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DNA FINGERPRINTING: A CONCISE COMMENTARY

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1. biology
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ABSTRACT

DNA fingerprinting is a powerful forensic tool for the use of human identification. However, it is not infallible and comes with its own set of complications and limitations. These drawbacks have generated some difficulty with the acceptance of DNA fingerprinting in the courts. The conclusion of this IQP is that after much study and refining, DNA fingerprinting techniques have progressed to a sound a reliable science.

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EXECUTIVE SUMMARY

DNA fingerprinting was introduced in 1984 by Alec Jeffreys, who is a geneticist at the University of Leicester in Britain. Jeffreys showed that small portions of the human DNA sequence are unique among different individuals by using RFLP analysis. Jeffreys named the procedure "DNA fingerprinting", a term which most forensic scientists believe is incorrect. The more accepted label is "DNA typing" or "DNA profiling" (Coleman and Swenson, 2000).

The two most widely used methods of DNA fingerprinting are restriction fragment length polymorphism (RFLP) analysis and polymerase chair reaction (PCR) analysis. RFLP analysis compares the lengths of VNTR strands between samples. A much smaller sample can be used during PCR analysis, because PCR amplifies a certain strand of DNA up to a million or more times. The copied DNA is then either analyzed with probes, or by a method similar to that used in RFLP.

During the collection of evidence it is crucial that everything and everyone involved in the collection is clean. The correct procedures for the collection, packaging, preservation, and documentation of DNA evidence

samples must be followed or the evidence may not be allowed into the courtroom. These procedures are also important for maintaining the original condition of the sample (Wade, 1999).

There are two central ways to collect samples for DNA analysis. The first is to collect the soiled object itself, and the second is the removal of the biological materials to a different piece of material (Inman and Rudin, 1997).

The first case solved by the use of DNA fingerprinting was the rape and murder of two young girls in 1983 and in 1986 in the small town of Narborough in Leicestershire, England. The police asked all males from three villages between the ages of 17 and 34 to voluntarily submit a blood sample. Four years after the first murder the police finally arrested Colin Pitchfork, and his sample matched that of the killer. His sample was the 4583rd sample.

During the next couple of years, there was a rush of forensic DNA analysis. DNA profiles were permitted in court cases without difficulty, and the private companies performing the analysis began an intense competition for the business and technology. Unfortunately, each company used different techniques and equipment, and results from

separate labs were not compatible (Coleman and Swenson, 2000).

Following the outcome of several landmark court cases, in 1996, the National Research Council issued its second report on DNA fingerprinting. It stated that the "technology for DNA profiling and the methods for estimating frequencies and related statistics have progressed to the point where the reliability and validity of properly collected and analyzed DNA data should not be in doubt" (NRC, 1996). It is the conclusion of the author of this IQP that using current government policy on the collection and handling of evidence, and using properly applied quality control and quality assurance measures, DNA evidence should have no trouble gaining acceptance into trial.

PROJECT OBJECTIVE

The purpose of this IQP was to examine a new controversial technology (DNA fingerprinting) and to determine its impact on society, especially the U.S. legal system. Techniques used for the identification of individuals using DNA fingerprinting were examined. The methods and procedures of DNA fingerprinting were described from the collection stage to the courtrooms. This was accomplished by reviewing relevant literature and court case reports. The research allows us to conclude that DNA fingerprinting is a very powerful forensic tool. However, like any powerful tool, it comes with its own set of complications that must be recognized and managed.

CHAPTER 1: DNA FINGERPRINT METHODS

1. Introduction

DNA fingerprinting was introduced in 1984 by Alec Jeffreys, who is a geneticist at the University of Leicester in Britain. Jeffreys showed that small portions of the DNA sequence were unique among different individuals by using RFLP analysis. Jeffreys named the procedure "DNA fingerprinting", a term which most forensic scientists believe is incorrect. The more accepted label is "DNA typing" or "DNA profiling" (Coleman and Swenson, 2000).

1.1 Genetic Background

DNA, or deoxyribonucleic acid, has been called the molecule of life. In every organism, DNA contains all the essential information to create and sustain life (Casey, 1992). DNA molds our physical characteristics, and according to some scientists, it even shapes our behavior. Each life form contains DNA, and it is passed on from generation to generation.

The most basic building block of every organism is the cell. The human body is composed of approximately 3 trillion cells. Most cells are composed of smaller components called organelles. The hub of each cell is its

nucleus (Inman and Rudin, 1997). Every cell that has a nucleus also has chromosomes, which are composed of DNA and its associated protein molecules (Casey, 1992).

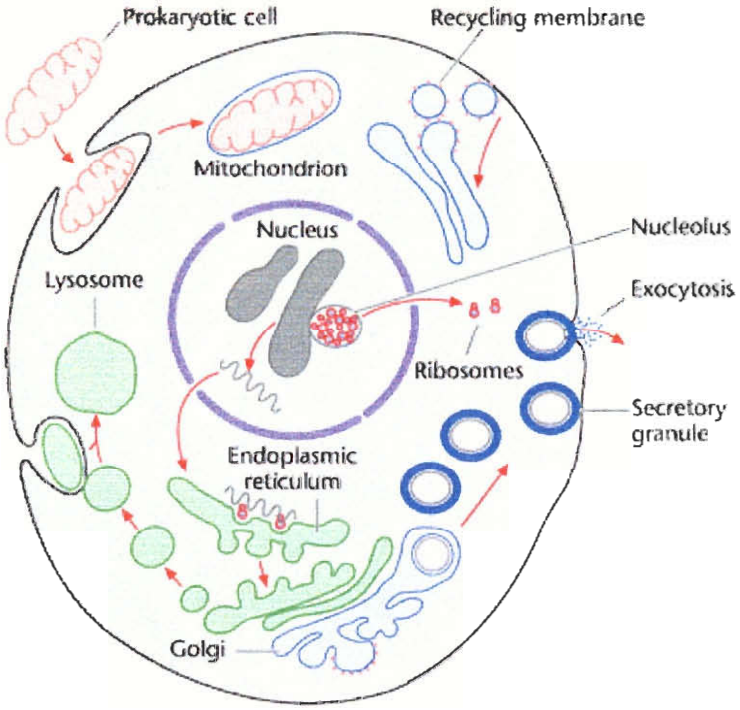

Figure 1.1: Diagram of a typical cell (Lane, 2001).

In humans and other higher organisms, DNA molecules have "two strands that wrap around each other to resemble a twisted ladder." This ladder is also termed the DNA "double helix." Each side of this ladder is made of sugar (deoxyribose) and phosphate molecules, which are connected by "steps" of nitrogen-containing chemicals called bases. There are four different bases in DNA: adenine, cystine, guanine, and thymine (or A, C, G, and T). The specific

order that these bases are arranged along the "sugar-phosphate backbone" is the "DNA sequence". This sequence is the distinct blueprint for that organism (Casey, 1992).

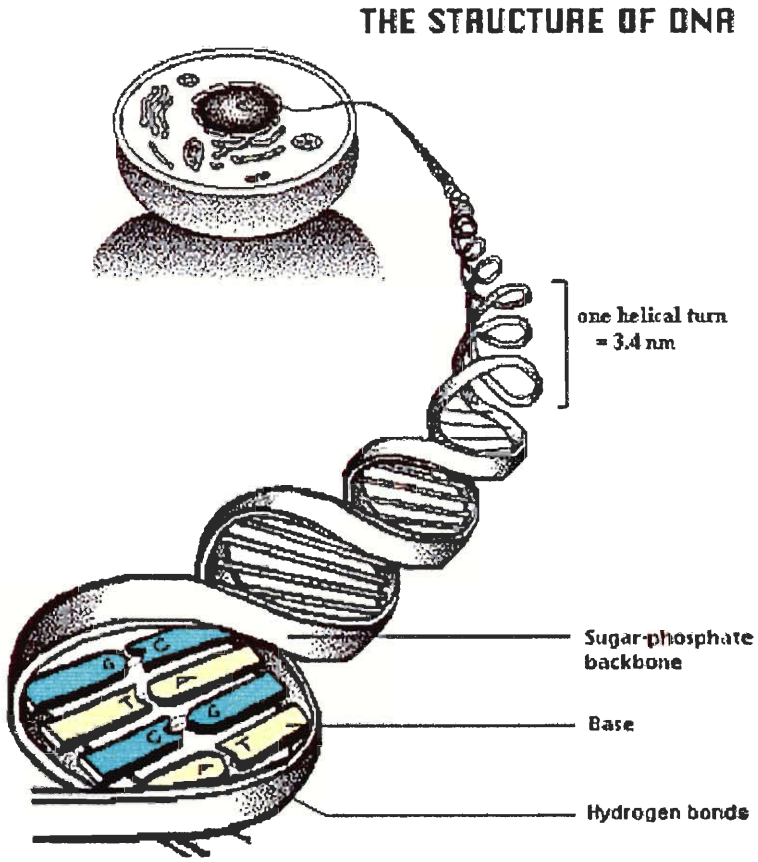

Figure 1.2: Structure of DNA (Access Excellence, 1999).

In the sequence of base pairs, A only pairs with T, and G only pairs with C. This specific pairing combination is called complementary base paring (Inman and Rudin, 1997). The bases are held together by hydrogen bonds, which are weak. Given that the bases connect the two

strands, the hydrogen bonds hold the double helix together. However, collectively they make the DNA molecules very secure. The weakness of the hydrogen bonds and their combined strength give DNA a good blend of stability and elasticity. Also, because the hydrogen bonds are weak, the two strands can easily be separated and then rejoined. This is important for much of DNA's functions (Dulbecco, 1987).

The complementary base paring is necessary for accurate replication (or copying) of DNA molecules (Inman and Rudin, 1997). Since the base pairs are enclosed in the middle of the DNA double helix, the genetic sequence cannot be read until the helix is divided (Dulbecco, 1987). During replication, specific enzymes "unzip" the DNA molecule, and the original is used as a "template" to create a new strand. The hydrogen bond between each base pair is broken, and new nucleotides are attached to the "backbone" of the new strand. As a result of the strict base pairing rules, the new strands are complementary to the original and to each other (Inman and Rudin, 1997).

Within the DNA are particular sequences called genes. Gene has two different, but related, meanings. The first is "the determinant of an observable trait or characteristic of an organism" (Millard, 2001). These

"traits" are also called an organism's phenotype. Examples of phenotype are things like hair and eye color, height, and facial appearances. The second meaning of a gene is "the DNA sequence that determines the chemical structure of a specific polypeptide molecule or RNA molecule" (Millard, 2001). These are the coding sequences of DNA and are termed exons; while, the noncoding sequences are termed introns (Krawczak and Schmidtke, 1998). Because each organism within and outside of a species has a unique phenotype, it can be understood that each individual has a different set of genes, and therefore a different set of DNA sequences. The human genome contains approximately 100,000 or more genes (Casey, 1992).

A human cell has 23 pairs of chromosomes, half of which are inherited from each parent. One pair out of the 23 chromosomes controls the gender of the individual. Males have one X and one Y chromosome, and females have two X chromosomes (Inman and Rudin, 1997).

A different form of the same gene is called an allele. If a specific locus of a pair of chromosomes has the same two alleles, then those alleles are called homozygous. The alleles are heterozygous, if the loci show differences between the pairs (Inman and Rudin, 1997).

With the exception of identical twins, every person has his or her own unique DNA. However, only 0.5% of the entire DNA code is unique from person to person. The other 99.5% is the same for everyone, and this is what makes us human beings (Inman and Rudin, 1997).

Genetic individuality is a factor of inheritance and mutation. In humans, and other diploids (diploid means that the organism has two pairs of chromosomes), one set of chromosomes is inherited from the father and another set from the mother. Inheritance causes uniqueness due to meiotic recombination, which is the exchange of random portions of the father and mother's chromosomes during meiosis. This swapping generally occurs between homologous chromosome pairs, and the segments that are exchanged normally match up and are compatible with each other. This maintains the proper DNA sequence. The second source of genetic individuality is mutation, which is the sudden change of the DNA sequence (Krawczak and Schmidtke, 1998).

Specific locations in DNA have been discovered, mostly through disease research, which vary between individuals more often than most locations. A specific site on a DNA molecule is called a locus (plural - loci) (Inman and Rudin, 1997). "If modifications of a gene exist at a specific locus in a population, the locus is polymorphic"

(Kirby, 1990). "Polymorphism refers to different forms of the same basic structure" (Kirby 24). When polymorphisms show high amounts of variation, they are termed "hypervariable" (Inman and Rudin, 1997).

Polymorphisms take place inside certain sequences or in the total length of a portion between two specific points. The first type is a sequence polymorphism. For example, these three strands, AATG, ATTG, and AGTG, show sequence polymorphism at the second base (by the correct base pairing rules, the matching pairs to these sequences can be determined). The second type of polymorphism is length polymorphism. These are differences in the numbers of repeated sequences. For example, the sequence AAGTCGTAA could be repeated anywhere from a few to thousands of times or more. These are called tandem repeats, and a locus that has a differing amount of tandem repeats is termed a variable number tandem repeat (VNTR) (Inman and Rudin, 1997). VNTRs typically contain repeat sequences between 8 and 35 bases long and between 100 and 1000 or more repeats. (Asplen and Samuels, 2000). They are also the basis of one type of DNA fingerprinting, called restriction fragment length polymorphism (RFLP) analysis, which uses the differing size of fragments between people to generate a DNA profile (Asplen and Samuels, 13).

The highly repetitive nature of VNTRs causes the chromosome to make more mistakes than usual during replication. These mistakes, or mutations, cause the VNTR to get longer or shorter, and can occur "up to 1% per generation". However, these length changes are usually only a few repeat units in size. The large amount of mutations also causes each allele to be rare (NRC, 1996).

1.1.1 Enzymes

Enzymes catalyze the creation or destruction of other "biological components" repeatedly. Enzymes that catalyze the growth of "components" are called polymerases. Enzymes that break down DNA into smaller pieces are called restriction enzymes. These restriction enzymes cut DNA at specific base sequences only. This allows every DNA molecule to be cut at the same places and into pieces of the same number and size (Inman and Rudin, 1997).

1.1.2 Hybridization

"Nucleic acid hybridization," which was introduced in the 1950s, is a useful technique in genetic research and in DNA fingerprinting. When DNA is heated to high enough temp, it can melt, which means that the hydrogen bonds between the base pairs break, and the two strands separate.

The hydrogen bonds are broken at the same time. If the temperature is dropped, the base pairs will join again to recreate the double helixes. This procedure is called reannealing or hybridization. However, when the strands come back together, random complementary sequences can form and the exact original double helix is not always produced. If the temperature were to be raised again, this "unstable" double helix would melt at a much lower temperature than the originals (Dulbecco, 1987).

Hybridization can be used to detect if two different DNA molecules have the same specific sequences, or are homologous. The more homologous the two DNA molecules are, the more stable the resulting hybridized molecules, and the higher the melt temperature (Dulbecco, 1987).

A single-stranded piece of DNA with a specific sequence of DNA can be manufactured to "target" that sequence. These are called probes, and when a radioactive atom is attached to them, the DNA molecules become visible to humans under ultraviolet light. Probes are used in order to identify specific genes or portions of the DNA sequence (Inman and Rudin, 1997).

1.2 Isolation of DNA

Before the actual DNA analysis can be done, the DNA must be separated from the other biological materials and purified from any non-biological materials. There are three main methods of DNA isolation for DNA typing: Chelex, organic, and differential extraction (Inman and Rudin, 1997).

1.2.1 Chelex Extraction

First the sample is boiled in a solution containing the chemical Chelex. This breaks open the cells, and releases the DNA. The Chelex also binds a good portion of the irrelevant materials. The Chelex is then removed, and the isolated DNA is left behind. The Chelex method of isolation breaks apart the two strands of the DNA sample, which makes this method more suitable when PCR analysis is to be performed. PCR analysis can be done on either single or double stranded DNA; whereas, RFLP can only be done on double stranded DNA (Inman and Rudin, 1987).

1.2.2 Organic Extraction

This method of isolation is more likely to maintain large pieces of DNA, and also provides a much more thorough cleaning. The sample, which could be a piece of fabric or

cotton, is first cut into small pieces and soaked in a warm solution. This frees the cells from the sample material. Next, another chemical mix is added, and mild heat is applied. This breaks open the cells, and the DNA is subsequently released. Organic solvents are then used to isolate the DNA. Lastly the DNA is cleansed and isolated even further by the use of special filters or by precipitation (Inman and Rudin, 1987).

1.2.3 Differential Extraction

This isolation method is used when sperm and other cells are present. These "other cells" are typically epithelial cells (e. cells), and include "saliva, skin, buccal, and vaginal cells, as well as those found in urine and feces" (Inman and Rudin, 1987).

First, the sample is soaked in a mild solution to remove the DNA from the substrate (or sample material). It is then incubated in a set of chemicals that break open only the e. cells. The liquid with the e. cells, which is now called the e. cell fraction, is removed. The DNA from the e. cell fraction is then isolated by organic extraction. The sperm cells are treated with certain chemicals, which remove the DNA from the substrate and break it open. This is now called the sperm fraction,

which is purified through organic extraction (Inman and Rudin, 1987).

1.3 RFLP Analysis

RFLP measures the size of DNA fragments cut by restriction enzymes. RFLP requires a good amount of HMW human DNA in order to be performed (Inman and Rudin, 1987). The most widely used restriction enzyme for RFLP analysis is called HaeIII, which cuts the DNA only when it encounters the sequence GGCC. HaeIII cuts this particular sequence between the G and C. The fragment lengths are controlled by the interval between each sequence of GGCC bases. This particular sequence appears millions of times in human DNA; therefore, the use of HaeIII creates millions of DNA fragments (NRC, 1996).

Once it has been decided to use RFLP, the first step is to put the DNA, restriction enzyme, and other components into a test tube and incubate it overnight in a warm bath. These items must be "carefully calculated and combined" in specific ratios. The overnight incubation allows the restriction enzyme to cut the DNA sample. This is called digestion. If some sites of the sample are not cut, the sample is said to be partially digested. To find out whether or not the sample had been fully digested, a small

amount of the reaction is removed and put on a digest gel, which is used to compare the sample with a set of "standard" digested and undigested samples of DNA. If the sample is found to be undigested, it is purified by performing parts of the extraction procedure again. This is done to eliminate whatever might be causing the restriction enzyme from working properly. Afterwards, the sample is once again incubated overnight with restriction enzyme (Inman and Rudin, 1987).

Once it is confirmed that the restriction enzymes have fully digested the DNA, a blue dye is added to the samples to make the sample visible on the gel. Each sample (if there are more than one, which there are almost always are) is added to a separate "lane" in an agarose slab. Agarose is a "gel-like" material extracted from sea kelp. Next, an electric field is applied to the agarose. Because DNA has a negative charge, it will slowly move toward the positive side of the gel. The positive end of the gel is on the opposite side as the DNA. This technique is called electrophoresis, and has been used in biology and genetics for many years before the introduction of DNA fingerprinting. The smaller sized fragments move faster than the larger ones; therefore, after time, the DNA is organized by size. This separation is enhanced further due

to "microscopic" holes in the gel. The smaller sized pieces move quicker through the holes. Typically, the electrophoresis is run overnight. The blue dye, which was added previously, indicates whether or not the separation actually occurred. If the electrophoresis is complete, the fragments will appear as a blue smear across the gel, signifying that they were separated (Inman and Rudin, 1987).

An ethidium bromide dye is generally added to the gel after the separation has taken place. This dye makes the DNA visible under ultra-violet light. Because the dye cannot differentiate between the DNA fragments, the result is a pink "smear". However, this is used as a control to ensure that the electrophoresis is complete (Inman and Rudin, 1987).

Through a process called Southern blotting, the DNA is transferred to a nylon membrane. Before this is done, the DNA must be separated into single strands. This is done to make the transfer of the DNA easier, and to allow probes to be hybridized to it later in the analysis. Next, the nylon membrane and some absorbent material are placed on top of the gel. The absorbent material attracts the fluid from the gel, and the DNA comes up with it. The DNA gets stuck

to the nylon membrane, and the DNA is adhered to it permanently (Inman and Rudin, 1987).

Probes that include radioactive atoms are now added to the nylon membrane. The probes hybridize to the target VNTR sequence, and then the excess probes are washed off. Next, the nylon membrane is put on an X-ray film. The emissions from the radioactive atoms on the probes deposit an image of the DNA fragments on the film. The film is now called an autoradiograph, or "autorad" for short. This process can take up to a week, because the radioactive decay of the film occurs slowly (NRC, 1996).

The probed DNA fragments appear as bands on the autorad. If two bands appear, the person is heterozygous at the locus. If only one appears, the bands are assumed to be overlapping, and the person is homozygous at that locus (NRC, 1996).

Performing RFLP analysis on one locus is almost always not enough to generate proper verification of a match. Therefore, it is necessary to test several loci. The major exception is a result that shows the two samples tested have come from different sources. In this case, it is not necessary to proceed in testing more loci. Generally, 4 or 5 loci are required to produce a statistically significant match. To do so, the previous probe is washed off, and a

new one is added to the membrane. This probe hybridizes to a different locus. Once again, an autorad is created (NRC, 1996).

The total time required to generate a profile of four or five loci can take weeks. However, many labs are switching from radioactive probes to chemiluminescent probes, which can shorten the required time from weeks to days. To perform chemiluminescent probing, the nylon membrane is covered with a substance that emits light due to a certain enzyme that is affixed to the probe. The film is exposed to light for a few hours to produce the same banding as in the autorad (Asplen and Samuels, 2000).

Using RFLP analysis to profile six different loci, a result can be generated in which the probability that two random Caucasian Americans will share is 1 in 100 billion (Asplen and Samuels, 2000).

There are a few advantages of RFLP analysis. First, there are several alleles per locus, and using many loci provides a high power of discrimination. Also, since there are many alleles per locus, RFLP can be used to work around a mixed sample. Lastly, an extensive database has been created for many populations (Asplen and Samuels, 16).

1.4 Polymerase Chain Reaction

PCR, or polymerase chain reaction, is a process of DNA amplification. PCR allows analysis of samples that would normally be too small or degraded for RFLP. Consequently, the samples generally used in RFLP examination are much too large for PCR analysis (Inman and Rudin, 1987).

Unfortunately, fewer loci have been produced for PCR analysis than for RFLP, and these loci generally show less distinction between samples. This makes PCR less likely to show that two samples came from different people than RFLP analysis (Inman and Rudin, 1987).

PCR uses an enzyme named taq polymerase, which copies DNA at high temperature. What makes this enzyme so extraordinary its ability to withstand relatively high temperatures and still function.

PCR can be performed after either chelex or organic extraction, and it is a repetition of 3 steps: denaturation, annealing, and extension.

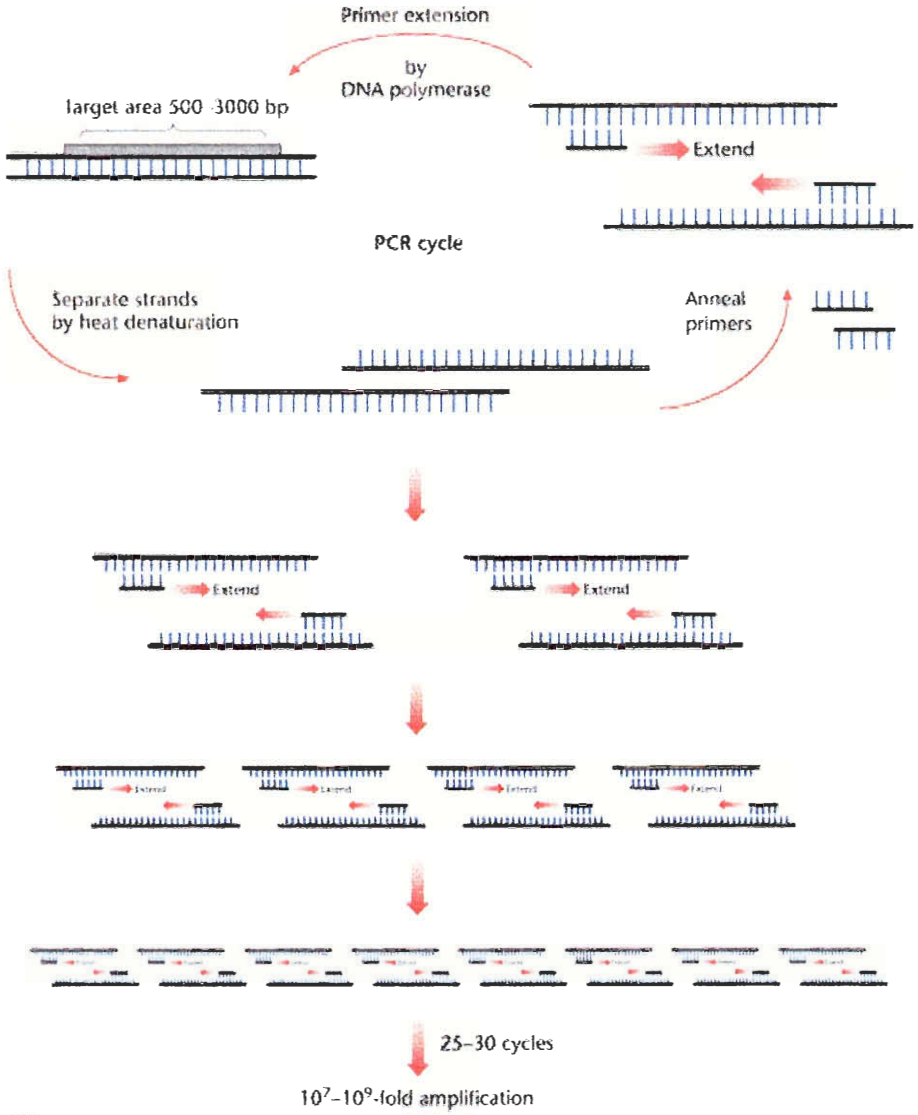

Figure 1.3: Schematic of PCR amplification (Caskey and Metzker, 2001).

Denaturation is the splitting the DNA double helix into single strands. Heating the sample breaks the hydrogen bonds and separates the two strands (Inman and Rudin, 1987).

Next, DNA primers, which are short, artificially produced fragments of DNA that hybridize to specific locations on the sample by complementary base pairing. The

primers mark the starting location of the synthesis of a new DNA strand; and, therefore, "prime" the reaction. Two different primers are used in PCR, one at the beginning and one at the end of the fragments of DNA that are to be amplified (Inman and Rudin, 1987).

Extension, the third step, entails adding nucleotides, which are paired to the original DNA by the Taq polymerase to create a new strand of DNA. This new strand is complementary to the beginning one, due to base pairing rules (Inman and Rudin, 1987).

The final result is double the amount of original DNA. These 3 steps are repeated until enough DNA has been duplicated to allow the analysis to proceed. The result of the PCR reaction is called the "PCR product" or "amplicon" (Inman and Rudin, 1987).

In order to verify that the PCR reaction is complete, a product gel is often performed. Sometimes, the Taq polymerase is prevented from amplifying the sample. If this is the case, the DNA can be cleansed by performing parts of the extraction again. The reaction can then be executed once more (Inman and Rudin, 1987).

1.5 Analysis of PCR

The PCR product can be analyzed with either of two methods, depending on the type of polymorphism present.

Sequence polymorphisms are detected using hybridization, and occasionally by examination of the DNA sequence itself. Length polymorphisms are detected using an electrophoresis method similar to the procedure used in RFLP analysis (Inman and Rudin, 1987).

1.5.1 Sequence Polymorphisms

The detection of sequence polymorphisms is based on the hybridization of probes to the amplified sample. A "commercially available" nylon strip, which includes a specific DNA sequence that comes from the same locus as the PCR product DNA, is compared to the PCR product. Each probe defines an allele and are called sequence specified oligonucleotides (SSO), or sometimes allele specific probes (ASO). If the probes are attached to a typing strip, the technique is called a reverse dot blot. The amplified DNA is profiled depending on which probe it hybridized to (Inman and Rudin, 1987).

The primers used during the PCR amplification have a biotin molecule attached to them. Once any left over probes have been washed off the strip, a streptavidin/horseradish peroxidase conjugate is applied, and it binds to the biotin. The biotin molecule has a strong attraction to the streptavidin, which is linked to

the horseradish peroxidase (HRP). The HRP turns blue after hydrogen peroxide and tetra-methyl-benzidine, have been added. Therefore, the dots (probes) that have bound with DNA, turn blue. The dots that turn blue match the alleles of the DNA. This method is used to analyze the loci in the polymarker test and the HLA DQα locus. Using basic everyday photography, the results are recorded and saved; and the strips are then thrown out (Inman and Rudin, 1987).

1.5.2 Length Polymorphisms

Using PCR to amplify length polymorphisms is more straightforward than RFLP analysis, because the specific DNA sequence has already been amplified to a quantity much larger than the other sequences present. This means that the PCR product is essentially the only DNA there. First, the PCR product is placed into a polyacrylamide gel, which is similar to the agarose gel used in RFLP, but more appropriate for PCR due to the small size of the PCR products. The PCR products are separated by length using electrophoresis. Probing and hybridization is unnecessary, because there is no irrelevant DNA present. A silver stain is added to the gel to allow the DNA to be seen. The gel is then dried and saved for records (Inman and Rudin, 1987).

The resulting bands for each sample are compared to each other and to a control sample, which has examples of all or most alleles from every locus used. Either one or two bands will be shown at each locus, and they symbolize the alleles present. If more than one locus from each sample has been amplified, each sample still gets its own lane in the gel (Inman and Rudin, 1987).

1.6 Significance of Results

After DNA analysis has been completed, the analyst comes to three separate conclusions about the genetic similarity of the samples: exclusion, similarity, or inconclusive. Exclusion means that the genetic profiles are dissimilar, and, therefore, could not have come from the same source. Similarity means that the results are alike and may have came from the same source. The third possible conclusion is that the result is inconclusive and it cannot be determined whether the samples are similar (Inman and Rudin, 1997).

If the samples have been found to be similar, it only means that no differences were found between them. They may have originated from different sources.

There are 3 possible explanations for genetic similarity: the samples came from the same source/person,

a coincidence caused a matched result between two different people, or an accident or error occurred during the collection, handling, analysis, and/or interpretation (Inman and Rudin, 1997).

Determining which of these possibilities is the correct one is very important. People's lives can be greatly affected by the outcomes. Nonetheless, a conclusion can be made based on population genetics. Statistics is used to calculate the regularity of alleles in a specific population. To find the chance that a random person from the certain population has the same DNA profile as that of the sample, the frequency of that profile in the population must be known (NRC, 1996). If many people in the population share the same DNA profile, then the chance of similarity of the samples is small. If very few people have the resulting profile, the chances of similarity are increased (Inman and Rudin, 1997). The frequency is usually determined by comparing the resultant profile to scientific data. However, this data resembles only a very small amount of the possible DNA profiles of that population. Therefore, the frequencies of distinct alleles are used to calculate the frequency of that particular profile (NRC, 1996).

To perform this task, the Hardy-Weinberg equilibrium and the linkage equilibrium are used. These theories approximate the genotype based on the allele frequency.

> "The Hardy-Weinberg model states that there is a predictable relationship between the allele frequencies and genotype frequencies at a single locus. This is a mathematical relationship that allows for the estimation of genotype frequencies in a population even if the genotype has not been seen in an actual survey.
>
> Linkage equilibrium is defined as the steady-state condition of a population where the frequency of any multi-locus genotypic frequency is the product of each separate locus. This allows for the estimation of a DNA profile over several loci, even if the profile has not been seen in an actual population survey" (Inman and Rudin, 1997).

The Hardy-Weinberg principle is based on a few assumptions: mating is random, the mating population is large, and migration insignificant. Especially in America, due to its diverse cities and towns, mating is not usually

random, and the mating population is not always large. Also, migration among populations is common. In large cities, regions of specific ethnic communities do exist. If a person of a certain ethnicity commits a crime in the community of a different ethnic group, the Hardy-Weinberg theory may be invalid. However, in this case, a more general population could be used instead of that specific one. In both theory and practice, it has been seen that limited compliance to Hardy-Weinberg and linkage equilibrium does not necessarily cause their use in calculating frequencies to be erroneous (Inman and Rudin, 1997).

1.7 Degradation

DNA becomes unstable when it is taken from its natural environment, which is inside the human body. Severe outside environments such as "time, temperature, humidity, light, and contamination (chemical and biological)," can cause degradation (Inman and Rudin, 1997). Degradation typically causes the DNA molecules to break into smaller fragments. The harshness of the environment determines the amount of degradation. A harsher environment will yield more degradation, which means there will be smaller, more numerous fragments. An important detail about degradation

is that it can only transform a sample from being able to give a result to being unable to produce a result. This means that degradation will not cause a false similarity or exclusion. At most it will cause the result to be inconclusive (Inman and Rudin, 1997).

After five days of being assaulted with "unleaded gasoline, motor oil, detergent, acid, base, salt, bleach, and soil," only the soil caused too much degradation and prevented a profile (Kirby, 1990). Therefore, any evidence sample may contain enough DNA to produce a result and should be collected.

A yield gel can be used to reveal the degree of degradation. The data can be used to establish whether RFLP or PCR analysis should be used (Inman and Rudin, 1997).

1.8 Contamination

A chemical or biological "agent" that is naturally found in the sample or its substrate can cause contamination. Typical agents are bacteria, soap, and deodorant. Incorrect collection, transportation, or analysis can also cause contamination by chemical or biological agents. Contamination can cause the DNA test to be inhibited, degradation of the DNA sample, or irrelevant

human DNA can produce an incorrect result. Contamination is more harmful to PCR analysis, because it could cause both the sample and the contaminant are amplified. Testing more than one locus makes it easier to distinguish the contaminant from the actual source (NRC, 1996).

The DNA analysis can be inhibited by any contaminant that impedes the enzymes involved from working properly. Clothing dyes and certain bacteria are known to do this. These types of contaminants are usually removed during the isolation process, and isolation can be done again if needed. The contaminants can also be counteracted during the analysis. A frequently used substance to do this is bovine serum albumin (BSA), which can be added during many of the steps of the analysis (Inman and Rudin, 1997).

Nonhuman DNA can also cause a problem with the analysis. Some microorganisms secrete enzymes that damage DNA, and this may prohibit a result from being formed. Typically, microorganisms do not create a problem, because the loci tested do not "cross-react" with those found in other organisms. However, the presence of nonhuman DNA can cause problems due to the analysis requiring the amount of human DNA present in order to work correctly. Therefore, analysts must figure out the total DNA and human DNA present to choose which test to perform and the amount of

reagents needed (Inman and Rudin, 1997). DNA profiles of police officers should be kept to refer to when human contamination is expected.

1.9 Problems with Analysis

In a DNA analysis laboratory, quality control and quality assurance measures should be implemented. Quality controls consist of "measures that are taken to ensure that the product, in this case a DNA-typing result and its interpretation, meets a specified standard of quality." Quality assurance involves "measures that are taken by a laboratory to monitor, verify, and document its performance." In other words, quality assurance is a safeguard on quality control (NRC, 1996).

Complications with "reagents, equipment, controls, or technique" typically cause no results or confusing results. Correct controls make erroneous matches or exclusion very rare (NRC, 1996).

Sometimes, samples loaded into a gel for electrophoresis can leak into the neighboring lane. This can lead to an incorrect result. To avoid this, the bordering lanes should be left empty if possible (NRC, 1996).

Also during the electrophoresis, some fragments may be so small that they fall off or spread apart at the end of the gel. This situation would produce a homozygous result instead of the correct heterozygous pattern (Inman and Rudin, 1997).

With RFLP analysis, two fragments of similar size often appear as one. A band 1050 base pairs long cannot be distinguished from one that is 1060 base pairs long. The resulting bands will show up as one rather than two. RFLP analysis is called a "continuous allele system", because alleles often cannot be told apart (Inman and Rudin, 1997). This requires similar sized bands to be categorized together in "bins." There are about 20 to 30 bins, as opposed to hundreds of alleles. The frequencies of these bins are available. A band is given a frequency depending on which bin it's in. This method is termed "fixed bin." A "floating bin" procedure exists, but it is much more complicated (Asplen and Samuels, 2000).

A problem concerning PCR analysis occurs when a sample has human DNA from more than one person. The original alleles may not be evenly amplified, which could cause a poor profile. Studies have shown that when a mixed sample with roughly equal DNA from two sources is profiled, the result will show a stronger pattern from one source. Equal

mixtures show reasonably similar intensities. If a variation in intensity is unusual, the analyst can identify one as the dominant profile (Inman and Rudin, 1997).

Another possible problem with PCR analysis is enhanced amplification of one allele over the other. This could even cause a homozygous pattern to appear when the individual is really heterozygous. This problem occurs more often during the early stages of PCR due to fluctuations in temperature during the denaturing step. Some samples were denatured less than others, and these were not amplified. However, this is less of a problem now, because most laboratory equipment is better calibrated and because of enforced quality control (Inman and Rudin, 1997).

CHAPTER 2: DNA FORENSICS

2.1 Collection of Evidence Samples

During the collection of evidence it is crucial, that everything and everyone involved in the collection is clean. The correct procedures for the collection, packaging, preservation, and documentation of DNA evidence samples must be followed or the evidence may not be allowed into the courtroom. These procedures are also important for maintaining the original condition of the sample (Wade, 1999).

There are two central ways to collect samples that are anticipated for DNA analysis. The first is to collect the soiled object itself, and the second is the removal of the biological materials to a different piece of material (Inman and Rudin, 1997).

The preferred approach is the actual collection of the material. This method poses less risk of degrading the sample, and places more control to the practiced (hopefully) lab technician (Inman and Rudin, 1997).

Removal of the sample is done by physically scraping the stain off of the material it was deposited on with a scalpel or forceps. This method can also be done by "re-hydrating" it in water or a "chemical buffer", and then

applying it to a cotton material. However, scraping the sample may cause it to fall and get lost during the collection, and re-hydrating can cause degradation by moisture. If the sample has been re-hydrated, it must be dried as soon as it is brought to a lab (Inman and Rudin, 1997).

However, before any evidence is collected, the stain should be tested to find out what kind of biological matter is there. Color tests can be used to distinguish between blood, semen, or saliva. This test can be performed right at the crime scene; however, as backup, the test should also be done at the lab. There is no sense in wasting the officers' or lab's time and money to analyze a meaningless sample (Inman and Rudin, 1997).

A good practice to exercise during the collection of evidence is to collect at least one "unstained" sample from the area surrounding the evidence. This is done to ascertain what may have been on the same material as the evidence before it got there (Inman and Rudin, 1997).

After the sample has been collected and transported to a lab, it should be dehydrated or else it may degrade. Freezing the sample also should be performed to protect it. The temperature and humidity of the lab should be kept

constant also to prevent DNA degradation (Inman and Rudin, 1997).

2.1.1 Blood

Liquid blood that is on a person can be absorbed onto a swab or cotton cloth. Dried blood from a human can be absorbed onto swabs or cotton cloths that have been dampened with distilled water. Part of the swab or cloth should be left "unstained as a control". After the sample has been collected, the swab or cloth must be air dried and stored in "clean paper" or in a sealed envelope. Plastic containers must never be used (Wade, 1999).

Liquid blood or blood clots deposited on surfaces are collected and stored using the same methods and procedures as the collection of liquid blood from a person. Bloodstains on objects that cannot be removed from the crime scene directly are to be collected with this same procedure (Wade, 1999).

Wet bloodstains on clothing are to be air dried and then wrapped in paper. A control sample should also be taken from the clothing and put into either clean paper or a sealed envelope. Again, never use plastic containers. If the piece of clothing cannot be collected directly, then

the stained portion along with a control sample can be cut from it (Wade, 1999).

2.1.2 Semen

Semen stains are collected using the same procedures previously discussed for bloodstains (Wade, 1999).

2.1.3 Miscellaneous Evidence

The procedures for collecting liquid and dry saliva stains and urine are the same as those used for blood and semen. However, it is important to realize objects such as cigarette butts, chewing gum, and envelope should be collected. These objects may contain enough DNA for analysis (Wade, 1999).

Hair is to be collected directly and placed in paper or a sealed envelope. Different bunches of hair samples should be collected and contained separately from each other (Wade, 1999).

Bone, teeth, and tissues must be collected with gloved hands or clean forceps. One to two cubic inches of red skeletal muscle, and three to five inches of bone are sufficient amounts. Teeth must be collected in a specific order. Tissue samples are to be put in an airtight container. Never use formaldehyde or formalin. Teeth and

bone are to be stored in clean paper or a sealed envelope (Wade, 1999).

2.2 Collecting Known Samples

Only "Medical personnel" should take blood samples from an individual. At least two 5mL tubes of blood should be taken, and EDTA, which is an anticoagulant, should be added to the blood. The tubes should be capped with a "purple-top" to distinguish them as evidence blood samples. When transporting the samples, they should be stored with cold packs and never dry ice. Before submitting the samples to a laboratory, they should not be frozen, only refrigerated. The tubes are to be packed separately in styrofoam or "cylindrical tube containers", and some type of absorbent material should surround them. The outside of the storage container must be labeled "KEEP IN A COOL DRY PLACE, REFRIGERATE UPON ARRIVAL", and "BIOHAZARD" if appropriate (Wade, 1999).

Medical personnel should collect evidence from someone who has been sexually assaulted. Sexual assault kits are commercially available to collect the evidence (Wade, 1999)

Salival cells are collected with buccal swabs. This is done by rubbing the inside of the cheek with the swab, and then either drying the swab or placing it in alcohol.

When drying the swab, it should be done by air. The dried swab should then be placed in either clean paper or a sealed envelope. The swabs do not need to be refrigerated (Wade, 1999). Collecting buccal samples is much simpler than the collection of blood samples, and just about any detective can do it (Budowle et al., 2000).

Both salival and blood samples should be labeled with the "date, time, subject's name, location, collector's name, and evidence number" (Wade, 1999).

DNA analysis laboratory reference samples are usually blood or buccal epithelial cells (or salival cells). For storage, blood samples should either be frozen or placed on filter paper. Blood can be frozen at between -20 degrees Celsius and -70 degrees Celsius in polypropylene screw cap tubes. The filter paper, which is commercially available, must be dried first, and then stored at frozen or at room temperature (Budowle et al., 2000).

FTA paper, which is commercially available, can be used to collect, transport, and/or store blood samples. The collection can be done by dropping the blood directly onto the paper by either "fingerpick" or using a pipet. The FTA paper traps the DNA, and the cell membranes are "lysed" in the paper's "matrix". This allows the proteins and other biological materials to be washed off, while the

DNA remains. Blood placed on FTA paper must be allowed to dry for at least one hour before storage. In order to prevent degradation, the FTA paper is infused with chemicals that denature bacteria and fungi and others that prevent UV harm and oxidation. FTA paper can be used to store blood and other samples either frozen or for relatively long times at room temperature (Budowle et al., 2000).

CHAPTER 3: LANDMARK COURT CASES

3.1 Case of Colin Pitchfork

The first case solved by the use of DNA fingerprinting was the rape and murder of two young girls in 1983 and in 1986 in the small town of Narborough in Leicestershire, England. The first, in 1983, was a 15-year-old girl. A semen sample was collected and analyzed for blood type. The sample was found to be from a person with type A blood. Unfortunately, this was not nearly enough to convict anyone (Coleman and Swenson, 2000; and FSS).

The second murder, in 1986, also produced a semen sample from somebody with type A blood. This, along with other evidence, led police to believe that the same person committed both murders. Their main suspect had confessed to the second murder but not the first. In order to confirm his confession to the police, the semen samples were brought to Alec Jeffreys. Through DNA profiling, he proved the suspect's innocence (Coleman and Swenson, 2000; and FSS).

However, the police were not done with their investigation. The semen samples did prove that the same man murdered both girls. The police asked all males from three villages between the ages of 17 and 34 to voluntarily

submit a blood sample. By May of 1987 over 3600 men had given samples and still the killer had not been found (Coleman and Swenson, 2000; and FSS).

Later that year, a woman overheard that one of her coworkers, Colin Pitchfork, had persuaded somebody else to take the test for him. The police arrested Colin, and his sample matched that of the killer. He gave the 4583rd sample in September of 1987. Colin Pitchfork was sentenced to life in prison, and a new age of forensic science began (Coleman and Swenson, 2000; and FSS).

3.2 Case of Tommy Lee Andrews

In 1987 Cellmark Diagnostics and Lifecodes Corporation began performing DNA typing in the United States. Lifecodes was the more successful of the two, and they did the analysis in the first US case that convicted a suspect through DNA profiling. Tommy Lee Andrews was convicted of rape, and his trial began in November of 1987 in Orlando, Florida. After RFLP testing of an evidence sample and a sample from Andrews, a Lifecodes scientist and an MIT biologist testified that Andrew's genetic profile could only be found in one out of ten billion people. Andrews was sentenced to 22 years in prison on November 6, 1987 (Coleman and Swenson, 2000).

During the next couple of years, there was a rush of DNA testing done on forensic evidence. DNA profiles were permitted in court cases without difficulty, and the private companies performing the analysis began an intense competition for the business and technology. Each company used different techniques and equipment, and results from separate labs were not compatible and could not be compared (Coleman and Swenson, 2000).

3.3 People v. Castro

In these early court cases, judges admitted the DNA evidence without fail, and the juries were dumbfounded by the impressive statistics of the results. One case commented that DNA fingerprinting is "the single greatest advance in the 'search for truth', and the goal of convicting the guilty and acquitting the innocent," (Wesley, 1988). It seemed like this new forensic technology could not be stopped. However, DNA fingerprinting took a serious hit in the New York State case People vs. Castro (Coleman and Swenson, 2000).

In February 1987, police found a man's pregnant wife and his daughter murdered, both had been stabbed repeatedly. Joseph Castro was the main suspect, and a small dot of blood was found on his wristwatch. Lifecodes

performed DNA profiling on the bloodstain using RFLP analysis. The scientists stated that the blood came from the victim, and that the resulting DNA profile could only be found in 1 in 189,200,000 people within the Hispanic population (Coleman and Swenson, 2000).

The defense team performed a detailed investigation of Lifecodes' methods and results. They found that the data was weak and was not even within Lifecodes' own match standards. Lifecodes had not used their own normal methods to interpret their results (Coleman and Swenson, 2000).

After having previously testified, four scientists, representing both the prosecution and the defense, together reviewed the evidence. They composed a two-page report on their dissatisfaction of the scientific results and the way the evidence was handled in court (Inman and Rudin, 1997).

The court found that "the testing laboratory failed in several major respects to use the generally accepted scientific techniques and experiments for obtaining reliable results;" therefore, the judge did not admit the DNA analysis (Castro, 1989). Joseph Castro was found guilty later in that year after confessing to the murders (Coleman and Swenson, 1997).

3.4 Frye v. US

Before any new scientific technology and evidence can be allowed in a court case, it must be permitted into the trial by a judge. This usually requires an admissibility hearing, before any evidence can be shown in front of a jury (Inman and Rudin, 1997).

The general standard for admitting new scientific evidence to the courts was established in the 1923 case, Frye v. United States. The case involved the decision of whether or not to include a lie-detector test (Inman and Rudin, 1997). The court ruled that "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, 1928).

3.5 People v. Kelly

In the California Supreme Court case, People v. Kelly, the standards for admitting a new scientific technique into the courts were reworked. The case resulted in a "two-step" approach for the admission of new science:

"The parties agree generally that admissibility of expert testimony based upon the application of a new scientific technique traditionally involves a two-step process: (1) the *reliability of the method* must be established, usually by expert testimony, and (2) the witness furnishing such testimony must be properly *qualified as an expert to give an opinion* on the subject."

"Additionally, the proponent of the evidence must demonstrate that correct scientific procedures were used in the particular case" (Kelly, 1976).

3.6 People v. Yee

In the 1991 case, United States v. Yee et al., the Federal Bureau of Investigation accepted RFLP analysis for the first time. The crime occurred on February 27, 1998, in Perkins Township, Ohio. The victim, David Harlaub, was shot several times by a member of the Hell's Angels while near his van. Hartlaub was actually not the intended target, but was mistaken for him. The police found blood inside his van that was assumed to have not belonged to the victim. The blood sample was given to the FBI for DNA analysis. The result showed that it belonged to John Ray Bonds, who was a chief suspect (Inman and Rudin, 1997).

During the Yee case, the DNA evidence was severely challenged and fought over. The statistical methods used were the main concentration of the conflict. The defendant's scientists mostly criticized the FBI for ignoring the possibility of subpopulations in their calculations. However, the judge accepted the DNA evidence. This case initiated argument about the population genetics involved in determining a result (Inman and Rudin, 1997).

3.7 People v. Axell

The 1991 case, People v. Axell, started a large debate over subpopulations and their affect on the statistical methods used in interpreting DNA results. The suspect, Linda Axell, was charged with the murder of a convenience store owner. In the victim's hands, the police found many strands of hair that had their roots intact. Hair samples can occasionally be analyzed by RFLP if there are enough hairs with relatively good quality roots. The hairs were analyzed by RFLP and similarity was shown between the results and a sample taken from Axell. Despite a long and fierce admissibility hearing, which concerned both the testing methods and the statistics used, the judge admitted the evidence (Inman and Rudin, 1997).

Soon after the Axell case, an article appeared in the journal *Science* about population genetics and the statistics involved. This caused a huge debate in the forensic science community over the methods used. The affects of subpopulations on estimating the frequency of the resulting DNA profile were particularly challenged (Inman and Rudin, 1997).

This resulted in a proposal for interpreting a DNA profile to be formulated by the National Research Council. These suggestions were published in 1992, and they proposed the use of a "ceiling principle" to put a limitation on the rarity of a specific genetic profile. This was made as a temporary solution to be used until data could be generated on enough subpopulations. This data was to be used to find out if the subpopulations had an affect on allele frequencies, and if so, what affect did this have on the current calculation methods. The legal system openly accepted the ceiling principle; however, numerous scientists disbelieved in its scientific validity (Inman and Rudin, 1997).

3.8 Later Years and PCR in the Courts

In the next few years after the NRC report, DNA fingerprinting results were often denied into the courts

due to the supposed lack of general acceptance in the scientific community (Inman and Rudin, 1997).

PCR has had much less trouble in gaining acceptance into the courts than RFLP had. "Ironically, part of the reason that the HLA DQα test, which was the only one available for many years, is not nearly as powerful as RFLP so the comfort level has been greater." This easier acceptance is also in part due to the DQα and D1S80 methods testing one locus. No arguments could be made about the calculation methods used in interpreting multiple loci (Inman and Rudin, 1997).

However, PCR has had its own kind of struggle in the courts. Whereas, RFLP had trouble due to population genetics and statistics, PCR has had difficulty with the scientific methods involved (Inman and Rudin, 1997).

In 1996, the NRC issued its second report on DNA fingerprinting. It stated that the "technology for DNA profiling and the methods for estimating frequencies and related statistics have progressed to the point where the reliability and validity of properly collected and analyzed DNA data should not be in doubt" (NRC, 1996). With current government policy on the collection and handling of evidence and using properly applied quality control and

quality assurance measures, DNA evidence should have no trouble gaining acceptance into trial.

CHAPTER 4: CONCLUSIONS

4.1 Final Thoughts

DNA fingerprinting is a very powerful tool in the identification of humans. It is most known for its use in the identification of criminals of violent crimes. However, DNA fingerprinting is not foolproof. Each sample requires a large amount of labor to generate a result. A good sample is necessary to begin with. If the sample is too degraded or not large enough, then analysis may not be possible. The analysis itself can take a long time to complete; up to five weeks or more, depending on the number of loci tested. Once a profile is generated, it must be compared to other crime scene samples. If two samples are similar, it only means that no differences were found between them. It does not mean that they came from the same person. This can never be determined to be true without any doubt. A series of complex statistical calculations and comparisons with population data must be performed in order to determine the probability that somebody else in that population will have the same profile.

A common misconception is that a match between two profiles assigns guilt to the suspect. This couldn't be

farther from the truth. The final result is a probability, not a certainty. Furthermore, if a match could theoretically be determined with one hundred percent accuracy, it would still only prove that the sample came from that particular person. It is up to the courts to determine guilt or innocence.

4.2 Future Trends

The future of DNA fingerprinting will show a transition from RFLP analysis towards PCR and STR (short tandem repeat) analysis. STRs are similar to VNTRs; they typically have three to five base pairs repeated seven to fifteen times. Due to the small size of STRs, they can be amplified with PCR and the detection of distinct alleles is possible (Asplen and Samuels, 2000). Currently, the FBI uses a particular set of thirteen STR loci as their standard in the Combined DNA Index System (CODIS). These thirteen loci give excellent discriminatory power. The chance of a match between two unrelated Caucasian Americans in a randomly mating population is 1 in 575 trillion. For any state or local law enforcement lab that performs DNA analysis, the FBI will supply software to help the use of the CODIS, along with installation, training, and technical support. The CODIS includes the Convicted Offender Index,

which contains DNA profiles of people who were previously convicted of violent crimes, and the Forensic Index, which contains DNA profiles from evidence found at crime scenes (Asplen and Samuels, 2000).

Another technique that will most likely be used more widely is analysis of mitochondrial DNA (mtDNA). Since each cell contains up to thousands of mitochondria and each one has its own DNA, only a small sample is necessary for analysis. This may allow DNA fingerprinting to be performed on old and degraded samples and on hairs and bone. However, the analysis of mtDNA has a fairly low discriminatory power, which would require a large database specifically for mitochondrial DNA (Asplen and Samuels, 2000).

A key trend in the future of DNA analysis will be increased speed of generating a profile. Automation of parts of the analysis will certainly become more widely available, and it also may allow for less human error. Another future trend in DNA fingerprinting will be the development of small, portable DNA analysis devices. This will allow a profile to be generated directly at the crime scene. However, one difficultly in implementing such a device is its use in an uncontrollable environment (Asplen and Samuels, 2000).

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