IQP-DSA-5593 52
IQP-DSA-6231

DNA FINGERPRINTING

An Interactive Qualifying Project Report:

Submitted to the Faculty

of the

WORCESTER POLYTECHNIC INSTITUTE

in partial fulfillment of the requirements for the

Degree of Bachelor of Science

by

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Date: April 24, 2003

APPROVED:

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ABSTRACT

The purpose of this project was to investigate the impact of DNA fingerprinting on society. We explored various DNA typing techniques as well as DNA forensics and the steps investigators are taking to prevent contamination. Landmark and sensational court cases were also researched to determine the legal ramifications of this interesting scientific technique. We conclude that DNA fingerprinting is a powerful technique that is extremely reliable if DNA evidence is collected, stored, and tested properly.

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EXECUTIVE SUMMARY

DNA fingerprinting is a widely used technique that analyzes hypervariable regions of DNA to determine if two samples come from the same person or related individuals. Scientists concentrate mostly on the hypervariable regions of DNA when trying to match because these areas vary the most from person to person, and it is very difficult to sequence an individual's entire DNA. Restriction enzymes are used to cut the DNA at specific sites where certain nucleotide patterns exist. Then, by using electrophroesis, scientists can have the different DNA segments separated by size. From there scientists can use probes to detect specific bands of DNA. Some typing techniques, like PCR, are better suited for dealing with small amounts of DNA, while others like DNA strand complementarity can help scientists deal with single-stranded DNA. Other techniques are also discussed.

DNA forensics is a powerful addition to the world of forensic science. The first part of understanding DNA forensics is knowing how to identify DNA sample sources and collect DNA samples without contamination. The crime scene investigation unit is responsible for correctly collecting and handling DNA evidence from crime scenes. Good sources of DNA evidence include blood, semen, saliva, urine, hair, tissues, bones, and teeth.

Every time a new sample is being handled sterile equipment must be used, this prevents previous DNA samples from being mixed into the sample currently being handled. Sneezing, coughing, or even talking near DNA evidence could cause contamination. Next, a known sample is collected. This is to compare the evidence to a

suspect and can either be a match in a DNA database or an actual sample collected from the suspect.

DNA forensics is not performed just out on the field but also in the lab. After samples arrive, scientists will still need to extract DNA from the samples. This is done with DNA extraction techniques. DNA extraction techniques discussed in this paper include Chelex extraction, organic extraction, and differential extraction. While in the lab, Quality assurance and Quality control are both being employed. Quality assurance includes laboratory audits, special reports, training personal and many other techniques to ensure everything is meeting high standards. Quality control involves ensuring that erroneous results are not released from the laboratory such as false negatives and false positives.

Chapter 3 explores numerous landmark court cases that have impacted DNA fingerprint evidence in the courtroom. Some do not explicitly involve DNA evidence, but instead involve the acceptance of another new scientific technique. These cases have been important factors in whether DNA evidence and testimony regarding the technique is allowed in legal proceedings. The 1923 Frye case, for instance, set an admissibility standard that a new technique must be generally accepted by the scientific community. Since then, other courts have set their own standards including a relevancy test suggested in the Downing case, and a three-prong test recommended in the Castro case. Others applied the Federal Evidence Rule 702 as the Supreme Court recommended in the Daubert case. The interpretation of each standard varies among courts, which can result in inconsistent rulings across courts. The Supreme Court's interpretation Rule 702's

procedure is extremely similar to the relevancy and three-prong test, and they found that it supercedes the Frye test.

A few of the most sensational cases involving DNA evidence are the subject of Chapter 4. These are the cases that have exposed the general public to DNA fingerprinting. It is apparent that DNA evidence is not only useful in present cases, but also in past cases. In the Anastasia case, DNA was collected from remains that were over seven decades old. In another case, Robert DeSalvo had been declared the Boston Strangler for thirty years until DNA later implied otherwise. Many believe that the truth behind both of these cases lies in the DNA. Others still question the reliability of the results.

The investigation of both the landmark and sensational cases also demonstrate the legal ramifications of mishandling evidence. The Simpson case, referred to as the "trial of the century," clearly reveals the importance of evidence collection procedures. If the evidence had been handled in the proper manner the outcome may have been very different. The importance of DNA testing standards becomes apparent in the Castro case where the testing laboratory declared a DNA match, but did not follow acceptable procedures. Overall, the cases in these chapters clearly show both the positive and negative aspects surrounding DNA fingerprint evidence, which affect society.

PROJECT OBJECTIVE

The overall purpose of this IQP was to investigate the technique of DNA fingerprinting and its impact on society. By researching landmark court cases involving the admissibility of DNA fingerprinting and new scientific techniques in general, we hoped to determine correct and incorrect legal uses of the technology and possible problems that arise. The majority of the general public's knowledge of the technique comes from sensational DNA cases, so we wanted to research those also. The objective was to investigate legal proceedings that would provide insight on both the positive and negative societal aspects of the technology.

Chapter 1: DNA TYPING

DNA fingerprinting is a widely used technique that analyzes hypervariable regions of DNA to determine if two samples come from the same person or related individuals (Brinton and Liberman, 1994). When trying to analyze DNA from two different sources, scientists look at certain regions of its base pairs since that is what differs from person to person. Since it is very difficult to sequence an individual's entire DNA, scientists concentrate mostly on the hypervariable regions of a person's DNA because those regions vary the most between people and therefore analysis is easier than if they looked at all the base pairs in a single person's DNA. DNA fingerprinting has a wide variety of applications, from paternity testing, to matching DNA from a murder scene to a suspected killer.

DNA can be extracted from biological sources that contain nucleated cells. Blood, semen, hair, and saliva are just a few examples of nucleated cells in the body. If one of these samples were to be analyzed, the DNA would first have to be purified by removing the DNA from the other parts of chromatin that it is surrounded in. Once DNA has been purified there are many different methods that can be used to analyze the DNA including restriction enzymes and electrophoresis, DNA strand complementarity, Southern blotting, dot blotting, polymerase chain reaction (PCR), DNA sequencing, restriction fragment length polymorphism (RFLP), combinations of some of the above methods, and capillary electrophoresis (Krawczak and Schmidtke, 1998, pp. 15). These techniques are used depending on the quality and quantity of the biological sample being analyzed.

Restriction Enzymes and Electrophoresis

Restriction enzymes cut DNA at specific sites where certain nucleotide patterns occur. Human DNA is acted upon by restriction enzymes in the exact same way as bacterial DNA when they exist naturally so it is easy to create a restriction map of where the enzymes are going to act on a certain set of nucleotides (Brinton and Liberman, 1994). After DNA has been cleaved using restriction enzymes it can then be separated by size using electrophoresis. Gel electrophoresis is a technique where DNA is put into wells in the top of a gel, agarose is often used, and an electrical current is applied. DNA is slightly negative so when the electrical current is run through the gel there is a positive charge at the bottom and a negative charge at the top. The DNA goes towards the bottom of the gel towards the positive charge. Molecular sieving then occurs where smaller pieces migrate faster through the gel and are therefore viewed farther down the gel. This allows the DNA fragments to be separated by size. The gel can then be stained with a fluorescent dye and specific areas of the gel, bands of DNA, can be identified using probes that have sequences complementary to those being amplified (Krawczak and Schmidtke, 1998, pp. 19).

DNA Strand Complementarity

DNA strand complementarity is another technique to analyze DNA that is related to restriction enzymes. DNA is made up of four base pairs: adenine (A), cytosine (C), guanine (G), and thymine (T). A can only base pair with T, and G can only base pair with T. DNA is double-stranded so if one strand has the base pairs GAATTC then the complementary strand has the base pairs CTTAAG (Krawczak and Schmidtke, 1998, pp.

19). This complementarity of DNA strands can be exploited in DNA analysis. After a size electrophoresis has been run, the DNA strands can be separated using alkaline solutions, allowing complementary probes (a little molecule of DNA that is complementary to the sequence that is being analyzed) to hybridize to its "sister" region. In a large mixture of DNA sequences, a single-stranded DNA oligonucleotide can find its complementary strand because it is the only strand it will bind to. This property of DNA exploited in many DNA analysis procedures.

Southern Blotting

Southern blotting is a method of DNA analysis that uses the above mentioned techniques for restriction digestion, electrophoresis, dentauration, blotting, then hybridization to a probe. First, the DNA is purified from the other material present in the sample using, for example, a detergent wash (Brinton and Liberman, 1994). Then, the DNA is digested with one or many restriction enzymes and run on a gel that will separate the DNA based on size. The DNA will then be denatured with an alkaline solution, which forces the DNA into single strands. The DNA on the gels is then transferred to a membrane and fixed there. The membrane is then incubated with a probe for several hours, and if the probe was a radioactively labeled then it will visualize the band of interest on X-ray film. The DNA that hybridized to the probe is then visible on the film as a black band (Krawczak and Schmidtke, 1998, pp. 20). Southern blotting shows whether the specific DNA fragment of interest is there or not, and also its size.

Dot Blotting

Dot blotting is similar to Southern blotting but it can only detect whether a certain sequence is present or absent in the DNA being tested. Dot blotting sidestep restriction digestion and electrophoresis. First, a small amount of denatured DNA is put onto a membrane and hybridized with a probe. If the specific DNA sequence matching the probe is present then it binds the probe and is detected (Krawczak and Schmidtke, 1998, pp. 21). Dot blotting is useful for sex determination and quantitation of PCR amplified products (Kirby, 1990, pp. 104).

PCR

Polymerase chain reaction is an in vitro method of DNA detection that can amplify specific target DNA sequences using only a small amount of DNA or genomic DNA (Krawczak and Schmidtke, 1998, pp. 23). First, the DNA is denatured by heating, to obtain single-stranded DNA. Deoxynucleotide triphosphates (DNA precursors) are added to the reaction mixture as is DNA polymerase, which is an enzyme that helps start the synthesis of DNA. Then the primers are annealed, which creates a primer extension. The DNA polymerase synthesizes a complementary strand. Then, the new double-stranded DNA molecules are denatured and become the beginning of another cycle of DNA synthesis. Many cycles can be performed for one target DNA sequence but they all include "annealing primer molecules to the template DNA, elongating the nascent strand and melting the double-stranded molecules to form two new single-stranded templates (Krawczak and Schmidtke, 1998, pp. 24)." The end result is that the DNA sequence

between the two primers is synthesized and amplified many times to the point where it is now visible on a gel.

Although PCR can only synthesize small DNA molecules with the upper limit around 10 kilobases, PCR has many advantages including the fact that it can be used on extremely small quantities of DNA, and samples and samples that have been badly degraded. DNA can be amplified using PCR from many different sources including "formalin-fixed paraffin embedded tissues, dried blood and semen stains, and hair follicles and shafts (Kirby, 1990, pp. 76)." PCR is also a good way to analyze DNA because it cannot recover damaged molecules only amplify those already intact (Kirby, 1990, pp. 76). PCR can analyze partially degraded samples including those that have been exposed to UV light, chemicals and humidity. Conditions often found in forensics. Samples that have been contaminated with microbial organisms can also be typed with PCR. Typing with PCR can be very quick, and PCR has a high discrimination potential that can analyze DNA from cigarette butts and envelope flaps, usually hard places to analyze DNA from (Forensic Analytical DNA Typing, 2002).

DNA Sequencing

DNA sequencing is another method of determining identity that is used despite the fact that it is not very efficient. The most widely used sequencing technique involves using DNA polymerase to synthesize a new double-stranded DNA from a primer. The single-stranded DNA template starts the cycles. This is very similar to PCR except that the sequencing involves the use of dideoxy analogs, which are mixed with the usual nucleotides to produce instant chain termination. Also, sequencing uses radioactively or

fluorescently labeled nucleotides, and four reactions are set up simultaneously. Each reaction has a different dideoxynucleotide added to it. During the reaction, the dideoxynucleotides are combined into the expanding DNA molecules randomly. This allows for each nucleotide on the strand having an equal chance of being substituted by a chain terminating nucleotide (Krawczak and Schmidtke, 1998, pp. 25). After a long reaction time, the mixtures are loaded onto a polyacrylamide gel to separate by size. The reaction results can be seen on the gel in the form of a ladder of bands that show the primary nucleotide sequence.

Mitochondrial DNA Sequencing

Mitochondria are organelles found in human cells outside of the nucleus and contain a genome separate from the nuclear genome. The human mitochondrial DNA (mtDNA) genome has already been completely sequenced. It has many characteristics that vary from nuclear DNA including that it is circular not linear, it is smaller (around 16.5 kb), it contains coding sequences for 2 ribosomal RNAs, 13 proteins, and a displacement loop of 1100 base pairs, and it is not recombinant (Budowle et al, 2000, pp. 189). The most important characteristic of mtDNA is that it is inherited maternally, so it is identical for siblings and relatives on their maternal side. This is useful in forensic cases involving unidentified bodies because the surviving relatives can be tested. Mitochondrial DNA also has a high copy number which means that in a small forensic sample mtDNA is more likely to be identified than polymorphic regions in nuclear DNA. MtDNA is very useful in the analysis of hair shafts because the sequence can be taken from as little as 1 to 2 centimeters of hair.

The mtDNA locus is the only locus where is it advantageous to sequence it because the region sequenced is small and well defined. The area also usually includes one allele per individual (Inman and Rudin, 1997, pp. 75). The procedure is very similar to nuclear DNA sequencing. To sequence the mtDNA (to determine the order of nucleotides), PCR is used to amplify the template. The template is then denatured and a sequencing primer is attached to the single-stranded DNA. Then, the primer is extended across the sequence of interest by the addition of the four deoxyribonucleoside triphosphate and one terminating dideoxyribonucleoside triphosphate analogs. The terminator is added to the chain by complementary base pairing to the template (Budowle et al, 2000, pp. 191).

After amplification, capillary electrophoresis can be used to quantify the mtDNA. By using capillary electrophoresis, the purified PCR products can be checked to see if any non-specific PCR products were created or if the PCR primers were not removed. The amplified products are then diluted with deionized water that acts as has a reference standard. This is injected hydrodynamically and is detected using a laser induced fluorescence (Budowle et al, 2000, pp. 193).

The presence of mitchondrial DNA is a large concern during nuclear DNA sequencing because mtDNA typing is much more sensitive than nuclear DNA typing. When typing nuclear DNA, negative controls are employed to track the mtDNA contamination and to ensure that it is less than 10% of the quantity of DNA observed. It has been determined that contamination levels less than 10% will not effect the results of the nuclear DNA sequencing. The sequencing involves the same quality control procedures as other forms of typing but there are additional controls because of the

sensitivity. The contamination level is monitored with reagent blanks and negative controls. If the contamination level goes above 10% then the procedure is started over. Bone and teeth extractions are done under an air filtration hood that is supplied with a HEPA filter which is changed every three months. Reusable items are autoclaved or exposed to UV light (Budowle et al, 2000, pp. 194).

RFLP

Restriction fragment length polymorphism (RFLP) is another DNA analysis technique that is well suited to forensic science. RFLP is most commonly used to differentiate between two samples from different sources by determining differences in the length of a known DNA fragment. RFLP produces DNA fragments of varying lengths by restriction endonucleolytic digestion. Because two people will have sequence length differences, at certain sites, restriction fragments of different lengths can be used to compare two people's DNA. Human genomic DNA allows for RFLP typing because it has certain regions that do not encode proteins and are highly variable (polymorphic). Variable tandem repeats (VNTRs) or minisatellite genetic markers are areas that are made up of tandemly repeated sequences of approximately 9 to 80 bases per repeat per unit. Among people, VNTRs show differences in the number of repeats for specific alleles. Most VNTRs that are used for DNA fingerprinting have over 100 types in a population, which means that the regions are very polymorphic (Budowle et al, 2000, pp. 59). Many labs today use five to eight different allele markers to differentiate between most unrelated individuals.

The RFLP technique is performed exactly as previously described for a Southern blot. First, both samples of DNA are digested with restriction enzymes, which will yield different DNA fragment sizes and numbers because each person has slight differences the their base sequences (Restriction Enzyme Digest Analysis, 2002). Then, the DNA samples are put in an agar gel and separated by size using electrophoresis. The DNA fragments are then denatured and fixed, in their single-stranded form, onto a nylon sheet by blotting. Then, the sheet is Southern blotted by washing with radioactively labeled DNA probes so that it hybridizes to its complementary sequence. When an X-ray film is exposed to the nylon sheet, one dark band will be seen at each spot where the probe hybridized with its complementary sequences. By looking at the positions of these bands, the two DNA samples can be analyzed to see if they are from the same person, related people, or different people.

However, one of the disadvantages of RFLP is that it requires a larger sample of DNA than PCR techniques, and the samples cannot be old or degraded in order to obtain an accurate comparison (Inman, 1997, pp. 37). RFLP can usually identify DNA pieces between 0.6 kilobases to 20 kilobases. However in degraded sample of DNA, the higher mw fragments might migrate to only around 2 to 4 kilobases. If that sample were analyzed using RFLP then it could result in a single banded pattern that would not necessarily reflect the individual that it came from. For degraded DNA samples, a yield gel should first be run to fully analyze the approximate fragment sizes so that decisions can be made about the type of analysis that is needed (Inman, 1997, pp. 103).

AMP-FLP

Amplified fragment length polymorphism (AMP-FLP) typing uses a combination of PCR and the discrimination of VNTRs to make the DNA fingerprint. In this procedure, the DNA is extracted and then amplified using specific PCR primers (Budowle, 2000, pp. 119). The part of the process that differs from PCR is the typing of alleles at a locus. This technique often uses the D1S80 locus whose variation is defined by the number of tandem repeats or VNTRs (Inman, 1997, pp. 47). Many different loci can be used for AMP-FLP typing, but the DS180 locus is one of the better loci because it can be amplified with PCR and it is one of the more well-defined VNTR loci (Budowle et al, 2000, pp. 122). This involves DS180 analysis VNTRs as the polymorphism is based on size (Budowle, 2000, pp. 119). The D1S80 locus has 16 bp repeat units with a first repeat of 14 bp. The locus creates DNA fragments that are much smaller than RFLP can analyze. The only disadvantage is that because it analyzes only one locus it is not as highly discriminating as RFLP (Inman, 1997, pp. 47).

PCR amplifies the genomic locus and then the sample is run through a polyacrylamide gel electrophoresis where the alleles can simply be detected using a general stain rather than film for radioactivity. Polyacrylamide gels are used instead of agarose because agarose gels do not provide enough resolution for typing AMP-FLPs. Polyacrylamide has many advantages including that it is inert and will not interact with DNA molecules, it has no charge, the pore size of the gel can be manipulated, and it can be cast very thin. The gel can be stained with silver, which has a higher degree of detection sensitivity when compared with ethidium bromide, a stain that is often used. The silver staining includes oxidation, silver incubation, reduction and a stop wash,

which is not very time consuming. To analyze samples using the DS180 locus, allelic ladders (which contain from 14 to 41 repeats) are put next to the DNA samples in question, and after they have been run the bands can be visually compared between the ladder and the samples. Unlike RFLP, the regions analyzed by the D1S80 locus and amplified by PCR do not have to be purified before the DNA is analyzed because they have already been purified (Inman, 1997, 47).

STR Loci

Short tandem repeat (STR) loci are a subclass of VNTR loci and are composed of repeat sequences that are 2 to 7 base pairs (Budowle et al, 2000, pp. 136). STRs are similar to the D1S80 system except that the repeats are shorter, which makes them useful for analyzing degraded DNA that is too small for RFLP analysis (Inman, 1997, pp. 48). STR loci are very small but they can easily be amplified with PCR for fragments up to 500 base pairs in length. STR typing can be done fast and with a high degree of exactness. PCR multiplexing is a relatively new technique that allows for the typing of 8 or 9 STR loci at the same time. This has many advantages over typing the loci one at a time including using fewer reagents, which saves money, and there is less of a chance of contamination because it is just one procedure instead of many.

DNA databases have helped STR typing become widely used as STR loci are the most descriptive PCR based genetic markers available (Budowle et al, 136). To implement a national DNA databank to help find people who commit violent crimes, each laboratory must use the same loci. Since STR loci are the most informative, databanks use approximately thirteen STR loci in many different testing situations. The

CODIS STR system is very useful because STRs can be rapidly determined using commercial kits, and the alleles act according to known principles of population genetics. The data that is input to the CODIS databank is digital, and easily analyzed via computer databases. The CODIS system is being added worldwide as more laboratories add information about the STR allele frequencies in different populations (What are the 13 core CODIS loci, 2000).

13 CODIS STR Core Loci Characteristics

STR Name	**Chromosome Location**	**Gene Association**	**Repeat Sequence Motif**	**9947A type**
CSF1PO	5q33.3-34	CSF-1 receptor	AGAT	10,12
FGA	4q28	Human alpha fibrinogen	$(TTTC)_3$ TTTT TTCT $(CTTT)_n$ CTCC $(TTCC)_2$	23,24
TH01	11p15.5	Tyrosine hydroxylase	$(AATG)_n$	8,9.3
TP0X	2p23-2pter	Thyroid hydroxylase	$(AATG)_n$	8,8
vWA	12p12-pter	Von Willebrand antigen	TCTA $(TCTG)_{3-4}$ $(TCTA)_n$	17,18
D3S1358	3p	Anonymous	TCTA $(TCTG)_{1-3}$ $(TCTA)_n$	14,14
D5S818	5q21-q31	Anonymous	$(AGAT)_n$	11,11
D7S820	7q	Anonymous	$(GATA)_n$	10,11
D821179	8	Anonymous	$(TCTR)_n$	13,13
D13S317	13q22-q31	Anonymous	$(GATA)_n$	11,11
D16S539	16q24-qter	Anonymous	$(AGAT)_n$	11,12
D18S51	18q21.3	Anonymous	$(AGAA)_n$	15,19
D2S11	21q11.2-q21	Anonymous	$(TCTA)_n$ $(TCTG)_n$ $[(TCTA)_3$ $TA(TCTA)_3$ $TCA(TCTA)_2$ TCCA TA] $(TCTA)_n$	30,30

(Budowle et al, 2000, pp. 136)

To type the amplified STRs, they are first separated into fragments using denaturing gel electrophoresis that separates the amplicons into single-stranded molecules. Then, the amplicons can be stained with silver, or the primers labeled with a fluorescent tag that combines with the amplicons during PCR. Then the sample can be scanned for the amplicons using a fluorescent scanner that can save an electrophoretic profile of the gel. The DNA samples can then be run though a PCR multiplex using several of the loci listed in the previous table and the results can be compared against the ladders (Budowle et al, 2000, pp. 136).

STRs have many advantages over other DNA typing techniques, especially RFLP. STR requires only 1 ng of DNA that can be partially degraded, whereas RFLP needs 25 ng of DNA that is in relatively good shape. DNA separation using STRs and capillary electrophoresis occurs in a short amount of time (Progression of DNA Typing Markers, 2001).

STRs and Capillary Electrophoresis

Capillary electrophoresis is an alternative to gel electrophoresis for STR typing. Capillary electrophoresis is a chromatography technique that has higher resolution than electrophoresis. Fluorescent labeling can be used to look at 3 or 4 loci simultaneously and the machinery involved in capillary electrophoresis can automate the result. This allows a faster identification procedure than many other typing procedures (Harris, 1997).

Capillary electrophoresis involves putting a fused silica capillary tube between two buffer reservoirs that each has an electrode. The electrodes are connected to a power

supply, and a detection window is located in the capillary to monitor DNA migration. First, the capillary is prepared and loaded into a medium and surrounded by buffer. Then, the DNA sample is injected, either by using hydrodynamic injection or electrokinetic injection. Hydrodynamic injection involves creating a pressure difference over the capillary whereas electrokinetic injection uses electrophoresis and electroendosmosis to insert the sample. However, hydrodynamic injections usually cause band broadening so electrokinetic injections are often used. After injection, the sample is separated and monitored using UV absorption (Budowle et al, 2000, pp. 159). In order to allow for UV detection, after electrophoresis fluorescent tags are covalently bonded to the 5' end of the primers on the single-stranded DNA molecule (Budowle et al, 2000, pp. 1, 158). UV absorbance is not easily detected in this system so fluorescence can be added during the PCR stage. DNA typing systems or kits, that utilize many of the above-mentioned loci, are then used to characterize the DNA that has been analyzed in the specimen. Many kits use multicolor dyes to allow for the examination of overlaying ranges which are looked at with a charge-coupled device camera that allows the signals to be seen as peaks in electropherogram. The electropherogram is then compared to allelic ladders for the STR's and the loci used. In capillary electrophoresis, as well as in other DNA typing techniques, the amelogenin locus is also used in allelic ladders because the gene is used in determination of sex from a DNA sample (Budowle et al, 2000, pp. 159).

Chapter 2: DNA FORENSICS

DNA forensics is a relatively new and powerful addition to the world of forensic science. The first aspect of DNA forensics is the proper collection of samples from crime scenes and from suspected criminals. These tasks need to be done correctly to prevent contamination. Next, the DNA samples need to be properly transported to testing labs. Analysts can then extract DNA fragments from the samples by using specific extraction techniques. When properly collected and handled, DNA can be better than any eye witness. It's not as easy to erase like a fingerprint, most of the time a suspect does not even know they have left DNA behind.

DNA Collection

The first step to using DNA in forensic analysis is properly collecting DNA samples. If the samples are not properly collected then they may be inadmissible in court, or the tests may not work as they are supposed to because biological activity can be lost. The crime scene investigation unit is responsible for properly collecting evidence which they believe will have DNA related to someone involved in the incident.

One aspect of correctly collecting DNA specimens is avoiding contamination. There are two types of contamination when dealing with DNA forensics, mixed sample, and contaminated sample. When a DNA sample is a mixed sample, it has two or more donors' DNA (whether it is human or animal) some of which may be pertinent to the incident being investigated. A contaminated sample is one which has been tainted with someone else's DNA after the incident. This can occur during an improper collection,

during transportation, or in the lab with DNA from another specimen (Inman, 1997, pp 12). To avoid contamination, the crime scene investigation unit has to follow many rules including wearing gloves at all times and changing them when handling different specimens. Also, investigators should use clean instruments to handle anything at the scene, and areas that may contain DNA should not be touched. Talking, sneezing or coughing near areas that contain DNA could also lead to contamination (Every Law Enforcement Officer Should Know, 1999).

Identifying good sources of DNA for analysis is also important. Blood, semen, saliva, urine, hair, tissues, bones, and teeth all contain DNA in nucleated cells (Handbook of Forensic Services, 1999). Urine can contain DNA if it has epithelial cells from the kidney and the urinary tract. Saliva contains DNA because it has mouth epithelial cells (Krawzcek and Schmidtke, 1998, pp. 17). Chewing gum, cigarette butts, and envelopes or stamps are good places to find saliva samples. UV Light can be used to identify semen stains when no clear evidence containing DNA is visible (Ultraviolet Light Use, 2000). It is also important to first perform tests on the substance to establish the type of biological material. This prevents time consuming DNA tests from being run on stains, such as food stains, that may not be important to the investigation. These tests can be done at the crime scene or in the laboratory (Inman, 1997, pp 14).

To properly collect samples, members of the crime scene investigation unit must follow a specific protocol. Failure to do so could cause the DNA results to be inadmissible in court. If the item containing the stain is small enough it is best to leave the stain on the item to minimize the handling of the stain. If stains are on larger objects such as couches, the investigation unit should cut away the fabric containing the stain

(Evidence Collection in Forensic Biology, 2002). If the stain is not on a moveable object then the stain must be transferred to cotton swabs. If the stain is dry then a cotton swab should be moistened with distilled water. An area of the cotton swab must be left unstained to be used as a control and also an extra cotton swab that has only been moistened with the distilled water will be used as another control in the collection process.

DNA has many different properties that are important to consider when doing a DNA test. When DNA degrades it is broken down into smaller fragments. Certain tests require DNA to have a certain fragment size; therefore, when DNA degrades below this DNA size it cannot be typed. When this happens the test is simply inconclusive (Inman, 1997, pp 11). Factors that cause DNA degradation include, time, temperature, humidity, light, and chemical or biological contamination (Inman, 1997, pp 11).

Collecting Known Samples

After samples have been collected, investigators need DNA from whomever they are trying to match to the samples found at the crime scene. These samples are called known samples. Blood, saliva, or DNA databases may be used as the source of DNA from the person being tested.

Approximately two 5-mL Blood samples should be collected from a person to test against a sample. A code, such as having purple tops on the collection tubes, may be employed to ensure proper identification as blood samples. EDTA should be added to prevent the blood from clotting. Each tube needs to be properly labeled with the subject's name, date, time, location, collector's name, case number, and evidence number or its

origin will be questioned. Blood should be refrigerated, not frozen (Handbook of Forensic Services, 1999).

When collecting saliva, clean cotton swabs should be used and rubbed against the inside surfaces of the cheeks and gums. DNA is actually located in the epithelial cells which originate from the cheeks and gums. Next, the swabs should be air dried and stored in clean paper, not plastic. Each sample should be correctly identified with the same information used to identify blood samples. Saliva does not need to be refrigerated but should still be submitted as soon as possible (Handbook of Forensic Services, 1999). Collection is very important because if the evidence is not properly collected, biological activity can be lost. If the evidence is not correctly packaged, contamination can occur. If the DNA evidence is not properly preserved, decomposition and deterioration can occur.

Transporting & Storing DNA

After the DNA sample is properly collected, it needs to be transported to a laboratory for analysis. To properly preserve the DNA, the sample should be air dried before transport to remove the excess moisture. This prevents further degradation caused by the moisture. Evidence should be packaged in clean paper or envelopes instead of plastic containers (Evidence Collection in Forensic Biology, 2002). Paper packaging will allow moisture to escape. Next, the package should be sealed so that it will be obvious if someone tampered with the sample. The package also needs to be clearly labeled so that it follows the correct chain of custody (Every Law Enforcement Officer Should Know, 1999). If the DNA is not clearly labeled and this is brought to the attention of the court, the origin may be questioned, causing the evidence to be inadmissible. Properly handling

the DNA during transportation also does not leave much room for tampering or contamination.

DNA Extraction Techniques

The first step to analyzing the DNA is isolating the DNA strands from the rest of the sample. Methods to accomplish this include Chelex extraction, organic extraction, and differential extraction. The extraction method used is chosen based on the type of evidence, amount of evidence and the kind of cells in the evidence.

Chelex extraction is used when there are trace amounts of DNA present. During this method the DNA double helix is split apart, eliminating many types of analysis. First, the sample is boiled with Chelex beads. The boiling breaks up the cells and releases the DNA. The Chelex beads bind to most non-DNA materials in the solution. The beads are removed with the non-DNA materials connected to them, leaving the DNA for analysis. PCR analysis may still be used after this technique as it can be done with either single-stranded or double-stranded DNA (Inman, 1997, pp 62).

Organic Extraction is more likely to keep the DNA in large pieces and maintains the double-stranded property. There are several variations of this procedure with the presence of sperm in the sample being the deciding factor on which is to be used. First, the cells are removed from whatever they were deposited on using a warm solution. Next, the cells are broken open using heat and a chemical solution. DNA is released and then isolated using organic solvents. To purify the DNA even more filters can be used. This resulting DNA can be used in any type of analysis because it still maintains its double-stranded property (Inman, 1997, pp 62).

Differential Extraction is used when donor sperm cells are mixed with someone else's epithelial cells. Epithelial cells can be found in saliva, skin, buccal, and vaginal cells. There are certain key differences between epithelial cells and sperm cells that allow their separation before isolating DNA. This can be useful for separating DNA evidence from a mixed sample. First, the cells are removed from the cloth they were on. Then, the sample is incubated in a set of chemicals. These chemicals break open the epithelial cells. The liquid that now contains the epithelial cells is called the epithelial cell fraction. This is separated from the rest of the sample and then extra chemicals are added to split open the sperm cells and remove the DNA. When both cell samples have been split, organic extraction is applied to both separately (Inman, 1997, pp 62).

The next step before performing an analysis on the DNA is to determine the quality and quantity of the DNA. Much of the DNA may be degraded. The DNA that is still intact and has a relatively long fragment size is considered to have a high molecular weight (HMW). Tests like RFLP depend on each trial using the same amount of HMW DNA for the results to be useful. This is called balancing the samples. This information is also needed to determine how much restriction enzyme is going to be used for certain tests. Too much DNA in each sample can be as bad as too little, so this step is necessary even when plenty of evidence was discovered.

Methods such as slot blot, yield gel, Southern blot and capillary electrophoresis can all be used for quanitation. A slot blot is used when an analyst thinks that there is very little DNA or it is degraded. This method of determining quantity and quality is the only method usable after a Chelex extraction. A Yield Gel can only be used with double-stranded DNA and needs higher quantity than the slot blot. When plenty of double

stranded DNA is present, using a yield gel and a slot blot produces more information, such as the percentage of the sample originating from humans. A southern blot can also be used in conjunction with a yield gel to asses the proportion of human DNA. If very little DNA is present then the analyst may just go forward with PCR testing.

Quality Assurance

Laboratories that are currently used for DNA typing adhere to a strict set of guidelines. The procedures and protocol that the labs use are based on standards set by the DNA Advisory Board, which works closely with the FBI Director (Budowle et al, 2000, pp. 160). Quality assurance includes quality control, laboratory audits by external agencies, maintaining clear protocol, preparing special reports on quality assurance, troubleshooting, maintaining and calibrating equipment, developing methodology, training personnel, continuing education of personnel, and preserving laboratory safety (Kirby, 1990, pp. 179). Each facility must have the same set of definitions for standards, a quality assurance program, organization and management, trained personnel, adequate facilities, and similar evidence control. Definition of standards includes determining set definitions of each key term in DNA forensics. For example, the set definition of analytical procedure is "an orderly step by step procedure designed to ensure operational uniformity and to minimize analytical drift (Budowle et al, 2000, pp.229)." Each lab should also have a quality assurance program that ensures that every aspect of the facility including evidence control and personnel training are looked at. The labs must have people fulfilling certain job titles including a technical manager and each person's responsibilities must be specified. There should be job descriptions, and the level of

education each job entails should satisfy these requirements before they work there. Each laboratory should be designed to have good security and to minimize contamination by having separate rooms for certain steps in the DNA typing procedure. Evidence control is another necessary standard for each laboratory and this includes documenting where the evidence goes to ensure chain of custody and to prevent contamination. These are just a few of the standards that are maintained at DNA forensics laboratories.

Quality Control

Quality control is also done at the laboratory level while the DNA samples are being processed to ensure the accuracy of laboratory results. When dealing with DNA fingerprinting, quality control also involves using the correct population allele frequencies in calculations (Kirby, 1990, pp. 179). Each laboratory that analyzes DNA must guarantee that the correct specimen is evaluated, the DNA is not degraded, contamination by other DNA is not enough to skew results, the procedure is controlled, the analysis of results is exact, and that there are no variations from standard laboratory protocols (Kirby, 1990, pp. 179). Quality control is used to ensure that errors in the system, including false negatives and false positives results, are not released from a laboratory. False negatives occur when two specimens' profiles do not match but they come from the same source. On the other hand, false positives occur when two specimens' match even though they originated from different sources (Kirby, 1990, pp. 180). Blanks are used often in laboratory procedures as a control. When a sample is processed a reagent blank is processed with it. This blank contains all of the reagents used in the experiment and it goes through the same procedures as the DNA sample.

Negative controls are also used during the amplification procedure. It contains all the reagents used in the amplification but there is no DNA sample added. Instead purified water is added and carried through the amplification and electrophoresis steps (Budowle et al, 2000, pp. 160). If these negative controls produce a positive signal then the experiment or machines should be looked at for contamination.

Positive controls are used for the amplification procedure by using a certain type of DNA, cell line DNA 9947A. It is put through the amplification procedure and when it is amplified the loci should show the right genotype. If it does not then it means there is contamination. DNA 9947A is also a female control for amelogenin and should show a band at 103 base pairs. Male controls can be used and they exhibit bands at 103 and 109 bases (Budowle et al, 2000, pp.160).

If contamination is not detected throughout the experiment, the results can show whether or not the sample was contaminated. In a locus-specific multiallele system, there should be no more than 2 bands in the resulting gel. If that occurs then contamination is most likely the reason, and the procedure should be repeated to find out where the contamination occurs. Contamination with microorganism DNA could be a problem in DNA typing. If cross reactivity occurred between the microorganism and the DNA then there would be an unusual banding pattern, which could be detected by hybridizing the assumed contaminants with the probe used. Then, the test membrane with the contaminants can be put in the hybridization container with other blots (Kirby, 1990, pp. 182). If the same banding pattern occurs then the suspected microorganism is the contaminant.

The equipment used in the DNA typing procedures should also be checked. The Thermal Cycler System 9700 is one of the most important pieces of equipment used in DNA amplification, so it should be checked regularly and kept up to standard. The sample wells and block cover should be cleaned quarterly. A temperature verification test should be run quarterly and the thermal cycler should be sent back to the manufacturer once a year for recalibration. The uniformity of the temperature in samples should be checked as should the cooling and heating system. Pipets and other such equipment should be exposed to UV light and cleaned with isopropanol or discarded. Also, the reagents used in the experiment should be checked before each procedure by comparing them to a positive result (Budowle et al, 2000, pp. 163).

Common tests such as RFLP and PCR use enzymes and therefore may be inhibited by additional agents that may interfere with the enzymatic function. Substances such as dyes (found in clothing), and some biological substances can have an effect on the reaction. These can be detected in the sample using tests such as product gel, and digest gel. When inhibitors are detected special beads or BSA (bovine serum albumin) protein can be used. Special beads can be used to bind dyes so that they can be physically removed. BSA proteins bind and disable inhibitors even during the reaction (Inman, 1997, pp 103).

In order to show a chain of custody, every specimen that passes through a laboratory dealing with DNA typing must have its own unique code. It is especially important in typing cases that confirmation of the sample origin is observed and recorded. Also, if the DNA quality of a sample is degraded it will be checked to see if there are

intact areas of the sample that need to be replicated present. If these regions are not detected then the sample will not be typed further (Kirby, 1990, pp. 182).

Chapter 3: LANDMARK COURT CASES

Frye v. United States of America (1923)

Frye is perhaps the most cited case for those court cases dealing with new scientific techniques, including DNA fingerprinting. The 1923 appeal set a standard for the admissibility of evidence acquired through new techniques. Frye had been convicted of murder in the second degree. The defense offered lie detector results and an expert witness as evidence that Frye was telling the truth in claiming his innocence. The defense even requested to conduct the test in the presence of the jury. The evidence was deemed inadmissible and their request was also denied.

Frye's defense acknowledged that no previous cases directly deal with this issue, but argued that the opinions of expert witnesses are admissible as evidence. In this case, those opinions could serve as a basis for analyzing the results of the test. However, the court found that a technique must first meet a general acceptance test before it may be admitted in a court of law. They found that it is difficult to determine when a technique is in the stages of first being discovered and experimented with and when it has demonstrated itself as a sound technique. The court determined that the only basis for this decision is whether the technique had gained general acceptance from the scientific community in which it belongs. The court further found that lie detector tests had not yet gained acceptance from the physiological and psychological communities and affirmed the original trial court's guilty decision.

United States of America v. John W. Downing (1985)

John Downing was convicted of mail fraud, wire fraud and interstate transportation of stolen property based solely on eyewitness testimony. The defense was not allowed to admit expert testimony concerning possible problems involved with eyewitness accounts. This district court had found that the testimony did not meet Federal Rule of Evidence 702 in that such testimony was not "helpful." However in 1985, the appeal court found that this is not always true, and that when applying Rule 702 two issues must be considered.

1. The potential helpfulness of the expert testimony versus the possibility that it may mislead the jury
2. The supporting scientific evidence behind the expert testimony

The court found that in cases such as this that depend solely on eyewitness accounts, the jury should be made fully aware of its possible problems. In some circumstances such testimony meets the "helpfulness" required under Rule 702. The court proceeded to explore ways for the district court to decide whether the specific testimony in this case should be admitted. The Frye test was criticized by the court and an alternative test proposed.

Rule 702 is found to reflect the same general acceptance criteria as in Frye because at the time the Federal Rules of Evidence adopted Frye as a dominant view. They did not believe that either standard is an appropriate standard to determine the admissibility of a new scientific technique. They observed that Rule 403 defines relevant evidence as evidence having any tendency to make the existence of any fact that is of

consequence to the determination of the action more probable or less probable than it would be without the evidence" (Downing, 1985).

Ultimately the court found the Frye test for general acceptance in the appropriate scientific community too vague and conservative. In the Frye case the court found "the thing from which the deduction is made must be sufficiently established to have gained general acceptance in the particular field to which it belongs" (Frye, 1923). The court observed that this could easily lead to the exclusion of useful and accurate evidence. A technique is noted to take time to gain acceptance within the scientific community. If the underlying theory behind the technique is shown to be reliable, then a general acceptance of the technique is sure to follow. The court also found that by focusing on general acceptance, the Frye test may allow evidence derived from unreliable principles to be overlooked. General acceptance should not be a necessary condition nor should it be the only condition of admitting evidence based on new scientific techniques.

Relevancy was determined to be the main basis of admissibility. The suggested relevancy test focuses less on inflexible general acceptance issues, and more on relevancy and reliability of the evidence. The court recommended the means in which to determine whether a technique is reliable if it had not yet gained general acceptance. This included how the new technique compared to other more accepted methods, the existence of literature on the subject, expert witnesses, and the nonjudicial uses of the technique.

The possibility that the evidence may confuse or mislead the jury was also found to be an issue. For instance, the shortcomings of a particular technique may be "unlikely to be effectively communicated to the jury" and result in an unwarranted "aura of reliability" (Downing, 1985). The court recommended that the trial courts assess this

misleading evidence issue along with the issue of relevancy, possibly in a pre-trial hearing. It was observed that the trial court in this case did not consider either of these issues, and it was remanded to do so. If the new pre-trial hearing deemed that evidence admissible in a new trial was misleading the conviction could be overturned.

Tommie Lee Andrews v. State of Florida (1988)

In 1988 Tommie Lee Andrews was convicted of aggravated battery, sexual battery and armed burglary in a case in which DNA was used as evidence against him. The victim had been raped, and a sexual assault kit was used in order to obtain semen that could be used by the State of Florida for DNA analysis. The analysis showed that the DNA found in the kit matched that of Andrews. In this appeal, as at the trial, the defense argued that the DNA fingerprint analysis should have been inadmissible. The appeals court addressed the admissibility of DNA evidence at three levels: a new scientific technique, DNA evidence in general, and the specific DNA analysis performed in this case.

The court first considered what standard should be applied to determine the admissibility of the relatively new DNA fingerprinting technique. They first had to decide upon the standard they would use in their determination. The Frye test was suggested in Frye v. U.S. and had been adopted in numerous court cases. But it had recently "come in for criticism by a number of judges and commentators as being too inflexible " (Andrews v. State, 1988). The alternative was the relevancy test initially recommended in Brown v. State and strongly adopted in U.S. v. Downing.

The determination of the court was that the Frye approach may lead to reliable evidence being deemed inadmissible simply because it was a new technique and had not yet gained general acceptance in the scientific community. The court further suggested that the relevancy approach should be the one adopted by the state of Florida. Adhering to Downing's recommendations, the court proceeded to evaluate various factors in order to determine whether the DNA evidence against Andrews was reliable.

The State had several expert witnesses that testified on behalf of DNA fingerprinting. The court observed that studies on the technique had come from "a research laboratory whose scientists did not initially desire to apply the techniques to actual forensic investigations" (Andrews v. State, 1988). Other scientists had been allowed to follow the protocols and make their own decisions concerning the technique. All of the research that was presented supported claims of DNA reliability. The court discovered that research had been done in this area for decades and that, for this reason, the technique would have most likely passed the Frye test too. For these reasons the court found that DNA evidence was admissible. They turned next to the specific testing done in the case.

Lifecodes, Inc was the laboratory that performed the DNA analysis. The company had been founded in 1982, and at the time provided paternity and forensic testing in addition to testing for genetic diseases. Lifecodes explained and defended the techniques that they implemented, and the techniques and results were reviewed by an independent scientist. The scientist agreed that the tests were performed correctly and were indeed accurate. They argued that the defense's contention against the reliability of the test was unfounded in part because controls were used in the testing process and also

because problems in the test usually lead to no result rather than an inaccurate one. The defense did not provide any expert witnesses to testify otherwise.

In applying the relevancy test, the court made the following observations in order to reach their decision. DNA testing is widely used in areas outside the realm of forensic science like disease diagnosis and treatment. It has been exploited for over a decade and evidence shows that it is highly reliable. The use of the technique in other areas also demonstrated its consistency. A large amount of literature exists on the subject. Its error rate is small, and if an error does occur there will often be no result rather than a wrong result. The frequency that given DNA bands occur in the population is based on sound practice using the Hardy-Weinberg equilibrium. The court found that the DNA fingerprinting evidence passed the criteria of the relevancy test, and upheld the initial trial court's decision and Andrews' conviction.

The People of the State of New York v. Joseph Castro (1989)

The court case of the People of the State of New York v. Joseph Castro in 1989 involved a pre-trial hearing referred to as "the most comprehensive and extensive legal examination of DNA forensic identification tests held to date in the United States" (People v. Castro, 1989). Castro's defense attorney said it was "unprecedented in the annals of the law" (Lewin, June 1989) and that it "put DNA typing as a whole on trial" (Lewin, July 1989). A pre-trial hearing was held to determine whether DNA tests presented by the People were admissible. The hearing took place over twelve weeks and yielded a five thousand-page transcript.

Castro was accused of murdering a pregnant woman and her daughter. Blood was found on his wristwatch and he claimed it was his own. The People wanted to introduce DNA evidence to show that it was actually blood from the victim. The hearing was ordered to test whether the evidence was admissible under the Frye standard (Frye v. U.S., 1923). Records showed that DNA evidence had been introduced in other cases and had never failed the Frye test. It was determined that the test alone could not fully determine the admissibility of the evidence. A three-prong test was introduced to deal with the main issues (People v. Castro, 1989):

Prong I. Is there a [generally accepted] theory in the scientific community, which supports the conclusion that DNA forensic testing can produce reliable results?

Prong II. Are there techniques or experiments that currently exist that are capable of producing reliable results in DNA identification and which are generally accepted in the scientific community?

Prong III. Did the testing laboratory perform the accepted scientific techniques in analyzing the forensic samples in this particular case?

The first two prongs tested the admissibility under Frye, but the third focused on the use of a particular technique in a particular case. The court determined that more attention should be given to prong three because DNA evidence had already been found to meet the Frye standard. Still, the Frye test was applied and the court found clear evidence that there was general acceptance of the theory involved in DNA identification.

It also established that there were dependable, existing techniques to determine whether two DNA samples match.

Then the hearing analyzed the techniques utilized by Lifecodes Corp., the laboratory that performed the DNA identification tests for the People. The hearing consisted of several experts on DNA fingerprinting who reviewed Lifecodes' procedures and the results from which Lifecodes made their conclusion that the blood on Castro's wristwatch was that of the victim. The experts found that the techniques used by the laboratory in the first three steps of DNA testing were acceptable. The three steps were cocktail hybridization of D2S44 and D17S79, DYZ hybridization, and autoradiographs 11 and 12 and DXYS14 hybridization and autoradiograph 5.

Lifecodes conclusion that the DNA matched was based on the results of the third step. However, the results showed that there were two extra bands on the sample from the watch. Often the third step is repeated to see if the bands reappear and further tests are taken to determine whether the bands are actually contaminants. The experts agreed that Lifecodes should have performed further tests before making any determination. Therefore, the People's evidence that the blood found on Castro's watch was that of the victim was deemed inadmissible. Castro's own DNA was also compared to that found in the blood on the watch because he had claimed that the blood was actually his own. Determining whether two DNA samples do not match is a much simpler process than proving that they do and Lifecodes' results did exclude Castro as the source of the blood. This evidence was deemed admissible and the prosecution showed that the blood was from someone other than Castro himself.

In addition to the conclusions on the admissibility of the evidence specific to this case, suggestions were made for future DNA hearings. The court recommended three procedures (People v. Castro, 1989):

1. Notice of intent to admit DNA evidence made as soon as possible.
2. The proponent must give adversary thorough documentation of the DNA tests and other relevant information.
3. The proponent has burden of proving that tests and such were conducted properly. The adversary has burden to prove why the evidence should be suppressed or modified.

At the time of this case there had already been over one hundred cases in the U.S. in which DNA evidence was admitted (Lewin, June 1989). Many lawyers did not know how to respond to it because it was usually admitted without objection. Castro's defense lawyer was troubled by Lifecodes' findings and requested the pre-trial hearing. There were no standards for DNA fingerprinting set forth by the scientific community, so the techniques had to be thoroughly investigated and analyzed. Eric Lander, a famous MIT population geneticist, was involved in the hearing and said that Lifecodes was not at fault as there was no guideline to follow. Others claim that a match should not have been declared in this case under any circumstances. However, Lifecodes stood by their findings. It was the hope of all those involved in the hearing that the case would spark a movement to establish a standard. As the trial came to an end, the FBI had already begun to institute its own standards. The ultimate consensus was that DNA fingerprinting was a powerful technique and could produce very reliable results if performed properly, but that is was not in this particular case.

United States of America v. Matthew Sylvester Two Bulls (1990)

DNA evidence was utilized by the prosecution in a case against Matthew Sylvester Two Bulls, in which he was convicted of aggravated sexual abuse and sexual abuse of a minor. The defense had requested that the evidence be suppressed, but in a pre-trial hearing the judge determined it admissible. However the judge made his decision based solely on the testimony of one government witness. The testing procedures employed by the FBI that analyzed the DNA were not considered. The judge based his decision on the general acceptance of the technique. The appellate court found that this was an error and that the FBI's procedures must be taken into account. Another trial was ordered in which there would first be a pre-trial hearing to determine the admissibility of the DNA fingerprint evidence.

The court had to decide what issues must be dealt with at the district court's pre-trial hearing. During the appeal the court explored DNA testing in general and it's admissibility under the Frye test, three-prong test and also Federal Rule of Evidence 702. Rule 702 states that "if scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or 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" (Two Bulls, 1990). The district court had allowed the expert witness to testify under Rule 702, and then deemed the evidence admissible under the Frye test because the witness showed that it was a generally accepted procedure. Two Bulls argued that the three-prong test recommended in People v. Castro should be applied.

Two main problems were found in the introduction of a new scientific technique. There may be too much caution in accepting it, which may prevent reliable evidence from being admitted. It may also be accepted as reliable without any question and later proven to be otherwise. The complexity a technique may conceal any problems in it. However it was found that the theory behind DNA tests is not controversial and was well accepted. General acceptance cannot be the sole basis of the decisions to allow DNA evidence into court.

The government argued that Castro's test is too strict, and that the Frye rule or Rule 702 should be applied. The court found that although each of these rules required general acceptance, "neither rule should permit speculative and conjectural testing which fails normal foundational requirements" (Two Bulls, 1990). Castro's recommendation of a pre-trail hearing shifts the focus from the admissibility to the weight of the evidence, which is commonly dealt with at the trial. The court found problems in allowing DNA evidence to be introduced at trial even if it is later suppressed. There is a high risk of prejudicing the jury against the defendant. The court believed that the weight of the evidence must also be determined beforehand.

The court agreed with another courts' statement "that once the trial court decided the evidence was reliable then it had to determine whether the probative value of the evidence outweighed its prejudicial effect" (Two Bulls, 1990). The court suggested three rulings that a judge should make in cases such as these:

1. Whether the DNA evidence is scientifically acceptable
2. Whether there are certain procedures that should be followed in conducting these tests

3. Whether these standards were followed in this case

This recommendation is essentially the same as Castro's three-prong test. In this particular case the court ordered the trial court to make five decisions in the pre-trial hearing:

1. Whether DNA evidence is generally accepted
2. Whether the testing procedures that were used in this case are generally accepted
3. Whether the test was performed properly in this case
4. Whether the evidence in the case is more prejudicial than probative
5. Whether the statistics used to determine the probability of a match is more probative than prejudicial

The People of the State of Illinois v. Reggie E. Miles (1991)

Reggie Miles was convicted of various counts including sexual assault and armed robbery. DNA evidence showed that the semen found on the bed sheet of the victim matched that of Miles. However there were several other incriminating pieces of evidence also presented in the case, including fingerprints found at the scene of the crime. The defense argued in this appeal that the DNA testing procedures employed by Cellmark Diagnostics were not proven reliable. They also argued that the testimony declaring the statistical probability of a random DNA match in this case was 1 in 300,000 should have been suppressed.

In 1988, immediately after the crime had been committed a stained bed sheet was collected from the scene and held as evidence. At that time only a blood test was run on

the semen stains. At the defendant's bail hearing in 1989 he requested a DNA test and volunteered hair and blood samples. The State admitted the results of this test as evidence and also introduced expert witnesses. The experts thoroughly explained DNA and the process of DNA testing. Each testified that the scientific community generally accepted DNA fingerprinting, that Cellmark had performed the tests properly and that the results were reliable. One of the experts commented that bacteria might erode samples kept at room temperature. The sheet had been stored in an evidence locker for almost two years, but the lab was able to obtain enough DNA for the test. The jury had been aware of each of these facts. Expert witnesses on DNA fingerprinting did not testify for the defense.

The defendant's appeal that the result of the DNA test was not shown to be reliable the court made two observations. First, that the defendant had originally requested the test. Second, that in addition to the DNA evidence the defendant's fingerprints were also found, including at the window used for breaking into the house. The court noted the statement it made in People v. Lipscomb in 1991: "Any question concerning the specific procedures used by the company or expert goes to the reliability of the evidence and is properly considered by the jury in determining what weight to give this evidence. If it is shown that the procedures used give an unreliable result, then the court may find it necessary to exclude this evidence entirely" (Miles, 1991).

After the Castro case the FBI had put together TWIGDAM, Technical Working Group—Interagency Working Group of DNA Methodology, and began follow their guidelines. Cellmark follows TWIGDAM's standards also. The defense pointed out that in the past Cellmark's results had been suppressed because they were so unreliable.

The court found that Cellmark had learned from their mistakes and that their adoption of TWIGDAM illustrated the soundness of their current procedures. The court also reviewed the procedures and found them to be sound. In addition, expert testimony was deemed admissible because DNA fingerprinting is not common knowledge of laymen.

In order to determine the probability of a random match, the defendants DNA was compared to Cellmark's database. The database is separated by ethnicity and only the appropriate part is used. Cellmark's African-American database consisted of 200-300 samples. A "product rule" was then applied to determine the probability. The defense argued that it multiplies "the frequency of occurrences of various bands at various loci to produce some large figure representing a small probability of a random match" (Miles, 1991) and may easily produce misleading information. The court found that because the probability statistics are based on DNA fingerprinting, and DNA fingerprinting is reliable, that the statistics derived from it are also reliable and admissible. It was found that the trial court acted appropriately in admitting the DNA evidence and upheld Miles' conviction.

William Daubert et al. v. Merrell Dow Pharmaceuticals, Inc (1993)

Petitioners, including William Daubert, sued Merrel Dow Pharmaceuticals alleging that the prenatal drug Bendectin that they market caused birth defects in their children. In the suit the respondent provided expert testimony that the drug has not been shown to cause birth defects. The petitioners had eight expert witnesses that testified otherwise. They testified that animal studies, chemical structure analysis and unpublished reanalysis show that Bendectin can cause birth effects. The respondent's

witnesses were admitted, but the district court found that the evidence given by the respondents did not pass the Frye test because it was not generally accepted. The court of appeals agreed and affirmed the district court's decision and the case continued to the Supreme Court.

In 1993 the Supreme Court made a decision concerning the standards by which the admissibility of this evidence should be determined and also commented on the admissibility of expert testimony in general. Much of the case focused on their interpretation of Federal Evidence Rule 702. In U.S. v Downing the court interpreted Rule 702 to be very similar to the Frye test in that it relied heavily on the general acceptance of a technique. As a result, they recommended and applied the relevancy test. Quite differently, the Supreme Court's interpretation of Rule 702 does not require general acceptance in any way. Their interpretation of Rule 702 is actually extremely similar to the relevancy test recommended in Downing.

The court first declared that the Federal Rules of Evidence superceded the Frye test and that its application is obsolete. They further observed that the Rules do not mention general acceptance or the Frye case in any way. Specifically, Rule 702 does not require scientific evidence to be generally accepted. Rule 702 was put into place in order to establish more lenient guidelines for expert testimony and the Frye test is much stricter. Rule 702 is interpreted as giving "the trial judge the task of ensuring that an expert's testimony both rests on a reliable foundation and is relevant to the task at hand" (Daubert, 1993). In Downing the court did not interpret the rule in this way and therefore established the relevancy test, which required the judge to complete the same task.

Their interpretation is based on the Rule's reference to "scientific knowledge" and to the requirement that the evidence must "assist the trier". They observed that "scientific" implies a grounding in science's methods and procedures, and "knowledge" connotes a body of known facts or ideas inferred from good grounds. The term "scientific knowledge" therefore implies reliability whereas the term "assist the trier" implies relevance.

The court named numerous factors that a judge may consider when making a decision regarding the admissibility of expert testimony. Although general acceptance is included as a factor, the court points out that it is certainly not required. The existence of general acceptance can only be used to enhance the notion of the technique's reliability.

1. Whether or not the technique can be subject to testing
2. Whether is had been reviewed by peers or in publication
3. The errors that may occur and the rate of such errors
4. General acceptance of the technique in the scientific community

Further, the court found that testimony that is deemed admissible could be appropriately challenged through the introduction of opposing evidence, cross-examination and requiring full burden of proof. The court threw out the appellate court decision and ordered the case to proceed in district court following the guidelines that the Supreme Court had set.

Chapter 4: SENSATIONAL DNA CASES

Anastasia/Anna Anderson

The Bolsheviks executed the last Czar of Russia and his family in 1918 during the Russian Revolution and hid their remains. Even though reports claimed the entire family had been killed, there were rumors that a son and daughter had lived through the ordeal. The fate of the Czar's youngest daughter, seventeen-year-old Anastasia, has been the subject of much controversy. Numerous women have claimed to be Anastasia, but one was more convincing than the others. In 1920 a woman with amnesia was hospitalized in a mental institution after jumping off a bridge into a canal in Berlin. The woman did not disclose her identity, so the hospital workers named her Anna Anderson. People were shocked when she finally disclosed her identity as Anastasia.

There were those that believed her, those that did not and those who were not sure. Some of Anastasia's friends and relatives believed that Anderson was Anastasia while others believed she was an imposter. In an attempt to provide proof, Anderson stated that she knew that the uncle of Anastasia, the Grand Deke Ernst of Hesse, had secretly visited Russia in 1916 while his country was at war with them. Only close relatives, such as Anastasia, would know such facts. Ernst declared that Anderson was an imposter and made a great attempt to discredit her. It had been suggested that Anderson was actually a Polish factory worker named Franziska Schanzkowska who had previously been institutionalized several times. Shortly before Anderson turned up in the institution Schanzkowska had disappeared. Ernst became a strong supporter of this theory. However, Anderson had many supporters that still believed she was Anastasia.

Anderson brought suit in a German court to prove that she was Anastasia and claim her inheritance. The case began in 1938 and did not end until 1970. Several experts testified on Anderson's behalf. The famous anthropologist Dr. Moritz Furtmayr studied the faces of each woman and testified that they were either the same person or twins. The handwriting of each was examined, and a handwriting expert testified that the same person wrote the samples. Anderson had a foot deformity like that of Anastasia. She also had scars that she claimed were from being shot during the attack on the royal family. Fingerprints of Anastasia could not be found for comparison. The court ruled that it had not been proved that Anderson was actually Anastasia. Her supporters note that the court did not rule that she was *not* Anastasia.

Anna Anderson died in 1984 and was cremated. In 1991 the burial site of the Czar and his family was revealed and the remains were exhumed. One of the daughter's remains was indeed found to be missing and presumed to be that of Anastasia as the rumors had suggested from the beginning. The bones were sources of the royal family's DNA. A DNA sample was also taken from a descendant of the family. The two samples were compared to ensure that the remains were indeed theirs. Because Anderson had been cremated, there were only three existing samples that could be used for testing. A pathology lab still had a sample of her intestine from a surgery she had in 1979. Her husband, John Manahan, had a lock of her hair. In addition, a glass slide was found in Germany that supposedly contained a drop of her blood. DNA tests were then performed on each of these samples and the remains. Two separate groups of scientists carried out DNA tests, one in England and the other in Germany.

None of Anderson's DNA samples matched that of the remains. Strangely, the DNA found in the sample of blood did not match that of the intestine and the hair. The DNA test found that Anderson could not be Anastasia. A DNA sample was also taken from the grand-nephew of Franziska Schanzkowska, the missing Polish factory worker, and compared to that of Anderson's samples. The test results showed that there was only a 1/300 chance that Anderson was not related the Fransiska's grand-nephew and therefore she must actually be Franziska.

Despite the DNA fingerprinting results there are many people that still believe that Anderson was actually Anastasia. Many believe in a conspiracy theory to disprove Anderson. Most of the royal family's descendants did not believe Anderson. They feel that this may have been biased because if she were Anastasia, the descendants' inheritance of the family's fortune would have to be turned over. Even though Anderson had died they still wanted to discredit her story and end the ordeal. Many believe that samples from Anderson had been tampered with. They point out that the sample of her intestine could not be found at one point and suddenly reappeared to be utilized in the testing (Welsh, 2003). There are also questions surrounding the sample of blood on the slide and to whom it belonged.

To those who did not believe Anderson's story from the beginning the DNA tests simply confirmed their opinions. Many believe Franziska simply took advantage of her similarities with Anastasia for her personal benefit. They also believe she simply "was the right person, at the right place, at the right time" and that her supporters "fed her information and encouraged her delusions" (Godl, 1998). Some go so far to say that she

may have had plastic surgery and studied the royal family in order to enhance her similarities to Anastasia.

The DNA fingerprinting results showed that Anna Anderson was not Anastasia, but still the controversy continues. Her supporters cannot directly dispute the DNA findings. Much of the non-DNA evidence implies that Anderson was Anastasia, and they do not think that the DNA evidence outweighs it. Even those that do believe the DNA results admit that Anderson was indeed similar to Anastasia and very convincing, but was really only one of the great "pretenders" of all time.

OJ Simpson

The trial of OJ Simpson in 1985 has been referred to by some as "the trial of the century" and by others as "the crime of the century." DNA fingerprinting played an important role in the trial, and the trial ultimately played an important role in the acceptance of the technique. The trial's international exposure introduced the technique to many people along with the debate surrounding it. In this case, much of the debate does not concern DNA fingerprinting in general, but the specific samples and procedures employed in this case.

OJ Simpson was tried for the murder of his ex-wife, Nicole Brown Simpson, and her friend Ronald Goldman. Numerous blood samples had been collected from the crime scene and from Simpson's home and bronco. DNA testing was performed on these samples to determine the person to whom the blood belonged. A total of forty-five tests were introduced. In the proceedings before the trial, the defense was unaware of all of the DNA evidence, and based on what it did know waived a hearing to determine its

admissibility. They soon became aware of additional evidence that would be introduced and made a motion for a hearing that the judge rejected. However the defense would certainly be allowed try to disqualify the evidence through cross-examination and other means.

Experts testified that proper procedures were followed regarding the collection of evidence such as blood. The defense ultimately claimed that the criminalists handled the evidence carelessly and therefore the tests were not reliable. When Dennis Fung of the Los Angeles Police Department (LAPD) took the stand, they presented a video that seemed to show him tossing the bloody glove onto the blanket that had been over one of the bodies, which could easily result in cross-contamination. The defense also questioned why Fung had placed blood samples in a plastic bag when it is known to cause bacterial growth. Fung responded that it was simply temporary.

The defense questioned the assistant director of the LAPD crime lab and led him to admit that mistakes may have been made in the collection of evidence. The defense proceeded to declare its theory that the evidence was tainted, and the possibility that Simpson was actually framed. Concerning the evidence found in the bronco, the prosecution showed that the person who had towed the truck had found it locked and, therefore, that evidence could not have been planted there. The defense argued that there were other possibilities.

Cellmark Diagnostics had performed most of the DNA tests and testified to their findings. They found both victim's DNA on a bloody glove found in Simpson's yard, as well as Simpson's own DNA in a sample taken from the crime scene. A sock in Simpson's bedroom had Nicole's blood and DNA on it. The defense responded that this

evidence could have easily been tampered with before it was sent to the laboratory for analysis.

Population statistics were used to determine the possibility that the blood found to be Simpson's had actually come from someone else was 1 in 170 million. It was further determined that Nicole's DNA was so unique and the blood that they found to be hers could not have possibly been from any other person in the world. The prosecution questioned the reliability of the statistics because Callmark's database contained only a few hundred samples. The jury would have to make it's own decision on the issues at hand.

The defense continued to raise doubt and pointed out that Cellmark had twice mistakenly declared a match because of cross-contamination in the laboratory. However, the prosecution also had some of the samples tested by the California Department of Justice's DNA laboratory that confirmed Cellmark's testimony. When their results were combined with Cellmark's the odds that blood samples were from people other than Nicole or Simpson increased further. In addition to DNA evidence, the prosecution also introduced hair and fiber evidence placing Simpson at the scene of the crime. The bloody shoeprints were shown to be the same size that Simpson wears, he was also shown wearing the same brand of gloves at a football game broadcast on television. Simpson's previous problems with Nicole were admitted as evidence, along with the problems surrounding his whereabouts at the time of the murders. The prosecution rested after presenting 58 witnesses and 488 exhibits.

An expert witness for the defense testified that the LAPD's procedures for collecting evidence were very lax and consistently resulted in problems with DNA

testing. Experts also testified that some of the ways in which the some of the blood was smeared was suspicious. The defense tried to that the blood had been planted. The defense closed by saying that detective Mark Furhman of the LAPD was prejudiced against blacks and that the LAPD assumed Simpson's guilt. They claimed that the investigators reached this decision at the beginning and became determined to win at any cost, even if it meant ignoring proper evidence collection procedures or planting evidence.

There was an enormous amount of evidence presented that implied Simpson's guilt. But the procedures surrounding the collection of the evidence by the LAPD and the procedures used in the DNA fingerprinting tests were continually questioned. The defense hoped that this would raise enough reasonable doubt for the jury to find Simpson innocent. Their tactic must have worked because thirty-seven weeks after the trial began the jury spent only four hours to find Simpson not guilty in the murder of Nicole Brown Simpson and Ronald Goldman. The case caused some to question the procedures involved in the collection of evidence and even the reliability of the DNA fingerprinting technique in general. Despite the problems with the sample collection, most consider the evidence against Simpson reliable and clear proof that he is actually guilty of the murders. Soon after the criminal trial, there was a civil trial in which it was found that Simpson had wrongfully caused the deaths of Nicole and Ronald, and was ordered to pay $33.5 million in damages.

Albert DeSalvo

Over less than a two-year span thirteen women were murdered by a serial killer named the Boston Strangler. From June 1962 to January 1964 these thirteen single women, who ranged from age nineteen to eighty-five, were strangled to death by an unknown killer. Generally, the several of the victims were much older than the latter half, but they all were sexually assaulted and strangled in very similar ways, and their homes had been ransacked to look like a burglary. Two of the murders are not official Boston Strangler cases because they were slightly different in nature from the others. In some of the cases semen was found at the crime scene, including that of the last victim Mary Sullivan.

Earlier, in 1961 Albert DeSalvo was arrested for breaking and entering, and admitted to be the "Measuring Man". The Measuring Man had went to women's homes claiming to be from a model agency and would offer to take their measurements for the agency to use in their model search. He spent about a year in jail and was released two months before the Boston Strangler cases began. In addition, in 1955, DeSalvo was arrested for fondling a young girl, but the charged were later dropped. He had also been arrested several times for breaking and entering.

In November of 1964 DeSalvo was arrested for breaking into a woman's house, threatening her with a knife, fondling her and then apologizing and fleeing. He was also charged with numerous sexual assaults of women in Connecticut. DeSalvo claimed that he had broken into approximately three-hundred homes, assaulted four-hundred women in four states and committed a few rapes. He was sent to Bridgewater State Hospital to

be observed. In a sudden turn of events, DeSalvo admitted to being the famous Boston Strangler.

There were doubts concerning the truth of his claim because he had been know to exaggerate in order to feel important. DeSalvo soon began to recount each of the murders and all doubts were quickly lifted. He indicated the specific circumstances around each murder, and the way in which he killed and left each of the women. He was able to describe the homes of the women and even specific characteristics like the color of a rug, the fact that a notebook was under a bed, or a specific brand of cigarettes on a nightstand. Investigators confirmed these details. Each murder was accounted by DeSalvo in an accurate and composed manner. F. Lee Bailey, DeSalvo's attorney, describes that he was convinced that "he was recalling scenes he had actually experienced" (Bardsley, 2003). Bailey believed that he was guilty and simply tried to protect him from later being executed.

Despite all the details he knew about the murders, some believed he was not the Boston Strangler, including everybody who knew him. There was no physical evidence that pointed to his guilt and he did not match the physical description given by witnesses. In fact, when a woman that had actually survived her attack visited the hospital in an attempt to make an identification she actually identified DeSalvo's roommate George Nassar as her possible attacker. There was speculation that Nassar was actually the Boston Strangler and he had told DeSalvo the details of the murders. DeSalvo was aware that he would be spending his life in jail and some believe that he made this claim in order to collect money for his family from book and movie deals, and also to be famous.

Soon, DeSalvo was convicted of the breaking and entering and sexual assault charges, and sentenced to life in Walpole State Prison. In 1973, before any charges involving the case were brought, DeSalvo requested a meeting with Dr. Ames Robey, a psychiatrist from Bridgewater hospital, and a reporter in order to tell them who the real Boston Strangler was. He made this request one evening, and by the next morning he had been stabbed to death. A week earlier he requested to be put in special lockup, but he was still murdered. Officials claimed that his murder involved the prison's drug trade, but many believe that someone within the prison did not want DeSalvo to have the meeting that he had set up. The controversy has never been settled.

The Boston Strangler cases involved the collection of bodily fluid evidence from the victims. At the time, DNA fingerprinting did not exist and it could not be determined whether it was DeSalvo's DNA at the crime scene. In 2000, the DeSalvo family began to fight to get the case reopened and DNA tests performed. The family of the last victim, Mary Sullivan, joined in this fight. They, too, questioned the guilt of DeSalvo and wanted to be sure that the Sullivan's killer is not still out there somewhere.

In order to obtain samples to be tested, both Sullivan's and Albert DeSalvo's bodies were exhumed in 2001. Samples were taken of DeSalvo's DNA and of the semen from Sullivan's body. James Starrs, a professor of forensic science at George Washington University, who had been involved with other high profile cases such as the kidnapping of the Lindbergh baby, was part of the team of scientists that performed the DNA tests. The results showed no match and it was determined that Albert DeSalvo could not have possibly been Sullivan's rapist (CNN, 2001). The families point out that if he is not Sullivan's rapist then chances are he is not her killer or the Boston Strangler,

and want an investigation of the other suspects in the case to continue. However, the DNA tests were done by a laboratory hired by the DeSalvo and Sullivan families and the State wishes to conduct it's own tests before investigating the case further. The families refuse to give the State evidence samples unless the State, in turn, gives them the crime scene evidence for their own review. The investigation remains open, but no new developments have occurred in the case.

Chapter 5: CONCLUSION

We conclude that DNA fingerprinting is a powerful scientific technique that, when performed properly, produces extremely accurate results. As standard procedures for collecting, storing and testing DNA samples continue to be implemented its reliability will only increase. Society directly benefits from the technique because it aids in proving a persons innocence or guilt, determining paternity and identifying people.

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