Graff live here?" The man mispronounced Graff's name, but the student did not seem to pay attention. The student said she lived on the floor below. Minutes later, the student heard the door open and shut on the floor beneath him and assumed he was let into Graff's apartment. Graff was phoned about ten minutes later by a friend, but there was no answer. (Bardsley and Bell, 2003; Kelly, 1995)

The last victim of the Boston Strangler occurred on January 4, 1964, over eighteen months from the first attack. Mary Sullivan, nineteen-years old, was strangled, raped, and left for her roommates to find. She was strangled with two stockings, a pink scarf tied in a large bow under her chin, and another pink scarf over the previous scarf. She was left in a seated position on the bed and a "Happy New Year's" card was placed against her foot. (Bardsley and Bell, 2003; Kelly, 1995)

The city of Boston and the surrounding area was fed up with the murders. The Boston Strangler case was handed over to Massachusetts Attorney General Edward Brooke on January 17, 1964. The Strangler case was very unusual, due to the fact that the case spanned five police jurisdictions. Brooke was in charge of putting together a special task force designed to work solely on the Strangler case, and the investigators could not be pulled off this case to work on other cases. There would be no withholding of evidence between the departments because of feuds or jealousies. Brooke selected his close friend, Assistant Attorney General John S. Bottomly, to head this task force, which was formally called, the Special Division of Crime Research and Detection, or later known as the "Strangler Bureau." The Bureau had to organize all the evidence before formally starting to look for suspects. The group called Peter Hurkos, the well-known Dutch psychic, to help find a suspect. Hurkos did identify a suspect, one who was previously investigated by the Strangler Bureau, but no evidence could be found to link the suspect to the murders. It was not until a man confessed to the murders that this case ended. The Strangler Bureau did, at least, provide some marginal comfort to the public, showing the area that they were trying to close this dreadful case. (Bardsley and Bell, 2003; Kelly 1995)

A few years prior to the Boston Strangler murders, a different series of sexual assaults occurred in the Cambridge area. A man, in his late twenties, would knock on women's doors and entice them to let him in; he would come to be known as the "Measuring Man." He would

say he was from a modeling agency and wanted to take some measurements. When he was done, he would say a "Mrs. Lewis" would contact them. This would not happen, and after some time the women started complaining. On March 17, 1961, the Cambridge police caught a man breaking into a house, this man was twenty-nine year old Albert DeSalvo. DeSalvo not only confessed to this breaking and entering, but he also confessed to being the "Measuring Man." (Bardsley and Bell, 2003; Kelly 1995)

DeSalvo lived in Malden, Massachusetts, with his German wife, Irmgard Beck, and their two children. He grew up in a broken family, with his father regularly beating him. DeSalvo grew up getting into trouble, and in 1948, he enlisted in the Army. While stationed in Germany, he met Irmgard Beck and married her. Eight years later, in 1956, he received an honorable discharge for disobeying orders. DeSalvo was arrested in 1955 for fondling a young girl, and in the same year, his first child was born. The baby, Judy, was born with a handicap which discouraged his wife from having another child. His wife, not wanting to get pregnant again, refused to have any sexual relations with DeSalvo, who was reported to have a very strong sexual appetite. During 1956 and 1960, DeSalvo was arrested for multiple breaking and enterings. In 1960, his second child, Michael, was born without any physical handicaps. Besides being a thief, people seemed to like him, except for the fact that DeSalvo wanted to top everyone. The police called him a "blowhard." DeSalvo, receiving sympathy from the judge, received an eighteen-month sentence for the "Measuring Man" crime. He was released in April of 1962, just two months before the first victim of the Strangler, Anna Slesers, was found. (Bardsley and Bell, 2003; Kelly 1995)

Almost three years after DeSalvo was released from jail, he was arrested again in early November 1964. On October 27, a newly married woman awoke to find a strange man in her home. Her husband had left for work and the woman was sleeping alone. The man entered her

home, gagged her, tied her to the bed, kissed her and fondled her. He told her to be quiet, apologized to her, and then left. The woman reported the assault to the police and compiled a sketch. The police recognized her attacker as the "Measuring Man," DeSalvo. He was brought in for questioning and was positively identified as the woman's attacker. DeSalvo was released on bail, until he was identified for a string of sexual assaults in Connecticut, coined the "Green Man" attacks because the assailant always wore green pants. DeSalvo confessed to breaking into four hundred apartments, three hundred assaults in four states, and a couple of rapes. Since DeSalvo loved to brag, and not all of the incidents were reported, the actual numbers could not be confirmed. (Bardsley and Bell, 2003; Kelly, 1995)

DeSalvo was sent to Bridgewater State Hospital for observation. It was here that DeSalvo met a dangerous man, George Nassar. Nassar was sent to the hospital after viciously executing a gas station attendant. He had a high IQ, was very manipulative, and was also DeSalvo's ward mate. During their time together, it is believed that Nassar and DeSalvo worked out a plan where DeSalvo would confess to being the Strangler and the two men would split the reward money Nassar would receive for turning DeSalvo into police. DeSalvo knew he would spend the rest of his life in jail, but thought the money he could get from the publicity of the case would help his family. On March 6, 1965, DeSalvo confessed to being the Boston Strangler. DeSalvo's confession was taped by Nassar's lawyer, F. Lee Bailey. In the confession, DeSalvo stated, "I know I'm going to have to spend the rest of my life locked up somewhere. I just hope it's a hospital, and not a hole like this [Bridgewater]. But if I could tell my story to somebody who could write it, maybe I could make some money for my family." (Bardsley and Bell, 2003) DeSalvo confessed not only to committing the eleven "official" victims, but two others as well; Mary Brown of Lawrence and Mary Mullen, an elderly woman who died of a heart attack before she could be strangled. Bailey, wanting to determine if DeSalvo was really the Boston Strangler,

called Lieutenant Donovan for some questions to ask DeSalvo. The details DeSalvo provided were accurate and numerous. Commissioner McNamara and the psychiatrist at Bridgewater State Hospital, Dr. Robey, became involved. Bailey convinced DeSalvo to cooperate with the police and to take a lie detector test. DeSalvo gave a very detailed account of each murder, and eventually the Strangler Bureau was convinced DeSalvo was guilty. (Kelly, 1995)

DeSalvo confessed to being the Boston Strangler, however, not everyone believed he was guilty. Those close to DeSalvo, his family, former co-workers, his lawyer, the prison psychiatrist, and some of the police force, felt that he was not capable of these gruesome acts. Everyone who knew him saw him as a "gentle, decent family man, who just happened to be an incorrigible small-time thief." (Bardsley and Bell, 2003) In her book, *The Boston Stranglers: The Public Conviction of Albert DeSalvo and the True Story of Eleven Shocking Murders,* Susan Kelly gives convincing arguments toward DeSalvo's innocence. The strongest reason she wrote was that there was "not one shred of physical evidence that connected him to any of the murders." (Bardsley and Bell, 2003; Kelly, 1995) Another reason was that no eyewitness could place DeSalvo at any of the crime scenes. Kenneth Rowe, the student who lived upstairs from Joann Graff, did not recognize DeSalvo as the man knocking on the door from a picture he was shown. Eileen O'Neil, who saw a strange man in Mary Sullivan's bathroom, also said the man was not DeSalvo. Thirdly, cigarette butts were found in an ashtray by Mary Sullivan's bed, but no one smoked in the residence. The same brand was found in Sophie Clark's toilet. DeSalvo did not smoke and therefore it is not likely that he murdered these two women. DeSalvo, quite possibly, might not have murdered any of the Strangler victims. (Bardsley and Bell, 2003; Kelly, 1995)

Two witnesses, Marcella Lolka and Gertrude Gruen, posed as visitors to the hospital to identify DeSalvo. Marcella Lolka lived in the same building as strangled Sophie Clark, and had

encountered the man who said he was there to paint. Gertrude Gruen was a survivor of a Strangler attack. Both women went to the visitors' area of the hospital to wait. When George Nassar walked in, Gruen became upset and said he seemed familiar to her. When DeSalvo entered, she said he was not the attacker. Later, Gruen told the police that Nassar reminded her of the man who attacked her. Lolka also eliminated DeSalvo, and became uneasy when Nassar walked in. (Kelly, 1995)

Was DeSalvo the Boston Strangler? Dr. Robey, the psychiatrist at the hospital, testified that DeSalvo had a remarkable memory and could have studied the very detailed crimes in the newspaper. DeSalvo also could have visited the crime scenes to get a good layout of the homes. The rest of the details could have been passed to DeSalvo by Nassar during their time together in the hospital. The last piece of evidence stating the DeSalvo was not the Strangler was that the police and other experts felt that the crimes were committed by more than one individual. The modus operandi, or method, was not the same in all the cases. Most serial killers stick within certain limits of one modus. DeSalvo was never charged with the murders due to a lack of evidence, but he was in jail for life on the "Green Man" charges which comforted the public. (Kelly, 1995)

Boston's citizens were satisfied with holding DeSalvo for the crimes of the Boston Strangler. DeSalvo was deemed competent to stand trial for the Boston Strangler attacks, but his confession was deemed inadmissible as evidence in court. On January 10, 1967 DeSalvo stood trial for the "Green Man" charges and was found guilty on all counts; he was sentenced to life in prison. In November of 1973, DeSalvo contacted Dr. Robey to meet with him; DeSalvo was going to reveal the true story of the Boston Strangler. The night before the meeting, DeSalvo was stabbed to death. It was later believed that DeSalvo was involved in a drug operation that led to his untimely death. (Lavoie, 2001)

In 2001, DNA Fingerprinting was applied to the case. The bodies of Mary Sullivan, the last victim, and Albert DeSalvo were exhumed for autopsy. Sixty-eight samples of hair, semen, and tissue were taken from Sullivan and tested against DeSalvo. Of the samples that were not Sullivan's, none were DeSalvo's. James E. Starrs, a professor of forensic sciences at George Washington University, stated on December 13, 2001, "We have found evidence and the evidence does not and cannot be associated with Albert DeSalvo…If I was a juror, I would acquit him with no questions asked." (Bardsley and Bell, 2003) Currently there is no DNA sample for Nassar, and no other physical evidence to associate him with the Boston Strangler. (Lavoie, 2001)

DNA Fingerprinting helped find DeSalvo not guilty of killing Mary Sullivan, and quite possibly he is innocent of all the other murders as well. The case, as of recently, is still not solved, and without a sample of Nassar's DNA, the case might never be solved. This is one of many highly publicized cases that involved DNA, and with the help of media coverage, made the public aware of this new technique that was not very well known.

The OJ Simpson Trial

On the evening of June 12, 1994, a barking dog alerted neighbors that something was wrong. Sukru Boztepe, who lived next door to Nicole Brown Simpson, followed the dog and came upon a horrible scene. Nicole Brown Simpson and her friend, Ron Goldman, twenty-five, were murdered. Goldman went to Simpson's home to return a pair of glasses left by her mother at the restaurant where he was waiter. Both were found dead, just inside the front gate, and covered in blood. The police responded and started a series of events that many called "the Crime of the Century." (Ramsland, 2003)

O.J. Simpson, former husband of Nicole Brown Simpson, immediately became the main suspect. Several key pieces of evidence were found at the crime scene and at O.J. Simpson's estate. Detectives going to his home to interview him found bloodstains on O.J. Simpson's Ford Bronco. O.J. was not at home and it turned out that he had just flown to Chicago. Simpson returned to Los Angles and agreed to answer some questions. Police questioned the cut on his left finger. At first, O.J. told conflicting stories, which caused problems. Also, the crime scene indicated that the killer was cut on the left hand. (Ramsland, 2003)

Several blood drops were collected from O.J. Simpson's white Ford Bronco, his driveway, the foyer of his estate, and the master bedroom. Blood drops were also found near footprints, at the crime scene, that matched O.J. Simpson's shoes, which were rare and expensive. Next to the two bodies was a bloodstained black glove containing traces of fiber from Goldman's jeans. The other mate for that glove was found on O.J. Simpson's property, stained with his blood. The blood found in O.J. Simpson's vehicle and home had traces of both victims along with his own. The blood collected on the center console of the Bronco contained both O.J. Simpson's and Goldman's DNA. The blood that was collected at the crime scene was analyzed and the results failed to match to the two victims. O.J. Simpson's blood was drawn for comparison, after all the blood was collected at the scene. O.J. Simpson's blood was analyzed and the results indicated that "the drops had three factors in common with Simpson's blood and only one person in 57 billion could produce an equivalent match." (Ramsland, 2003)

Forensic serologists from the California Department of Justice, along with a private contractor, collected all the blood samples and did the DNA testing on the samples. Around the time the blood evidence was being tested, the testimony of the limousine driver, who took O.J. Simpson to the airport, was reveled. The driver stated that he arrived at Simpson's estate around 10:30 P.M., but no one answered the door. The driver also stated that he saw a "black man cross

the driveway and go into the house." (Ramsland, 2003) O.J. Simpson claimed that he overslept and that is why the driver was unable to reach him on the intercom. Beside the blood evidence, matching shoe print, and the gloves, there was more evidence against O.J. Simpson. There were diary entries, from Nicole Brown Simpson, that alluded to O.J. Simpson being an abusive husband and capable of harming her. (Ramsland, 2003)

On June 17, 1994, just before O.J. Simpson was going to be arrested, he fled in his white Ford Bronco with his friend, Al Cowlings, and O.J. Simpson hinted in a letter that he might kill himself. When the police finally arrested him, they found a passport, fake beard, and thousands of dollars in cash in the vehicle.

O.J. Simpson then hired a team of celebrity lawyers for his defense and was arraigned in court on July 22, 1994, where he pleaded "absolutely 100 percent not guilty" to all the charges. (Linder, 2003) Before the trial started, the defense was going to call for a pre-trial hearing on the DNA evidence collected. The defense team was going to challenge the DNA in every way but decided to drop the matter. They knew that whatever happened could set a "dangerous precedent" and the long trial process could turn the jury against them. The defense waived the pre-trial proceedings and went on with the trial. (Ramsland, 2003)

The trial began on January 24, 1995, with the prosecution's opening arguments. The prosecution, presented by Christopher Darden and Marcia Clark, portrayed O.J. Simpson as an abusive father and husband as well as being very jealous of his ex-wife, Nicole Brown Simpson. Their point was to make it convincible that O.J. Simpson murdered his wife so no other person could be with her. After this argument, the prosecution presented all the evidence against O.J. Simpson. They were going to try their best to prove that Simpson was guilty of the murders of Nicole Brown Simpson and Ron Goldman. (Linder, 2003)

Jonnie Cochran, one of O.J. Simpson's attorneys, made his opening statement in Simpson's defense. Cochran stated that he was going to prove that the evidence against Simpson was "contaminated, compromised, and ultimately corrupted." (Linder, 2003) Cochran presented a very confusing timeline stating that Simpson had arthritis and that it was impossible for Simpson to have committed the double murder. The prosecution counter-argued, presenting their own timeline which indicated that Simpson had plenty of time to kill Nicole Brown Simpson and Ron Goldman. (Linder, 2003)

During the very long trial, many witnesses testified to O.J. Simpson's abusive behavior. Most notably were the friends and family of both O.J and Nicole Brown Simpson. Denise Brown, Nicole's sister, testified that on several occasions, she witnessed O.J. Simpson abusing his wife. On the day of the double murders, Denise Brown saw O.J. Simpson and testified that he "looked like a madman." A close friend of O.J. Simpson, Ron Shipp, testified that Simpson confided in Shipp that he had dreams of killing his wife, Nicole. The 9-1-1 operator was also called to the stand. She had answered a call from Nicole calling to report spousal abuse. The tape was played in court and O.J. Simpson was heard yelling and swearing in the background. (Linder, 2003)

The prosecution next called on witnesses to elaborate on the timeline on the night of the murders. The limousine driver was called upon. The driver was supposed to pick up O.J. Simpson at his home and drive him to the airport, on the night of the murders. The driver attempted to pick up Simpson around 10:30 P.M. but there was no answer. O.J. Simpson came out of his house around 11:00 P.M. and explained that he overslept. Simpson apologized and then the driver tried to help Simpson with a black bag and he refused the driver from touching. (Linder, 2003)

Kato Kaelin, O.J. Simpson's house guest on the night of the murders, was called to the stand next. Kaelin testified that he and O.J. Simpson went to McDonalds that night and returned to the house a little after 9:30 P.M. Charles Cale, O.J. Simpson's neighbor, testified that he walked his dog by Simpson's house between 9:30 and 9:45 P.M. and did not see Simpson's white Bronco. (Linder, 2003)

The last set of witnesses testified on all the physical evidence. Three different crime labs performed the DNA analysis. All three labs determined that the blood samples matched O.J. Simpson's DNA using two different tests for the analysis. The first test, known as RFLP (discussed in Chapter-1), gave a 1 in 170 million match, and the second test, known as PCR (also discussed in Chapter-1), gave a 1 in 240 million match, both to O.J. Simpson. The defense tried to suggest that the blood evidence was planted by the police, but the prosecution counter-attacked that suggestion. The prosecution called forth Detective Mark Furman to testify that the evidence could not have been planted. The defense counter-argued that Detective Furman was a racist and it was likely that he could have planted evidence. The defense asked Furman is he ever used the "n-word," Furman lied and said no. This hurt the prosecution's case as well as Furman's reputation; he was proved to be a racist and thus likely to plant evidence. (Ramsland, 2003; Linder, 2003)

The next evidence presented to the court was the notorious pair of gloves. The prosecution gave one glove to O.J. Simpson and asked him to try it on. Simpson obliged and the glove did not seem to fit his hand. The glove either shrank or Simpson's hand was swollen, but the jury saw that the glove did not fit and this quite possibly affected their decision, ignoring the fact that one glove was found at the crime scene and the other in O.J. Simpson's home, even if the gloves were not his, it strongly links him to the crime scene. The prosecution moved onto other evidence. They presented evidence that Simpson's hairs were found at the crime scene and

fibers from Simpson's Bronco were also at the scene. By presenting more evidence quickly, the prosecution hoped the jury would over look the fact that the glove did not fit on O.J. Simpson's hand. (Linder, 2003)

The prosecution rested its case and the defense took over to try to disprove all of the prosecution's claims in regard to the motive, to show that Simpson was incompetent to commit the murders, to show that the prosecution's timeline was flawed, and to show that the technical evidence was contaminated, planted, or both. O.J. Simpson's daughter, mother, and sister all testified as character witnesses. Simpson's doctor also testified that Simpson was not in good health, but the prosecution replied with a video showing Simpson physically active and in better shape than what he lead on to his doctors. The defense then called on two key witnesses that helped seal the case for them. The first witness called was a screenwriter, Laura Hart McKinny. She testified that in several interviews between herself and Detective Furman, he excessively used the "n-word" and even admitted to planting evidence, in the past, to guarantee a conviction. This hurt Furman's reputation, it hurt the prosecution's case against O.J. Simpson, and the statement also proved Furman was indeed a racist. The other key witness was the highly renowned criminalist Dr. Henry Lee. He testified that there appeared to be "something wrong" with the way the blood was packaged. The defense then proposed that the samples could have been switched. The defense also claimed that the blood had been degraded by being stored in a lab truck. The prosecution counter-argued with a DNA expert of their own, Harlan Levy. He stated that the "degradation would not have been sufficient to prevent accurate DNA analysis." (Ramsland, 2003) Levy also pointed out that the control samples were used and should have shown any supposed contamination. The defense suggested that the control samples were also mishandled and quite possibly contaminated. (Linder, 2003; Ramsland, 2003)

In closing arguments, the prosecution tried to demonstrate that there was no police corruption or planting of evidence, despite Furman being a racist. The jury deliberated for four hours and reached a verdict, O.J. Simpson was not guilty. The prosecution's case was unraveled by the infamous glove and the endless explaining of the complex procedure involved in DNA analysis. The defense kept all the explaining to a minimum and simple, befriending the jury.

After the criminal trial, O.J. Simpson was brought back to court for a civil trial in Santa Monica. In this case, the decision is judged on the preponderance of evidence, or solely on the evidence, not judged on the "beyond the shadow of doubt" standard of a criminal trial. The judge called O.J. Simpson forward to testify and disallowed the defense to present any theories similar to those in the criminal trial. The jury deliberated and after using the "preponderance of evidence" standard allowed in the civil trial, found O.J. Simpson guilty of the wrongful deaths of Nicole Brown Simpson and Ron Goldman, and awarded the plaintiffs $8.5 million dollars in compensatory damages. The court ordered O.J. Simpson to turn over all his assets, including his 1968 Heisman trophy. (Linder, 2003)

Even though the DNA proved that O.J. Simpson had something to do with the murders of Nicole Brown Simpson and Ron Goldman, the prosecution made errors in presenting the evidence. The defense put doubts into the minds of the jury and made a mockery of the DNA analysis. This did some damage to the credibility of DNA fingerprinting in the eyes of the public, but the highly publicized trial did, at least, make the public aware of this type of testing.

The Concetta "Penny" Serra Case

The murder of Concetta "Penny" Serra, in the summer of 1973, baffled the police and the residences of New Haven. The case went cold, with the lack of forensic technology available at

the time. It was not until 1994, when a man was arrested for beating his fiancée that the Penny Serra case would get a break.

The New Haven area was shocked by the murder of Concetta "Penny" Serra, twenty-one years old, on July 16, 1973. She took the day off from the dentist's office, where she worked as a dental assistant, to run some errands and used the family car, a 1971 blue Buick Electra 225. Around 12:42 PM, Serra drove into the Temple Street Garage and parked. She was chased through several levels of the parking garage before she was stabbed to death in the tenth level stairwell. Serra was found around 1:00 PM by an employee of the New Haven Parking Authority as he was retuning from his lunch break. The employee immediately called the police. As he was walking toward his station, he found a brown wig on the ninth level and he turned it over to the police as evidence. The Buick was found on the eighth level, parked at an erratic angle. The car had blood on the door handle, the door's surface, the car's steering column, the floor on the driver's side, and on the aluminum trim of the car, both inside and outside. A pair of gold-rimmed glasses was found on the passenger seat and a Temple Street garage parking ticket was stamped 12:42 PM, the time Serra entered the garage. The floor behind the driver's seat had more blood on the carpet and on a tissue box. Blood smears were on the box, and several tissues were removed. A car mechanics rag and a white envelope, covered in blood, were also found in the backseat of the car. (Lee, 2004)

Police found one blood trail from Serra's car to where her body lay still. On the floor of the seventh level, police found a set of car keys, covered in blood, and a man's white handkerchief, also covered in blood and red paint. Technicians followed another blood trail that lead from the car to the fifth level. Blood evidence was also collected from the Buick's gas pedal as well as the carpet and floorboard around the pedal. More blood was found on the railing in the stairwell between the ninth and tenth floors. A Jeep was found on the tenth level that also

had blood on it. The enormous amount of blood evidence was collected and preserved. (Lee, 2004)

The Buick was then dusted for fingerprints. Several partial prints were lifted from the tissue box. Latent prints, not visible to the naked eye, were also lifted from the interior of the car and the front seat ashtrays. Several cigarette butts were found in both the driver's and passenger's ashtray and in the backseat ashtray as well. (Lee, 2004)

While technicians were collecting all the physical evidence, other investigators were interviewing eyewitnesses and garage employees. The man who worked in the ticket booth remembered receiving a parking ticket that was moist with what appeared to be blood. When the ticket was handed over to the police, the employee told police that the man spoke with a foreign accent. After he left the garage, the man drove his car erratically through the exit. The driver was described as a Caucasian having long, dark hair and a thin build. He was a young man, but not a kid. (Lee, 2004)

Another garage employee was interviewed. He remembered seeing a young woman running through levels five and six with no shoes on her feet. She was being chased by a thin white male with longish black hair who appeared to be in his late twenties. Another witness stated that he saw a young man with dark hair and a moustache chasing a young woman through the eighth level. The witness, a young man who worked across the street from the garage, was in the garage to smoke marijuana on his lunch break. He omitted telling investigators that he was stoned during the interview, but this fact became known at a later date. (Lee, 2004)

By 4:30 that afternoon, Penny Serra's body was transported to Yale-New Haven Hospital for an autopsy. Faint traces of semen were found on her slip and underwear but there were no signs of sexual assault. Scrape wounds were found on her right wrist and on her left knee and left ankle. There was a knife wound to her right little finger and a cut above her right eye. An

abrasion was found on the right temporal region of her head. The fatal blow was a single stab wound to her chest, between the fifth and sixth ribs that penetrated the right ventricle of the heart. The murder weapon could not be identified at the time but was small; a maximum width of one and one-eighth inches and three inches in length. The blade appeared not to be serrated, since no evidence was found of a "sawing" effect of that type of blade. (Lee, 2004)

Philip DeLieto, Penny Serra's former fiancée, emerged as the prime suspect. DeLieto was a distant cousin of the then chief of police, Ben DeLieto. This fact hit home with the average citizen of New Haven; DeLieto's relationship with Serra seemed shaky and fueled more police and public suspicion toward him. DeLieto did have an alibi; he worked all lunch hour at his family's restaurant. Customers corroborated DeLieto's story; one customer was a Navy Commander who saw DeLieto working during the lunch hour. The public did not want to hear about an alibi for DeLieto. Once he was excluded as a suspect, the police hit a stone wall. Nothing was taken from Serra's wallet, and the killer left the two gold chains around her neck. There was no effort to steal the Buick, there were no signs of sexual assault, and her clothes were not ripped. With the limited forensic evidence and the technology of that time, police did not know what direction to take the investigation in. (Lee, 2004)

Four additional experienced detectives were assigned to the case. Since a mechanics rag was found in Serra's car, the investigators were going to interview owners and managers of all the gas stations and car repair shops in New Haven County. This was going to be a daunting task, but investigators did their best. They did not, however, travel to Waterbury, the second largest city in New Haven County. This would be seen as a regrettable oversight later on. (Lee, 2004)

In October of 1973, police received the result of the blood typing tests. It was confirmed that the blood evidence, found in the tenth level stairwell, was type A, Penny Serra's blood type.

The assailant's blood type was found to be type O. The latent fingerprints were compared to the Serra family. Most were identified, except for the bloody fingerprints on the tissue box. The bloody prints did not originate with the Serra family or with Philip DeLieto. The fingerprints were sent to the Connecticut State Police Identification Unit and to the FBI's fingerprint-identification division, but unfortunately, no identification was made. (Lee, 2004)

While investigators looked for more suspects, John Serra started a campaign of his own to catch the killer. He started an advertising campaign about his daughter's murder and stated that the killer might possibly be in the New Haven area still. The case went cold with no suspects and with the limited DNA testing and forensic technology available.

Things started to turn around in 1984. Anthony Golino went to high school with Penny Serra during the sixties, and knew her; he also could have possibly dated her. Golino married in 1977 and after the couple's son was born in 1982, they divorced. Golino's wife went to the police and told them she was beaten up on several occasions and he threatened to kill her, essentially saying, "I will do to you, what I did to Penny Serra." (Lee, 2004) Golino was brought in for questioning and was questioned about a scar on his left hand, one which he was unable to explain. Detectives also pursued witness accounts of Golino and Serra dating in the summer of 1973. (Lee, 2004)

In the summer of 1984, New Haven police were granted an arrest warrant charging Anthony Golino were the murder of Penny Serra. The arrest occurred in 1984, but the prosecution was not ready for trial until the middle of 1987. Golino's blood was never tested for the type. On the eve of the trial's opening, Golino's blood was tested. His blood was not type O, it was type A, similar to the victim's. The murder charges were dropped but his name was already tarnished. (Lee, 2004)

During the 1980s, New Haven police also pursued another suspect, Martin Cooratal. His dental bill was found on the dashboard in Serra's car. He was Albanian and worked at a luncheon restaurant. The ticket booth employee stated that the bloody ticket was given to him by a man with a foreign accent. Cooratal also resembled witnesses' descriptions of the man who chased Penny Serra through the garage. New Haven police applied for an arrest warrant for Cooratal, but was refused for lack of evidence. (Lee, 2004)

The case was not going anywhere and pressure was building for a wider investigation. In 1987, police decided to reconstruct the crime scene. The discussion caused a stir in the media. The show, *60 Minutes*, sent Mike Wallace, their star reporter to review the Serra murder. In February 1988, the show aired and was seen by an estimated twenty million Americans. CBS producers mainly focused on Philip DeLieto, Serra's former fiancée. The show proved beneficial since it widened public interest in the case. (Lee, 2004)

Finally on September 10, 1989, the crime scene was reconstructed in the Temple Street Garage. The evidence was reviewed and compiled into a report. The report focused on the blood evidence in and on the Serra family car. The blood was all human and was type O; only the assailant's blood was present in and on the car. Penny Serra's blood was found only in the stairwell on the tenth level, which was type A. This showed that Serra struggled with her assailant by her car and injured him. This left the blood trail to the tenth level stairwell where Serra was stabbed to death. According to witnesses, the assailant got into Serra's car on the ninth level and drove it down to the eighth level and parked it. The assailant went down to the seventh level, leaving a blood trail, to his car and drove out of the garage after paying and handing in the blood-smeared parking ticket. Based on the timestamp of the ticket, the assailant left the garage at 1:01 PM. While getting into his car, on the seventh level, that assailant dropped the blood stained keys and a white handkerchief. The report also covered the brown

wig that was found by a garage employee. Two hair fragments were found that were not consistent with the other hairs found in the wig. It later discovered that the wig had been moved around before being collected as evidence. The hairs could have been picked up from the garage from multiple contacts. An enormous amount of significance was placed on the four sets of unexplainable latent fingerprints found either in the car, on the car, or on objects in the car, i.e. the tissue box. Three sets were completely unidentifiable and the fourth was only a bloody smear print. (Lee, 2004)

During the late 1990, a break finally occurred on the case; computer imaging became a reality. The latent prints were scanned into a computer and visualized on a high-resolution monitor, then were passed through filters and gray-scaling programs to produce the best possible images. The fingerprints were later sent to the Automated Fingerprint Identification System, also known as AFIS. Several police agencies searched their files. The agencies included Metro Dade County (Miami) Police, Baltimore Police, the California Department of Justice, the Royal Canadian Mounted Police and the FBI. No identifications were made, but all the agencies indicated that they would search the database regularly. Advances in latent fingerprints and DNA were improving dramatically and it would be only a matter of time before the killer of Penny Serra would be brought to justice. (Lee, 2004)

Human behavior does not seem to change over time and a person will often repeat a previous crime, which is what happened with Edward Grant. In the summer of 1994, Edward R. Grant of Waterbury, CT beat his then-fiancée so fiercely that she was hospitalized. The woman reported Grant's assault to the local police and he was arrested. Grant was fingerprinted upon his arrest and the full set of prints was filed into the state AFIS system. The assault charges were later dropped but the fingerprints were already in AFIS. In 1997, a Connecticut/Rhode Island fingerprint database got a hit on Grant's left thumbprint: it matched the bloody partial print that

was lifted off the tissue box in Penny Serra's car. Grant worked for his family's successful auto repair and towing business, which would explain the mechanic's rag in Serra's car. Grant's age, occupation, and track record violence with women further intrigued police. Grant was arrested and read his *Miranda* rights. He could not account for his print at the crime scene and police asked for a sample of DNA. Grant refused and this further intrigued the investigators. The police asked and were granted a warrant for Grant's DNA. The blood was sent to the Connecticut State Police Forensic Science Laboratory in Meriden, CT for DNA analysis. The DNA evidence came back and indicated that there was a "300-million-to -1 chance that the type O blood found at the Serra crime scene was Edward Grant's." (Lee, 2004) Grant was then formally charged with the murder of Penny Serra in June of 1999, and his bail was set at $1 million, which with help from his family, he was able to post. While sitting in a police car after Grant was initially arrested, he commented to an officer next to him that the arrest of a railroad serial killer in Texas was caught "from a single thumbprint, too." (Lee, 2004) John Serra died in November of 1998 before Grant was formally charged. Mr. Serra did, however, know that the fingerprints matched Grant and police were gathering DNA evidence to further incriminate the suspect. (Lee, 2004)

Even though John Serra had passed away before justice was served, Grant went to court to stand trial for the murder of Penny Serra. Grant's defense team made motions to dismiss the charges, citing one major fact that the storage of the blood evidence was inadequate and the samples could have degraded over time. The prosecution disputed these motions stating that all the scientific evidence, at that time, had been preserved to the best of the abilities of the forensic laboratory. The defense's motions were dismissed and the trial came to order on April 29, 2002. The defense had a hard time proving Grant's innocence, the only arguments they had stated did not seem sufficient enough to prove innocence. The defense stated that there was more evidence

against a previous suspect, the timeline of the murder proved that Grant was out of state, and the main argument was on the forensic evidence, the blood and fingerprints, did not match the other suspects and that Grant was innocent despite his DNA and fingerprints matching the evidence found at the crime scene. The prosecution, on the other hand, had two powerful examples to help prove Grant's guilt. A scale model of the garage was built and a computer-controlled display visualized for the jury all the evidence against Grant. This made all the complex talk of DNA and fingerprints easier to understand and quite possibly helped convict Grant later on. (Lee, 2004)

The DNA was questioned the most; mainly citing the significant amount of time between the murder and the conviction. The DNA analysis of the blood evidence at the scene matched Grant. Dr. Carll Ladd, a lead criminalist at the Connecticut State Police Forensic Laboratory, confirmed that the results were indeed Grant's stating, "there is a 300-million-to-1 chance that the blood samples are not those of Edward Grant." (Lee, 2004) On May 9, 2002, Dr. Henry Lee, a famous criminalist, and Dr. Edward Blake, a California geneticist, were called to testify on all the forensic evidence. Both Lee and Blake confirmed that the DNA evidence had not been corrupted and did not decay overtime. The fingerprint evidence was not allowed in court since the prints came from charges that were later dropped. Even though the charges were dropped, Grant's statement to the police officer about the Texas railroad serial killer was caught "from a single fingerprint, too" added to Grant's guilt. There were no prints, but Grant incriminated himself with those words since they were uttered after his *Miranda* rights were read. (Lee, 2004)

On May 16, 2002, the jury was lead on a tour of the crime scene and a few days after, the defense opened their case for Grant. The defense tried to have the blood evidence thrown out and tried to include the semen evidence, however, there had been no signs of sexual assault; the semen evidence was not allowed due to the fact that it was unrelated.

On May 22, 2002, the jury had heard all the evidence and it was time to begin their deliberations. A few days went by and people were concerned that there would be a hung jury, since shouting was heard outside of the deliberation room. On May 28, the jury had finally reached a decision; Grant was guilty of first degree murder. When the jurors were interviewed, several stated that their decisions were based solely on the DNA and fingerprint evidence. On September 27, 2002, Grant was sentenced to a twenty-years-to-life term. This did finally bring closure to the Serra family, but as the old adage states, "Justice delayed is justice denied." (Lee, 2004) It is sad that John Serra, Penny's father, did not live to hear of Grant's sentence, but it is comforting for Rosemary Serra, Penny's sister and the only living member of the Serra family, that Grant will be spending the rest of his life behind bars. (Lee, 2004)

Chapter Conclusion

With the advances in forensic technology that are exploding now, it is only a matter of time before the final pieces of evidence are put together to solve other previous cases. New techniques are being created that will eventually make solving crime easier, so that justice will not be delayed or denied. The cases discussed in this chapter are just three of the many highly sensational cases in which DNA fingerprinting has played a role in the outcome of the trial. These sensational cases bring DNA fingerprinting and forensic technology to the public's attention, pointing out the power of the new technology. DNA fingerprinting and other forensic technology has benefited from the exposure in the media as more people hear about it in the news. DNA fingerprinting and other types of forensic science have been a benefit to the judicial system as the evidence has incriminated guilty persons, and has helped to exonerate innocent persons. Hopefully with the media coverage of DNA and forensics almost everyday, society will see this technology as useful to the judicial system and will help to fund these methods, and work

harder to understand them during trials. The funding will enable these methods to be fine-tuned, which will better serve the public and the judicial system. DNA fingerprinting has made a difference in cases, whether they are highly sensational or private cases, to help bring the guilty to justice. DNA fingerprinting has taken enormous steps since it was discovered, and will continue to grow until fully accepted by the public and the courts.

CHAPTER-5: DNA DATABASES

Introduction

DNA Databases play a crucial role in the success of DNA fingerprinting as a crime solving tool at the national, state, and local level. At a crime scene, biological evidence such as saliva, hair, or sperm from a rape kit are collected and taken to federal, state or local forensic laboratories for analysis. The information is then entered into a DNA database and searched against DNA profiles of convicted violent crime offenders or profiles from other crime scenes. Laboratory personnel look for matches and notify officials of their findings, giving them a lead in their investigation. Since its discovery, this method of solving crimes has been highly successful.

It was in September of 1984 that a British scientist named Alec Jeffreys discovered unique genetic markers for individual human genetic identification (Newton, 2004). In 1986, Jeffreys used his new technique to help British police solve the murder of two young women in Narborough, England. Later on the UK authorized the creation of the world's first national DNA database in April of 1995 (Key Dates, 2007). By then, this new technology was in use in the US, convicting and overturning convictions, like that of Gary Dotson who in 1989 after spending 8 years in prison for rape, had his conviction overturned on the basis of DNA evidence. As in Britain, U.S. DNA databases were formed, and in 1994 FBI officials received authorization to create its own combined DNA identification index (CODIS) (Walsh, 2006) which has become the world's largest DNA database.

Today DNA databases have become more sophisticated and widespread. However, with the advancement of DNA technology, especially of DNA Databases and their expansion, come concerns and questions about the potential misuse of such information. Who has access to the

DNA databases? Are they secure? Are there privacy policies? What if insurance companies obtain information pertaining to predisposition to illnesses? There is a sense of fear that eventually, we will lose our liberties to databases. These questions and more are at the heart of the arguments against the expansion of DNA databases. Although important to be addressed, and will be addressed in this chapter, most of these fears are unnecessary since there are policies, securities, and measures in place over DNA databases used for criminal justice purposes.

What Are DNA Databases Used For?

DNA databases store DNA profiles of individuals ranging from convicted felons to the victims themselves, depending on the type of database. The main reason for the creation of a DNA database is to solve violent crimes in which there are no suspects, specifically sexual assault crimes. However, DNA databases serve other purposes, including allowing military officials to identify missing soldiers or their remains, or aiding researchers to find causes and treatments for dozens of genetic diseases (Fisher, 2004). Databases currently have various types of indexes, such as the convicted offenders index, forensic, missing persons, and unidentified human remains indexes. In addition, there are private groups- research institutes, businesses, and religious organizations- that manage their own DNA databanks which citizens voluntarily submit their DNA (Rosen, 2004).

But the main reason for a DNA database is best described by what it can do for crime solving. By matching DNA profiles from suspects or an existing crimescene to those entered from previous offenders or crimescenes, DNA databases link suspects to victim(s), suspects to crime scene(s), or crime scene to crime scene, providing officials with a “cold hit”. A “cold hit” is defined as a match which provides the police with an investigative lead that would not

otherwise have been developed if a database search wasn't performed (Brown & Niezgoda 1995). An example of a "cold hit" is in the case of Debbie Smith.

> On a Friday March 1989 afternoon in Virginia, Debbie Smith, wife of police officer Robert Smith, was forced from her home kitchen into the woods by a man she had never met before. There, he raped her and threatened her not to tell, saying that he knew where she lived and would come back to kill her. When local police got the case, they found a suspect and sent his blood sample for a conventional serological exam that cleared him. Still the evidence was preserved for the then new DNA fingerprinting examination. Then in 1994, Debbie and Robert's residing county experienced an outbreak of sexual assault and rape crimes. Police obtained a new suspect and sent his blood for DNA analysis and also resubmitted Debbie's evidence, thinking there might be a link. The suspect was cleared; however, the laboratory now had a DNA profile of Debbie's rapist. Meanwhile, the state of Virginia was undertaking developing a databank containing DNA profiles of convicted felons, and periodically they searched that database against evidence from unsolved cases. In the summer of 1995, the DNA profile from Debbie's case was searched against the database and got a "cold hit". Norman Jimmerman, a two time felon incarcerated for abduction and robbery was identified as the raper of Debbie Smith. After six long years, Debbie's rapist was finally caught and put behind bars to serve a 161 year sentence. (Brown & Niezgoda, 1995)

The FBI uses the "cold hit" metric to measure the success of its COmbined DNA Index System or CODIS program, established under the DNA Identification Act of 1994 (Rosen, 2004). Beginning in 1990 as a pilot project with 12 states and local forensic laboratories, CODIS describes the software used to maintain and run DNA databases and provides support software for Federal, state and local forensic laboratories (Adams, 2002).

There is a CODIS hierarchy which consists of three levels; national, state, and local (see Figure-30). When local crime labs want to share information, unknown profiles from the Local DNA Index System, or LDIS, must be forwarded to the state level. Then at then state level, profiles from different local labs are inter-searched in the State DNA Index System, or SDIS, of each state. To share this information with the rest f the CODIS community, states must forward their profiles to the national level where the profiles can be searched against the National DNA Index System, or NDIS (Brown & Niezgoda, 1995). Matches made through CODIS are known

as “hits” (Adams, 2002). As of 1995, more than 10 years ago, CODIS had generated twenty-eight case-to-case hits and twenty-five case-to-offender hits, as well as tying twenty-five offenders to seventy-nine victims (Brown & Niezgoda, 1995).

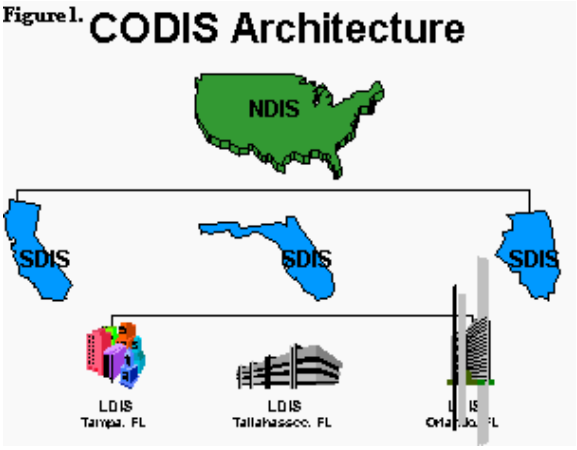


Figure 30: The CODIS Hierarchy. (Source: Promega.com, 2007).

It is important to mention how the probability of these databases producing the “cold hits” or matches between two DNA profiles is calculated. Typically, a DNA database will compare a DNA sample from a crime scene with a suspect sample. A DNA profile is a series of numbers generated by analysis of a specific location on a chromosome called a locus (Adams, 2002). A consistent set of loci are analyzed in each DNA sample. Usually, in order for DNA profiles to be searched at the national level they must contain information on 13 Short Tandem Repeat (STR) loci, termed the 13 core loci. These 13 loci are approved for use in the FBI’s CODIS program and were chosen because they are not directly linked to any genetic code or medical condition but only serve as identification markers (Adams, 2002). To determine the probability that two DNA profiles match solely by chance is done by using the product rule. For each locus, the frequency of the type of gene sequence at that location (allele frequency) must be known. Depending on the size of the database, the allele frequency, how many times it is observed in a given population, is obtained for each allele and its frequency is multiplied by the

frequencies of the other loci analyzed to get the overall profile frequency (see Figure-31) (Brenner 2004). This tells the chance of a coincidence occurring where an unrelated person, by chance, had the same DNA profile as that found at the crime scene and not the suspect. Clearly, the larger the database, the more accurate the matches become, because the more accurate is our estimate of each locus allele frequency. The more loci analyzed, the probability that a match was made by chance will decrease because there will be more variation as the size of the database continues to increase.

DNA Profile		Allele frequency from database				Genotype frequency for locus	
Locus	Alleles	times allele observed	size of database	Frequency		formula	number
CSF1PO	10	109	432	p=	0.25	$2pq$	0.16
		11		134	q=		
TPOX	8	229	432	p=	0.53	p^2	0.28
THO1	6	102	428	p=	0.24	$2pq$	0.07
		7		64	q=		
vWA	16	91	428	p=	0.21	p^2	0.05
						profile frequency=	**0.00014**

Figure 31: Calculation of Probability of a Match. Example of how the probability of a match occurring by chance is determined, in this case when 4 loci are analyzed. (Source: http://dna-view.com/profile.htm)

Who Should Give DNA Samples for Database Entry?

Currently every state in the US collects samples for profiling from certain individuals. However, who those individuals are that must provide a DNA sample for databasing differs greatly among the states (see Figure-32) (Puri, 2001).

	Sex Offenses	Offenses Against Children	Murder	Assault & Battery	Robbery	Kidnapping	Burglary	Attempts	Juveniles
Alabama	x	x	x	x	x	x	x	x	
Alaska	x	x	x	x	x	x	x	x	x
Arizona	x	x	x			x	x	x	x
Arkansas	x	x	x	x	x	x	x		x
California	x	x	x	x	x	x	x	x	x
Colorado	x	x	x	x	x	x	x	x	x
Connecticut	x	x				x			
Delaware	x	x						x	
Florida	x	x	x	x	x	x	x	x	x
Georgia	x	x	x	x	x	x	x	x	
Hawaii	x	x	x			x			
Idaho	x	x	x	x	x	x		x	x
Illinois	x	x	x		x	x	x	x	x
Indiana	x	x	x	x	x	x	x		
Iowa	x	x	x	x	x	x	x	x	
Kansas	x	x	x			x	x	x	x
Kentucky 4	x	x	x	x	x	x	x	x	x
Louisiana 5	x	x	x	x		x		x	x
Maine	x	x	x	x	x	x	x	x	
Maryland	x	x	x	x	x	x	x	x	
Massachusetts	x	x	x	x	x	x	x	x	
Michigan	x	x	x	x	x	x	x	x	x
Minnesota	x		x	x	x	x	x	x	x
Mississippi	x	x				x			
Missouri	x	x	x	x		x			
Montana	x	x	x	x	x	x	x	x	x
Nebraska	x	x	x			x		x	
Nevada	x	x	x	x	x	x	x	x	
New Hampshire	x								x
New Jersey	x	x	x	x		x		x	x
New Mexico	x	x	x	x	x	x	x	x	x

New York	x	x	x	x	x	x	x	x	
North Carolina	x		x	x	x	x			
North Dakota 7	x	x	x	x	x	x		x	
Ohio	x	x	x			x	x	x	x
Oklahoma	x	x	x	x	x	x	x		
Oregon	x	x	x	x	x	x	x	x	x
Pennsylvania	x	x	x					x	x
Rhode Island	x	x	x	x	x	x	x		
South Carolina	x	x	x	x	x	x	x	x	x
South Dakota	x	x	x	x	x	x	x	x	x
Tennessee	x	x	x	x	x	x	x	x	x
Texas 9	x	x	x	x	x	x	x	x	x
Utah	x	x	x	x	x	x	x	x	x
Vermont	x	x	x	x	x	x	x	x	x
Virginia	x	x	x	x	x	x	x	x	x
Washington	x	x	x	x	x	x	x	x	x
West Virginia	x	x	x	x	x	x	x	x	
Wisconsin	x	x	x	x	x	x	x	x	x
Wyoming	x	x	x	x	x	x	x	x	
Department of Denfense	x	x	x	x	x	x	x	x	
District of Columbia	x	x	x	x	x	x	x	x	
Federal	x	x	x	x	x	x	x	x	
Puerto Rico	x	x	x	x	x	x		x	
Total	54	51	50	43	40	50	40	43	28

Figure 32: Federal and State Qualifying Offenses for Entry in the CODIS DNA Database. (Source: http://www.fbi.gov/congress/congress02/adams051402.htm)

States have become more inclusive over the years forcing more criminals to provide DNA samples because of the success of DNA databases. Today, all fifty states collect DNA samples from certain convicted sex offenders, and have DNA database statutes directed at violent offenders (Walsh, 2006). Thirty-four states require DNA samples from individuals convicted of any felony, both violent and non-violent. At least thirty-four states include some misdemeanors

as qualifying offenses for entry into their DNA databases. For example in New Jersey, individuals convicted of 'any crime', which means being sentenced to six months or more imprisonment are qualified for DNA sampling (Walsh, 2006).

Even juveniles are not exempt from DNA sampling, although this is limited. In twenty-eight states, juveniles 'adjudicated delinquent', which means that they were adjudicated by the State juvenile system for what would constitute a qualifying offence if they were an adult, are also DNA profiled (Walsh, 2006). More inclusive states such as California, Texas, Louisiana, and Virginia, take DNA samples from individuals *arrested* for a violent felony, even pre-conviction. Some states will eliminate the profiles if the arrestees are subsequently proven innocent, but only in California will those DNA profiles be kept in their DNA database as of 2009 (Walsh, 2006).

The states of Virginia and California have the most inclusive DNA databases of all the states. With a database started in 1989, Virginia became the first state to implement a criminal DNA database. The qualifying offences for the database originally included just certain classes of convicted sexual offenders, but now includes all sexual offenders, adult felons, juveniles over the age of fourteen who commit serious crimes, and all persons arrested for any violent felony or burglary (Rosen, 2004). Recently California made some legislative changes to their DNA database laws. In November of 2004, "The DNA Fingerprinting, Unsolved Crime and Innocence Protection Act" also known as "Proposition 69" allowed for the profiling of all persons, including juveniles, convicted of any felony offence, any sexual offence (including misdemeanors), who are in prison or on probation or parole with a record of a past or present conviction of any of the qualifying offences, and in 2009, all adults arrested for any felony offence. Moreover, suspect samples may now be searched against any database, for any crime,

once taken. Finally, it becomes the burden of the individual to request the removal of his or her DNA profile from the database if proven innocent (Walsh, 2006).

As a resident of the state of Massachusetts it is important to discuss qualifications for inclusion into the Massachusetts DNA database. Like all states, Massachusetts collects DNA samples from all convicted sex offenders. According to Chapter 22E of the General Laws, "Any person who is convicted of an offense that is punishable by imprisonment in the state prison, and any person adjudicated a youthful offender by reason of an offense that would be punishable by imprisonment in the state prison if committed by an adult shall, within 1 year of such conviction or adjudication, submit a DNA sample to the department" for entry into the State database (Massachusetts General Laws, 2003). In addition, individuals who are on probation or on parole as a result of such a conviction or adjudication, who have not previously submitted a DNA sample to the department must submit one within one year of the law going into effect, in 2003.

At the National level, originally the only DNA profiles contained in the NDIS were profiles from convicted sexual offenders and those convicted of violent crime (murder, assault and battery, etc.) (Walsh, 2006). However, under the Justice for All Act of 2004, it can contain any DNA profile which a state chooses to collect, except that of arrestees who have not been charged in an indictment, and samples voluntarily submitted for elimination purposes (Walsh, 2006). Another class of individuals required to give a DNA sample are military personnel. They are required to give genetic samples to the Department of Defense for entry into the Pentagon's DNA database (Rosen, 2003), for the purpose of identifying missing soldiers or their remains in war casualties. It actually has been considered to have all babies provide a DNA sample at birth, however this stringent criterion is highly controversial, and no state has adopted it yet.

When it comes to whose DNA should be in a database, the debate continues. What's settled is that convicted sex offenders and violent offenders must be profiled. On the other hand,

critics oppose the DNA testing of non-violent offenders, suspects (arrestees as well), and most of all innocent people, even consenting ones (Etzioni, 2003). In addition, most don't agree with the profiling of juveniles, the mandatory profiling of military personnel, and the idea of profiling children at birth. The basis for these arguments include innocence until proven guilty, and suspects aren't guilty. Also, pertaining to innocent people, they feel that even voluntary samples might not actually be voluntary, but that the people where coerced by officials to provide a sample. They also worry that testing and entering DNA profiles of these individuals might become mandatory procedure. When it comes to testing juveniles, critics feel as if the government is abandoning its juvenile reform policies and just treating youths as hard criminals.

Personally, the author of this chapter feels it is crucial for all convicted sex offenders to provide a DNA sample. Also all individuals who are convicted of a felony, both violent and non-violent, should provide the state with a DNA sample. These individuals commit crimes which are most likely to leave behind DNA. Also, they tend to strike again having similar recidivism rates (Kaye & Smith, 2003). Moreover, research in England and the United States in recent years has shown that violent criminals such as rapists frequently commit felonies such as burglary before they turn to violence (Etzioni, 2003). For that reason, convicted burglars (felons) should provide a DNA sample. All those arrested for a felony should give a DNA sample for testing. Testing is another tool which officials could use to prove guilt or innocence. If the individual is cleared, it should be the responsibility of the state to remove the information from the database. It should not be necessary for individuals guilty of misdemeanors to provide a DNA sample. Most are just petty crimes that don't carry much jail time and are solvable by paying a fee. However, repeat offenders should be considered for profiling depending on the offences committed. Although there is a juvenile reform policy, having DNA profiles of adjudicated juveniles is necessary since they too can commit very serious crimes. Finally, having

a military database is a necessity. There are many unidentified human remains due to war, meaning that there are many families with missing loved ones. Having such a database gives these families closure, they can be certain of the fate of their loved one. On the contrary, there are no great obvious benefits of taking DNA samples from babies at birth. It is as if they are being labeled suspects before they can even walk.

Concerns Regarding DNA Sampling

There are many concerns regarding the expanding use of DNA databases, and who provides samples. Some argue that it is a violation of the Fourth Amendment, which protects citizens from unreasonable search and seizure, to take DNA samples. The Fourth Amendment has been interpreted by the Supreme Judicial Court as any search by public employees that intrude 'unreasonably' on matters of personal privacy (Walsh, 2006). The Fourth Amendment clearly applies to DNA testing since obtaining a DNA sample involves intrusion of the body (giving blood or mouth swab). The Supreme Judicial Court decided that taking a DNA sample is a form of search and seizure (Puri, 2001).

Nevertheless, the physical intrusion required to obtain the sample is minimal. While blood was still considered minimally invasive, DNA samples can now be obtained simply with a mouth swab. Thus the earliest cries of "cruel and unusual punishment" for being forced to provide blood samples has disappeared. In addition, the courts maintain that prisoners have lesser expectations of privacy compared to freed persons and that government interests outweigh the degree of intrusion that an individual is subject to for DNA sampling (Walsh, 2006). Therefore, obtaining a DNA sample is not considered 'unreasonable'.

Another argument regarding obtaining a DNA sample is that the individual is subject to cruel and unusual punishment when having to give blood for DNA testing. This is

understandable since there are many people who are afraid of needles or 'can't see blood'. Also some argue that the detention in administrative segregation until consent is given to take a DNA sample is a form of cruel and unusual punishment. The courts nonetheless have shown no sympathy for this general argument and view a refusal to consent as failure to comply with administrative order (Walsh, 2006). Regardless, as stated before, DNA samples are now collected by buccal swabbing, or cheek swabbing. A Q-tip is rubbed against the cheek and the rubbing motion collects loose cells in the inner cheek lining. Since DNA is the same in every cell in the body, a cheek cell contains the same DNA as blood cells. A cheek swab is effective, quick, and painless (DNA Diagnostic Center, 2006). The cheek swab method for DNA sampling clearly removes the argument of cruel and unusual punishment. One could then say it is cruel and unusual for parents to force their kids to brush their teeth!

Concerns Regarding the Use of DNA Databases

There are various trepidations about the use and expansion of DNA databases. These concerns all deal with the effect of DNA databases on privacy and liberty, and the potential for abuse.

Privacy and Liberty

In a society that cherishes freedom for all, DNA testing and databasing, according to critics, pose a threat to the people's privacy and liberty. On the liberty stance, critics argue that DNA databasing is another increasing governmental surveillance of the individual, the Big Brother argument. In response to that argument, the idea that "one day a future government might..." (Etzioni, 2003) use this technology to track individuals is mainly paranoia but not completely unrealistic. Most DNA databasing is done by private sectors, and the focus should

not be strictly on the government. In addition, the government has laws regulating its use of DNA databases more than privately managed databases. Regulations were passed under the Privacy Act of 1974 banning several kinds of information, and their uses and new regulations were introduced in 1998 concerning the protection of the privacy of children, and in 2000 concerning the protection of financial records; large-scale regulation of medical privacy, including genetic privacy, is expected (Etzioni, 2003).

In terms of privacy, yes, DNA testing and databasing is invasive, physically and informationally. Critics argue that it is an invasion of privacy because the individual's body is physically entered to obtain the DNA sample. As stated before, the courts rule that this invasion is minimal (simple mouth swab) and not significant in terms of the government's interest. Privacy is not severely invaded by a cheek swab. People see inside of your mouth while you speak. Also, a DNA sample could be obtained without ever making contact with the individual. Cigarette butts, straws, cups, etc. all contain DNA of their user, and once discarded, especially in a public trash, is not private matter.

One thing that's certain is that DNA has revealing capabilities, more so than medical records. DNA encodes the genes that make you who you are, from your phenotypic characteristics, to who you are related to. Also, DNA can reveal ones predisposition to certain diseases (Rosen, 2004).

This brings up the issue of genetic privacy. Take Alzheimer's for example. Alzheimer's disease (AD) is a progressive, neurodegenerative disease characterized in the brain by abnormal clumps (amyloid plaques) and tangled bundles of fibers (neurofibrillary tangles) composed of misplaced proteins (NINDS, 2007). It is known that mutations in any of the three genes APP, PS1, or PS2 can cause Alzheimer's disease (Adams, 2000). As a result, one argument critics put forth is that insurance companies might get a hold of this information leading to genetic

discrimination where they would raise premiums or not extend coverage for these people (Puri, 2001). Critics also predict employment discrimination. This could also occur with other diseases known to have genetic links, such as breast cancer.

These hypothetical situations are unlikely however. They are more like scare tactics that cloud the facts about DNA databasing in crime solving. The standard 13 core loci used for DNA analyses were specifically chosen because there is no genetic link to any traits or predisposition to diseases in the information they contain (Adams, 2002) and because they offer so much variation in the human population. The genetic markers used are just "junk DNA" that differ between each individual and are just for identification purposes. So, crime lab DNA databases don't contain information about a person's susceptibility to a certain disease. Recently there has been some evidence that some "junk DNA" codes for a new form of regulatory protein that affect the function of the genes near them (Junk DNA …2004). Even so, there is no evidence yet that the loci being used by databasing crime labs have these functional characteristics. The issue still at play here is the type of protections crime labs should have with what to do with the physical DNA sample (blood, swab, etc.) once the profile has been created. If it is agreed to destroy the physical samples after obtaining the forensic profile, then genetic information can no longer be obtained, and the argument for genetic privacy can thus be silenced. Unfortunately this is not currently the case since some states allow the DNA samples after databasing to be used for "humanitarian reasons" such as medical research (Lazer, 2003). In those cases, concealing the identities of the donors and having some form of documented informed consent from the donors, even convicts, allowing the use of their DNA in research are good privacy measures. Additionally, Congress in 2003 passed a health care reform bill, The Kassebaum-Kennedy Bill, banning insurers from discriminating on the basis of genes or other preexisting conditions (Fisher, 2004). Finally, there are genetic privacy protections provided by many institutions for

children and adolescents that refuse parental requests to test children for late onset diseases when no medical intervention is available to prevent or alleviate the disease (Annas, 2003).

Potential for Abuse

Tying in with privacy and liberty concerns are those regarding the possible abuse of DNA database technology. As DNA databases expand, critics are concerned that some sort of 'function creep' will occur (Walsh, 2006). That is, databases will become a universal identifier rather than the crime solving tool it was initially intended to be. Some argue that Nazi-like policies will form where people are killed or discriminated against because of their genes (Etzioni, 2003). This "threat of eugenics" argument holds little ground because as Amitai Etzioni (2003) notes, Nazi-like regimes formed before the discovery of DNA testing, profiling, and databasing so these techniques are clearly not the sole cause for their formation. In addition, he agreeably notes that one can pretty much argue a similar argument against any new technology that is developed, specifically those involved in communications and transportation.

It is vital to discuss quality, maintenance, and accessibility to DNA databases, mainly involving crime labs, as this is also a potential source of profiling error and abuse if in the wrong hands. In the 1980's, the FBI conveyed a group of Federal, state, and local forensic scientists, known as the Technical Working Group on DNA Methodology (TWGDAM) that developed guidelines for a quality assurance program that virtually all labs performing DNA analysis adopted (Adams, 2002). The DNA Advisory Board, convened by the FBI under the DNA Identification Act of 1994, added another set of guidelines, *Quality Assurance Standards for Forensic DNA Testing Laboratories*. Both standards have been approved by the FBI director, and both have been in effect since April of 1999 (Adams, 2002). Laboratories must abide to these standards in order to receive government funding. These standards also have good

measures in place regarding the facilities, personnel, analytical procedures, reports, and safety, just to name a few, that control and limit access to the lab. As mentioned before, DNA is revealing and not just anyone or any company (i.e. insurance companies) should have access to that information. We don't want to see a situation like that in Iceland where a biotech company, deCODE, has exclusive rights the country's genome (Kahn, 1999). DNA samples used in criminal investigations should not be accessible by non-governmental organizations for purposes other than crime solving. Those samples should be accessible to them (researchers, insures, etc.) only through direct voluntary submission, not having gone through the hands of crime labs so that critics can't claim that the database is being abused.

The Universal Database

As DNA databases continue to expand, so is the idea of creating a universal database, particularly in England. As the leader in DNA database technology, the English database includes far more classes of offenders and even individuals just appearing in court, even if acquitted (Wendling, 2003). Due to all these samples, the database averages between four hundred and seven hundred matches a week, as opposed to the approximate six hundred total matches of the United States database as of 2001 (Puri, 2001). The Chairman of the Police Superintendents' Association, Kevin Morris, projected the idea of creating a universal database for the U.K. in 2003, collecting DNA samples from all of its residents (Welding, 2003). The basis for this was the numerous cases helped using the database and also the possible deterrent effects it might have on committing crimes. Also, it eliminates discrimination because as Alec Jeffreys put it, "If we're all on the database, we're all in exactly the same boat- the issue of discrimination disappears" (Rosen, 2004).

Critics oppose this idea as well for the reasons previously stated. Personally, the author of this chapter believes there are drawbacks to this idea, at least in the United States if it ever took this initiative. First of all, the larger the DNA database becomes, the more similar the samples become at the chosen loci, so unless more loci are chosen for analysis the result can't be always guarantee a unique match (Wendling, 2003). In the case of the United States, a universal database is not something that the country is ready for. One reason for this is the backlog issue that databases are facing. As of 2004, there were about 1.5 million convicted felons who qualified to be entered into a database but were not yet entered (Puri, 2004). This is mainly because state and local laboratories do not have the full capacity to analyze all biological evidence submitted to them. Rather they must prioritize their cases based upon court dates or whether a suspect was identified (Adams, 2002). This results in many unanalyzed evidence locked in storage for cases without suspects. So commencing a universal database would just add to the backlog issue.

If there were to be a universal database though, it should be on a voluntary basis. That means it is not mandated by the government to provide a sample, and there are no penalties for not providing one. In addition, this would mean that babies would not be sampled at birth since they can't make the decision for themselves yet, nor would parents be allowed to make it for them. By publicizing and educating the public about the benefits of DNA technology and showing proof of safeguards and privacy protections, it is certain that people will be willing to give DNA samples for the cause.

Future of DNA Databases

DNA databases are continually expanding as shown by California's "Proposition 69" that will be in full motion in 2009. Although there are still some issues, the FBI and law makers are

working hard to settle them because they recognize the amazing opportunities there are with DNA databases and crime solving. Senator Orrin G. Hatch (R-Utah) in October of 2003 introduced the "Advancing Justice through DNA Technology Act of 2003" (Hatch, 2003). This bill would provide over $1billion in improving lab capacities, training and assisting personnel, and addressed the backlog problem in crime labs. In addition it would enact the Innocence Protection Act which addresses dealing with post-conviction testing to compensation for the wrongfully accused (Hatch, 2003). This act was passed in 2004 under a new name, "The Justice for All Act of 2004" and has been in process since. There is no bill that could ever silence all the concerns of DNA database critics, but they are moving in the direction that protects the public interest while still expanding DNA databasing technology to its full potential.

CONCLUSIONS

In the early 19th century, there were no cars in the street, no cell phones, no computers, and certainly no internet. Today, however, these things are common and have become integral parts of the society. They are true examples of how technology affects society because now transportation to anywhere, communication with anyone, and accessing information about anything is relatively easy. In the same way, DNA fingerprinting technology has had a profound effect on our society. DNA fingerprinting technology can help identify the unknown parent of a child, identify unknown human remains, or solve crimes, both current and "cold cases".

Before DNA fingerprinting, a crime was solved based mainly on circumstantial evidence and eyewitness testimony, two highly unreliable sources of information. However, DNA is a much more reliable source of information. It is unique to each individual, hard to prevent its deposition at a crimescene, and its sequence can not be altered as evidence (Chapter 1). When used for forensic purposes, DNA often helps seal the case (Chapter 2), and the procedures for collecting and storing DNA have improved substantially to prevent contamination or tampering. It took some time for DNA evidence to be accepted in court because the technology was new and in some cases the technique was not properly controlled for degradation or contamination (Chapter 3). Standardized procedures are now in place for collecting and storing evidence, and for using controls during the analysis. Pretrial hearings are now used to determine whether these standardized procedures were followed for each case. Although the public may not be aware of landmark DNA courtcases, they likely are aware of some of the sensational cases discussed in Chapter 4, each of which used DNA evidence in a different way. The creation of DNA databases has been a major force for helping solve crimes and helping to identify repeat offenders (Chapter 5). Each state enacts its own laws governing who must provide DNA profiles

to CODIS, the world's largest DNA database. Most states currently require all convicted felons and violent misdemeanors to contribute, but some are considering expanding this requirement to include all arrested individuals, opening up arguments over rights to privacy, the constitutionality of databases, and whose DNA profile should be in a database.

From the research performed for this IQP, the authors conclude that DNA databases serve as a powerful system for helping solve crimes, and we agree with the current Massachusetts legislation requiring all convicted felons and violent misdemeanors to provide DNA samples to the CODIS database as the current best standard for determining database admittance. Co-authors Jennifer and Kevin agree with the current Massachusetts legislation that arrestees should not be required to provide DNA samples, while co-author Soana believes all arrestees should be required to provide samples, under the condition that if the arrested individual is subsequently found innocent then the DNA profile must be removed. When a DNA sample is taken, all three co-authors agree that the original sample should be destroyed following the analysis of its 13 core forensic loci, to prevent any future opportunities for obtaining medical predisposition information from that sample at its non-forensic DNA loci.

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