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Recovery of DNA Profiles from Fingerprints on Paper
after the Application of Ninhydrin or DFO
Given Certain Time Periods

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ABSTRACT

Recovery of DNA Profiles from Fingerprints on Paper
after the Application of Ninhydrin or DFO
Given Certain Time Periods

Marco Colin Lovejoy

This study examined the recovery of DNA profiles from fingerprints on paper. This examination occurred in three phases: initial determination of DNA profiles on paper, recovery of DNA profiles given certain time periods, and recovery of DNA profiles after the application of the chemical enhancement techniques of ninhydrin or DFO given certain time periods. Phase II and Phase III paper sheets were exposed to the environment to simulate a house setting. All samples from the phases were extracted with phenol-chloroform. Samples from Phase I and Phase II were amplified with AMPF*l*STR® Identifiler® amplification kit while samples from Phase III were amplified with Promega Power Plex ® 16 amplification kit. Phase I samples contained, on average, the most amount of DNA with 21.05pg/μL. Phase III samples contained, on average, the lowest amount of DNA with .56pg/μL. It was found that no profiles were recovered from the samples after analysis. It is not recommended to perform DNA analysis after the application of ninhydrin and DFO on paper unless all other options have been exhausted.

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I.   <u>Literature Review</u>

The discipline of forensic biology has been around since the early 20th century.

Examiners would determine the characteristics of bodily fluids and determine a person's blood

type if blood was present. Blood types could be categorized into classes. However, the

characteristics of the bodily fluids and blood type classes were not enough for individualization.

Fortunately, each bodily fluid contained a small molecule called deoxyribonucleic acid or DNA.

The DNA molecule could be typed and used for individualization. The first explanation of DNA

typing occurred with Alec Jeffreys in 1984 when he first discovered that certain areas of DNA

sequences repeat over and over again [1]. He also found that these sequences could vary in the

number of repeated sequences. It is known that a cell is the basic unit of life. A eukaryotic cell

is composed of a nucleus that contains 92 strands of deoxyribose nucleic acid or DNA. These

DNA strands contain the blueprint on how to build and maintain the cell with various amounts of

enzymes and have the capability to store genetic makeup from the past and transfer it to the

future [2].

The entire genetic makeup of DNA is called a genome. The basic structure of DNA is

made up of three parts: a nucleotide, a sugar, and a phosphate group. There are four nucleotides

in DNA: adenine (A), guanine (G), thymine (T), and cytosine (C). These four bases are paired

up with its complementary base depending on hydrogen bonding and Van Der Waal forces [3].

For example, adenine bonds to thymine with two hydrogen bonds while cytosine bonds to

guanine with three hydrogen bonds. These pairings of bases allow the DNA structure to curve

into its easily recognizable double helix formation as founded by Dr. Watson and Dr. Crick [3].

Humans have about 3 billion nucleotide positions in the DNA structure [2]. These bases can line

up in any combination at any position to give over a vast amount of combinations, possibly in the

trillions. It is no wonder that with the exception of identical twins, there are no two genetic

profiles that are the same.

DNA strands are packaged in the form of chromosomes. In humans, there are 46 total

chromosomes or 23 pairs of chromosomes: 22 autosomal and 2 sex-determining chromosomes.

Humans receive half of their chromosomal makeup from their mother and half from their father.

Chromosomes can be broken up into coding regions, called exons, and non-coding regions or

introns. For DNA analytical purposes, analysis occurs in the non-coding regions or the introns.

The coding regions will be the same inside every individual. In fact, "about 99.9% of the

genome is identical between any two individuals" [4]. The coding regions are the same for every

individual because these regions are transcribed and translated into proteins, enzymes and other

materials needed for survival. The .1% of the genome is where the individuals are different. It

can be calculated that there are about three million sites at the .1% of the genome that can make

every individual unique, with the exception of identical twins [4]. The .1% of the genome is

made up of the non-coding regions. Analysis will occur at specific places in the non-coding

regions. The areas of significance are called loci (singular: locus). A locus is a physical location

on a chromosome.   An example of a locus is D3S1358, a commercially used marker in DNA

analysis kits. At each locus there are two specific locations or alleles. For every one locus there

are two alleles. Variations of these alleles, such as different numerical sequences, lead to

uniqueness of a profile.

Over the past 25 years, DNA analysis has evolved from a very slow process to a more

rapid one. Dr. Jeffreys discovered variable number of tandem repeats or VNTRs. VNTRs have

repeat sequences between 15 and 35 base pairs [4]. VNTRs were examined with restriction

fragment length polymorphism or RFLPs [5]. RFLPs were used because the analysis "involved

the use of a restriction enzyme to cut the regions of DNA surrounding the VNTRs" [2]. RFLPs were highly discriminating; sometimes more discriminating than current method, yet the process was very slow. The process for results was about 6 to 8 weeks and needed a significant amount of DNA sample to even consider it to be analyzed [2]. The finished product was run on a yield gel to quantify. The yield gels separate the size of the DNA fragments; small fragments will travel farther in the gel than larger fragments [4]. Larger fragments would be found higher in the yield gel while small fragments would be found lower in the yield gel. VNTRs were the first polymorphisms to be successful in analysis and in the courtrooms during the late 1980's and early 1990's [4]. The RFLP statistics were sound and was admitted into courts steadily. Shipp, et al looked at RFLP analysis after bloodstained white cotton cloths were either superglued, exposed to a high energy source light, or both [6]. They found that an RFLP profile could still be obtained after superglue fuming, but UV lighting had deleterious effects on DNA. VNTR analysis is rarely used at present time because of DNA analysis growth and advancements. As stated by Kaye, "VNTRs…does not measure the fragment lengths to the nearest number of repeats" [4]. This limitation left the examiner to estimate the number of repeats of the fragment. The analysis known as HLA DQ-α typing was a faster process than RFLP analysis, but results were not as discriminating as RFLP [2]. HLA DQ-α requires a reverse dot bot system and a colorimetric detection system where the probes were placed onto a nylon membrane and a biotin labeled product was hybridized with the probes [7]. HLA DQ-α typing was examined on simulated and casework envelopes, stamps and cigarette butt type evidence and questioned documents; it was determined that sufficient DNA was possible for HLA DQ-α, even after latent print and ESDA examinations [8]. DNA analysis has evolved to the present procedures today

through two important developments: polymerase chain reaction (PCR) and short tandem repeat (STR) analysis.

The technique of PCR analysis was conceptualized by chemist Kary Mullis in 1983 and published under the former Cetus Corporation [9]. The technique is the basis for DNA replication as millions of copies of DNA can be created in a few hours. A small sample between .5 and 2.5ng of extracted DNA is needed for optimum results [10]. The sample is placed into a specified thermal cycler and the sample is amplified, or multiplied, to create over millions of identical copies. STR analysis is the most widely used technique in today's forensic biology laboratories. STR analysis allows the examiner to observe a core repeat region that can be between 2 and 7 base pairs long and alleles correlating to the repeat region can be between 50 and 350 base pairs long [4, 10]. Currently, biology examiners analyze four or five base pair regions with a number of different allelic markers. STR analysis can also be created to copy the areas of interest for PCR, thus decreasing the amount of DNA needed for PCR [4]. In contrast to VNTRs, STRs can determine the number of repeats found at a particular locus and it does not require a significant amount of sample to process. At a locus that is homozygous, the two alleles will have the same number of repeat sequences. At a locus that is heterozygous, the two alleles will have different number of repeat sequences. When using an analysis kit, the more loci analyzed, the more unique a profile will become.

When a crime is committed where there is bodily contact, such as homicide, assault, and rape, DNA analysis should be considered. The most common samples for DNA analysis are blood, saliva, semen, vaginal swabs and hair. DNA analysis will usually start with documentation and photography of the evidence sample. If suspicious stains are visible to the naked eye or through the use of an alternate light source, a presumptive test is applied to

determine if the stain could be blood, semen, or any other bodily fluid. One of the most common presumptive tests for blood is the Kastle-Meyer test [11]. In this test, the stain is swabbed with a wet cotton swab. A drop of alcohol is placed onto the swab. Phenolphthalein is then added to the swab. Finally, a drop of hydrogen peroxide is added to the swab. If blood is present on the swab, the hemoglobin will react with hydrogen peroxide and the phenolphthalein will become oxidized to produce a dark pink or "permanganate" color [10, 11]. Another common presumptive test is the acid phosphatase test which tests the presence of semen [12]. Acid phosphatase is found in higher concentration in seminal fluid, but can be found in other bodily fluids [12]. The stain is swabbed with a wet cotton swab. A few drops of a sodium alpha naphthylphosphate and Fast Blue B solution are added to the swab. If acid phosphatase is indicated, a purple color will be visible [2]. A definitive test for the presence of semen is the "Christmas Tree" stain test. A cutting of the swab or a clothing sample is taken and extracted. The cuttings are applied to a microscope slide. Heat is applied to fix the cells, or to not make the cells move. The "Christmas Tree stain" is then applied to the slide. The solution is made up of aluminum sulfate, nuclear fast red, picric acid, and indigo carmine. [7]. When viewed under a light microscope, the head of the sperm cell will stain a light red or pink color; the tail will stain a yellowish-green color [7]. Once it is indicated that the stain could be blood or semen, another sample is taken and extracted, either by sterile swab or cloth cutting.

The extraction method can be of the following: phenol-chloroform, DNAIQ™ Isolation System, and Chelex® 100. Of course there are several other methods available, but these methods are the most common in forensic laboratories. Phenol-chloroform dissolves the proteins in the DNA sample, and separation of the protein from DNA is possible. Phenol must be applied at least a couple of times until the protein precipitate has separated out from the aqueous solution

[13]. The DNA can then be precipitated with a number of buffers. The one drawback to using phenol-chloroform is that it is toxic and numerous tube changes. The tube changes can cause a lower DNA concentration yield. The DNAIQ™ Isolation System is a product of Promega, Inc. and it is a safer method compared to phenol-chloroform. After extraction of the swab from the tube, silica beads are added to the solution [10]. The DNA binds to the silica beads. The solution would be placed onto a magnetic bench where the magnetic attraction leads the beads to one side. Extraction of the liquid from the solution will leave the DNA and beads. The DNA can be extracted after several steps. The Chelex® 100 is a simple process as well where the solution is added to the DNA swab and incubated [14]. After incubation, Proteinase K is added to the solution. Proteinase K will digest the proteins in the solution. The solution is then centrifuged after Proteinase K has been extracted and the DNA solution is left in the test tube. It has been found that PCR is less inhibited when the Chelex ® 100 extraction is used [14]. If the evidence sample is seminal fluid, Proteinase K will not be able to break the disulphide bridges formed between the cysteine amino acids found in the acrosome of a sperm [10]. Thus, another chemical is needed to break these bridges: dithiothreitol or DTT. DTT can easily break the disulphide bridges between the cysteine amino acids and release the DNA into solution for extraction [10].

The next step in DNA analysis is quantitation. Quantitation is the process where the concentrations of the DNA samples are determined. Quantitation allows the examiner to adjust the concentrations of the DNA samples to be within range for amplification. There are various methods to determine the concentration of the samples. One early method is through the visualization of agarose gels or yield gels [10]. The gel is placed in an electrophoresis buffer and the DNA is loaded into each wells. A charge is applied across the gel and the DNA migrates

across the gel. DNA is generally negatively charged in nature due to its phosphate groups

attached to the nucleotides [10]. A negative charge is placed near the wells while a positive

charge is placed at the other end of the gel. The DNA molecules will migrate to the positive side

of the gel and will only migrate depending on its size in the sample. If a sample has a large

concentration of DNA, then it will not travel as far as a smaller concentration of DNA. Stein

(1996) used gel electrophoresis for RFLP analysis to determine a DNA profile from razor blades

and glass slides after each sample was processed with cyanoacrylate, ninhydrin and gentian

violet [15]. They found that "this examination showed no influence of used chemicals on DNA

extraction, DNA quality, and DNA typing of samples subjected to dry storage at room

temperature." Even though the process is thorough, it is very time-consuming and requires a

significant amount of DNA. Forensic laboratories have been making the switch over to real time

PCR. In real time PCR, one can visualize amplification as it is occurring. The instrument and

quantitative analysis kit used can determine the quantity of DNA present in a sample. For

example, Applied Biosystems has developed the Quantifiler analysis kit with the application of

an assay known as TaqMan®. Butler states that the TaqMan® probe is a fluorogenic 5' nuclease

assay [16]. The probes contain two fluorescent dyes: a reporter dye and a quencher dye. The

reporter dye attaches to the 5' end of the probe while the quencher dye attaches to the 3' end of

the probe. The reporter dye is not able to fluoresce because it is suppressed by the quencher dye.

The probe attaches to the DNA strand at a specific location between the two PCR primers.

When polymerization occurs in quantitation, the probe starts to become displaced from the DNA

strand. The *Taq* polymerase will cleave the reporter dye away from the rest of the probe,

increasing the reporter dye signal [16]. Once released, the reporter dye will begin to fluoresce

because it is away from the quencher dye. When more reporter dyes are released into solution,

more PCR product has occurred [10]. Figure 1 illustrates how the TaqMan® probe works in real-time PCR. Once the quantitation results are reported, the sample may need to be concentrated or diluted to fit into the range for amplification.
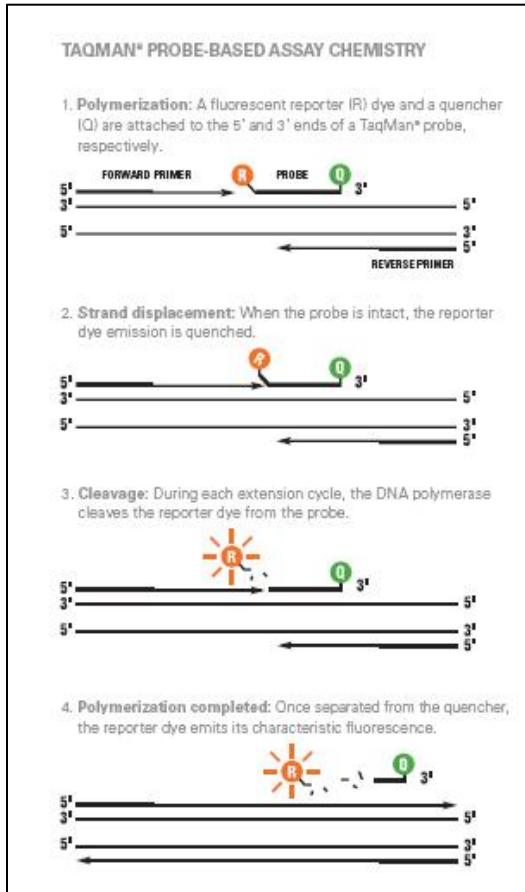
Figure 1: Overview of TaqMan®- Probe-Based Assay Chemistry (Courtesy of Life Technologies and Applied Biosystems™) [16].

Amplification is the process where the DNA sample is "multiplied" to an exponentially large amount. Amplification is possible with the process of polymerase chain reaction, or PCR. As stated before, PCR was conceptualized by Kary Mullis in 1983 [9]. PCR is able to focus and multiply several specific DNA regions. It also allows minute sample sizes to be amplified. PCR imitates the replication of DNA outside the nucleus of the cell. There are many components needed for PCR. These components are the DNA template, a DNA polymerase, nucleotide

triphosphates, primers, magnesium chloride (MgCl₂), and buffers. The DNA template is the

DNA of interest or target DNA strand. The DNA polymerase is the enzyme used to replicate

DNA and usually the *Taq* Polymerase is used in this instance. The *Taq* Polymerase was

discovered from the bacterium *Thermus aquatics* [9]. This certain bacterium is able to tolerate

and survive high temperatures. The polymerases from *Thermus aquatics* do not degrade under

high temperatures [9]. The nucleotide triphosphates are free standing molecules that are added

to the solution. PCR will use the molecules to build copies of the original DNA template. The

primers flank the region of interest and allow the *Taq* Polymerase to bind to the DNA template.

It acts to identify the region of DNA to be amplified [2]. Magnesium chloride is used to help

stabilize the process. The buffers are used to maintain pH and salinity balance. These

components are mixed into solution to create the 'master mix' which is added to each sample for

amplification.

PCR has three main steps in order to amplify DNA strands and occur in the following

order: denaturation, annealing and extension. All of these steps occur at different temperatures.

Denaturation occurs at 94°C and allows the double stranded DNA to break the hydrogen bonds

that hold the double strand together [10]. For example, the two hydrogen bonds that link adenine

and thymine together will break. However, the phosphodiester bonds that hold the sequence

together do not break because of stronger covalent bonds. Once denaturation has occurred, the

temperature will drop to the range of 50 to 60°C and annealing will occur [10]. The primers in

solution will bind to the newly formed single-stranded DNA. Once annealing has occurred, the

temperature will rise to 72°C and extension will start [10]. The *Taq* polymerase found in

solution will bind to the primers and will move along the single stranded DNA placing

complementary nucleotide triphosphates to the DNA. For example, the *Taq* polymerase will

place a thymine in the new strand and bond it to an adenine nucleotide. The polymerase will form the hydrogen bonds between the pairs and continue on the path. The PCR mechanism would have gone through one cycle once extension has been completed. A normal PCR reaction will be between 28 and 32 cycles. After 28 cycles, there would be over 67 million copies of DNA; after 32 cycles, the number increases to over 1 billion copies [2]. Completion of amplification will allow the DNA sample to be analyzed and visualized for a DNA profile.

Analysis is the final step of DNA profiling. The analysis of a DNA sample will depend on what type of analysis kit is used and what kind of instrument separates the DNA samples into its respective peaks. A general analysis kit will look for certain DNA markers found in the sample. These markers, also called loci (singular: locus), are located at different regions on the chromosomes. Each locus has a specific name that corresponds to the location of it. For example, the locus D3S1358 is located on the short arm of the third chromosome. Each loci located in a kit will look for a set of repeating sequences and the end result will come up with a quantitative number to tell how many repeats there are at the alleles. If the specific locus is homozygous, or has the same size alleles, the number of repeat sequences will be the same. If the specific locus is heterozygous, or the alleles are different in size, then the number of repeat sequences will be different. Each kit used in analysis will have specific fluorescent dyes that will glow when it is struck by a source light [4]. In comparison to yield gels, genetic analyzers will detect the shorter fragments first and the largest fragments last.

Capillary electrophoresis, or CE, completes PCR STR analysis [17]. A buffer solution is pumped through a capillary. This buffer solution is a water-soluble polymer, such as POP-4 from Applied Biosystems. The samples are injected at specific injection times and go through the capillary. Larger DNA fragments will interact more with the medium than the smaller DNA

fragments, thus separating the samples out [4, 17]. The samples will pass through a fluorescent detector and an electropherogram will be produced [17]. The electropherogram will recognize allele separation at each locus, if applicable. The analysis instrument used will recognize most analysis kits through software application. In today's forensic biology labs, there are over a dozen DNA analysis kits. The most common kits used in laboratories are created by Applied Biosystems or Promega. Applied Biosystems have created the kits named AmpF*l*STR®. There are different variations, such as Profiler™, Profiler Plus™, and Identifiler™ [18]. Promega has created kits named PowerPlex® and some variations include PowerPlex® 16, PowerPlex® 2.1, and PowerPlex® HS [19]. Each aforementioned kit does have a slight variance to it; different loci may be analyzed. No matter which kit is used, the same kit will be used to process all DNA samples in one particular lab.

In 1994 the United States passed the DNA Identification Act. This act allowed the establishment of the Combined DNA Index System or CODIS in 1998 by the Federal Bureau of Investigation (FBI) [20]. CODIS is a federally held DNA database; it allows the federal, state and local laboratories to link DNA profiles and has expanded over the last 15 years [20]. When a person's information is loaded into CODIS, it can contain a laboratory identifier, a specimen finder, the DNA profile and the integrity of the DNA record [10]. CODIS uses 13 STR loci for the national DNA database. The 13 STR loci are the following: CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, and Amelogenin, the gender determining gene [2]. STR analysis from all laboratories allows CODIS to be a powerful tool for missing person profiles or for developing a suspect. As the FBI states, "Based upon a match, police from multiple jurisdictions can coordinate their respective

investigations and share the leads they developed independently" [20]. The kits developed by Applied Biosystems and Promega contain these 13 core loci.

In the 21st century, forensic biology examiners have been focusing research on making DNA analysis more sensitive by decreasing the amount of DNA sample needed for analysis. Generally, examiners only need between .5 and 2ng of DNA to analyze [10]. However, low template number DNA (LTDNA) testing is found to be less than .1ng or 100pg (picograms) [21, 22]. Newer instruments have been created to detect more sensitive and lower amounts of DNA. Examples of such low amounts of DNA are in fingerprints, skin (or epithelial) cells, and secondary transfer. Low amounts of DNA can be found on anything that has been touched by an individual. Low template DNA is essentially the same analysis as normal PCR STR analysis, but there are a few modifications. The cycle number for DNA amplification can be increased from a normal amount of 28 cycles to a maximum of 34 cycles. Kloosterman (2003) determined and validated the application of 34 cycles should be used when normal PCR conditions cannot create a DNA profile [23]. Multiple amplifications of the same sample should occur. Samples should be run in triplicate, or run on the same amplification three times [21, 24, 25]. This increases the chance of confirming an allele to be present in a profile and eliminate alleles that appear in one sample. Negative controls should be used with every analytical test [24]. Van Oorschot (2003) has also suggested that through improved collection, extraction and quantitation techniques, more available trace amounts of DNA can be obtained [26].

There are some drawbacks to low template DNA analysis. With the increased sensitivity of amplification, the sample could start to develop stochastic effects. Stochastic effects are not the analyst's fault; they occur in amplification from improper or lack of annealing and extension. The most common forms of stochastic effects are heterozygote imbalance, allele drop-out, allele

drop-in, stutter peaks, and near threshold peaks [21, 27, 28]. Several authors and researchers

have found stochastic effects inevitable because the small amount of DNA available for

amplification increases the risk. Heterozygote imbalances are not as significant as allele drop-

outs or drop-ins. Cowen (2011) states that a heterozygote imbalance occurs when the

distribution of the ratio of peak areas is increased and one allele peak is significantly larger than

the other allele peak [28]. However, both peaks are still present and a proper conclusion can still

occur. Allele drop-outs occur when an allele area peak is not higher than the threshold level

[28]. The allele is not absent in the template DNA, but is hidden in the background noise located

beneath the threshold level or missed entirely during the first rounds of amplification [27]. An

examiner could decrease the relative fluorescent units (RFU) to increase the possibility of

observing the allele. An allele could also not amplify at all and not be observed; this is called an

extreme allele drop-out. Allele drop-ins will also occur. These alleles are not represented by the

profile of the donor [28]. Extraneous or contaminated DNA could be amplified and be observed

in the profile. Stutter peaks are the most common stochastic effects. They arise during PCR

because of strand slippage. This means that the strands are typically 4bp shorter than the main

alleles. Sometimes, stutter peaks can occur larger than they really are. This proves that although

increasing sensitivity would be beneficial for more profiles to be recovered, it also increases risk

of stochastic effects in the sample. Low template DNA analysis should not be on the same level

of analysis as conventional DNA analysis. It should be noted as well that since low template

DNA analysis is more sensitive than conventional DNA analysis, the stochastic threshold must

also change [29]. Puch-Solis used statistical data called the "tail method" to determine the

change in the stochastic threshold. The equation is:

where p_1 represents the proportion of heights smaller than 50 RFU out of all heights of heterozygotes, p_2 represents the proportion of alleles whose heights are greater than a cut-off point C out all alleles where the partner allele has a height smaller than 50 RFU and p_3 represents the probability that a height of an allele is greater than T out of all heights greater than cut-off point C and whose partner allele has a height smaller than 50 RFU [29].

Fortunately, researchers have been able to determine that touch DNA is able to be analyzed. Van Oorschot and Jones (1997) reported that they were able to correctly obtain DNA profiles and yield DNA samples from 2 to 150ng from swabs directly taken from the palm of a hand [30]. The examiners also produced profiles of multiple alleles of various intensities from different objects [30]. Findlay and Frazier (1997) used Oorschot's results and tested it against six forensic STR markers [31]. They did not state what STR markers were used in analysis, but did find favorable results after micromanipulation procedures and 34 cycles of amplification [31]. Renterghem (2000) was able to develop a full DNA profile from fingerprints placed on a microscope slide with no application of processes [32].

Gill (2000) found full profiles when the PCR step was set to 34 cycles while using the AMPF*l*STR® SGM Plus™ analysis kit [21]. He stated that the amount that can be fully profiled is from 25-50pg, or 4 to 10 cell nuclei. He also stated the *Taq* polymerase enzyme used in amplification becomes inefficient above 34 cycles. Finally, he also stated that one cannot get any profiles when the DNA sample contains less than 25pg [21]. Wickenheiser (2002) determined that some surfaces are able to hold more DNA bearing cells than other surfaces [22]. He stated that "epithelial cells sloughed through active handling onto a porous and jagged substrate should comprise a good portion of the DNA yielding cells [22]. He also stated that

surfaces that do not develop good fingerprints will obtain a better DNA profile while surfaces that do develop good fingerprints after processing do not develop a DNA profile [22].

Several researchers have modified various parts of the amplification and analysis processes. Caragine and company (2009) used protocols and interpretation guidelines to validate low template DNA testing for AmpF*l*STR® Identifiler® [33]. The samples were amplified in triplicate under enhanced PCR conditions to produce "robust and reliable" results. They created quality control, testing, and interpretation protocols [33]. Weiler (2012) concluded that it is possible to improve DNA recovery by increasing the annealing time during amplification [27]. Even though the entire PCR process was increased to 10 hours, the process does not require hands-on tweaking and can be included in the PCR process. It has shown less allelic drop-outs and increased peak heights compared to the conventional DNA amplification [27]. Davis (2011) included proofreading enzymes to the PCR master mix to improve DNA recovery and profiling [34]. Proofreading enzymes would edit and replace the correct bases the *Taq* Polymerase mistakenly placed during extension. However, they discovered that adding a proofreading enzyme did not improve STR results for such a low amount of DNA. They also recommended not to use proofreading enzymes for low template DNA work [34].

Fingerprints are impressions of a person's dermal ridges placed onto a surface or substrate. This substrate can range from glass to wood to metal. A fingerprint is deposited onto a surface from the perspiration and oils that are secreted from the skin. The sweat and oils can also contain DNA bearing cells and can be used for analysis [22]. A fingerprint can be found as a visible print or as a latent print. A visible print is a print that is observed with the naked eye without any assistance from detection techniques. A latent print is invisible to the naked eye and requires some assistance from detection techniques. Fingerprint examination is a very important

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area of forensic science; it was at one time the only identification method before DNA was used for forensic purposes.

The examination is based on two main principles. The first fingerprint principle is that the fingerprint is persistent [35]. Dermal ridges are formed on the fetus's fingerprint around the 10th to 16th week of gestation [36]. There has been some research done to determine if certain genes located in the genome can slightly cause dermal ridge development [37]. However, more research is needed to confirm this. Once the dermal ridges are formed on the fingers of the fetus, the dermal ridges are permanent and remain unchanged for the duration of the individual's life [36]. The only way the individual can change his or her fingerprint is if an injury cuts deep into the dermal layer and a scar is formed.

The second fingerprint principle is that a fingerprint is unique [35]. There are three general classifications of fingerprints: arches, loops and whorls. In addition to the general classifications, a fingerprint will have several different ridge characteristics, or minutiae. These ridge characteristics can include ridge endings, bifurcations, and enclosures. When looking even deeper at fingerprint, a fingerprint's ridges and pores could potentially be present. The combination of the general classifications, the position of the ridge characteristics, and the shape of the ridges and pores will make a fingerprint unique.

A fingerprint, whether a visible or latent print, will have a different appearance on various surfaces. There are two general classifications of surfaces: porous and non-porous. Porous surfaces will include wood, paper, cardboard and fibers (cloths). Porous surfaces will "hold" the fingerprint on top of its surface for a short amount of time. Eventually, some of the substances in the fingerprint will be absorbed by the surface, leaving a residue. The fingerprint

could be difficult to develop for fingerprint examination, but it could be extremely useful for DNA analysis. Non-porous surfaces will include glass, tile, ceramic, metal, and plastic. Unlike porous surfaces, non-porous surfaces will have the fingerprint residues stay on top of the surface for a significantly longer time. There are other classifications of surfaces, such as semi-porous and soft prints (which could include putty, soap and wax), but these surfaces have not been researched in depth as the two main surfaces.

When a fingerprint is suspected to be on a particular surface during a crime scene investigation, the examiner will document the print with notes and photography, recover the evidence, if possible, and return it to the laboratory for development. The examiner will choose a fingerprint detection technique depending on the surface. Of course, there are different fingerprint detection techniques for porous and non-porous surfaces.

Fingerprint detection techniques for porous surfaces include iodine fuming, physical developer, silver nitrate, ninhydrin and 1,8 diazafluoren-9-one or DFO. Iodine fuming occurs when crystalline iodine, when heated, is sublimed. Latent prints are developed with iodine when the item of interest is placed into an enclosed area and gaseous iodine is produced [38]. The fingerprints will interact with the iodine molecules and will produce a purple print. However, this purple print will disappear over time, so processing and photography must be quick. Physical developer is used when other porous surface processes have not developed a sufficient fingerprint. Physical developer can also be used for a fingerprint that was once wet [38]. However, physical developer will wash away any proteins that were part of the fingerprint. This could have deleterious effects on DNA Analysis. Silver nitrate is also used on porous surfaces. When applied, the silver nitrate reacts with the sodium chloride, or salt content, in the fingerprint residues [38].

Ninhydrin was first introduced as a fingerprint reagent for porous surfaces in 1954 [39]. Ninhydrin is another porous surface process where the chemicals of ninhydrin react with the amino acids that are in the fingerprint. This reaction will form a blue-purple fingerprint color called Ruhemann's Purple [38, 39]. Ninhydrin has become one of the most popular choices for fingerprint development on paper. Items of interest are dipped into a ninhydrin solution and left to dry for 24 to 48 hours [39]. The process of ninhydrin can be accelerated with the application of heat; the simplest method is with an iron. A ninhydrin chamber can be used where temperature and moisture are set for a certain amount of time [39]. DFO, available in 1990, is a more sensitive process for porous surfaces where it has replaced ninhydrin in some cases [39]. The fingerprint is developed by reacting to the amino acids in the fingerprint residues on dry porous surfaces [39, 40]. The finished product of DFO needs heat and the fingerprint is observed using a specialized light source. DFO was used in research for potential threat mail before being used for DNA analysis [40]. They did find that DFO did not interfere with DNA analysis and a successful DNA profile could be obtained.

Fingerprint detection techniques for non-porous surfaces include powders, cyanoacrylate or superglue, gentian violet and amido black. The most common application of non-porous surfaces is powders. There are various powders: black, fluorescent, and magnetic. Black powder is the most traditional powder where a camel hair or fiberglass brush is dabbed into the container and gently dusted onto the fingerprint [38]. Black powder works most effectively when applied to white or light colored surfaces. Fluorescent powders are bright colored powders that are applied to the surface the same method as black powder, but require an alternate light source to see a detailed fingerprint. Again, some alternate light sources can degrade DNA and have an impact in DNA analysis. Different colored powders work most effectively at different

wavelengths. Magnetic powder is applied like black and fluorescent powder, but the magnetic brush is a wand with no bristles [38]. The wand is magnetized to attract iron fillings along with other fillers and it is passed over the fingerprint. Some of the fillings will adhere to the print, leaving the impression. This reduces damage or destruction to the fingerprint. Research has been specifically done with powders on fingerprints to determine if a DNA profile can be produced [41]. Eleven powders ranging from white powders, black powders and magnetic powders were used on fingerprints that were deposited on glass and wooden plates. They found that the DNA peak heights for glass were lower than for wood. They also found that application of a brush can potentially wipe away any DNA material on the surface. Finally, it was shown that five powders were useful and did not interfere with DNA analysis; of particular interest was magnetic powder [41].

Cyanoacrylate, or superglue, fuming is a popular method for processing fingerprints on most non-porous surfaces. The chemical is mostly cyanoacrylate ester and it interacts with the residues in the fingerprint [38, 39]. The fumes of superglue can be applied by the application of heat. Superglue fuming chambers are typically designed to process multiple items. A heat plate and a small volume of superglue are added to the chamber and the reaction takes place. The end product will have a white-colored fingerprint. Zamir used cyanoacrylate fuming for the enhancement of fingerprints on adhesive tape and then processed for DNA profiles [42]. The results showed that cyanoacrylate fuming or the fingerprint processes after it did not interfere with DNA analysis. Gentian violet is a process that develops prints on the adhesive side of tape [38]. When the application is applied, the print will become purple in color. Amido black develops prints that have been placed in blood or bloodstains [38]. It is sprayed on the print to

develop the ridges; then a rinsing with tap water washes away the residues that are not associated with the print.

Research has been performed as to whether these fingerprint detection processes affect DNA analysis. One of the earliest research articles written by Shipp used argon light lasers, alternate light sources and superglue on bloodstains [6]. RFLP analysis was done after the application of one of the three processes. They found that no effects to the DNA sample while using superglue or a laser, but had some deleterious effects on DNA while using the alternate light source, especially UV [6]. However, one has to remember that the DNA process done in this research was RFLP analysis which is rarely done in laboratories anymore. Alternate light sources were observed on thin bloodsmears to determine if these sources deteriorate DNA [43]. The examiners used four different light sources: argon-ion laser, a Polilight, a Superlite, and a shortwave UV source. After PCR amplification, it was found that shortwave UV light should be avoided because it degraded the DNA samples. However, the other three light sources used did not have any effect on PCR-STR analysis [43]. In other research fingerprints were deposited in blood and processed on paper, glass, bags, tape, and steel blades while using the processes of UV light, DFO, physical developer, ninhydrin and cadmium, luminol, cyanoacrylate, gentian violet, powders, multimetal deposition (MMD), and amido black [44]. They used aged bloodstains (1 week, 1 month, and 3 months) with the fingerprint processes and it was determined that the only processes not to use for DNA analysis were MMD, UV radiation, and magnetic powder[44]. This is contradictory to what was found from Van Hoofstat [41] because he found that magnetic powders do not interfere with DNA analysis. Another study was used to determine if magnetic powder, along with soot powder and scotch tape inhibit DNA profile production [45]. It was

determined that there was a small amount of DNA present after extraction and quantitation (<.01 to .3ng) and only some fingerprints created DNA profiles.

Some researchers have focused on obtaining low template DNA profiles from various surfaces. Pesaresi (2003) applied fingerprints to glass, metal, and wood by preparing them through pressure for 30 seconds or rolling them [46]. Fingerprint powders were sprinkled onto the samples. Favorable results were found as DNA profiles could be obtained after analysis. The most favorable conditions were with metal surfaces [46]. Alessandrini (2003) also attempted to develop DNA profiles from glass, metal and wood [47]. They tested a total of 374 samples and discovered that the amount of DNA recovered can vary in different experiments from the same donor. This amount ranged from no DNA to tens or hundreds of picograms. They did determine that the quantity of DNA recovered depends on two factors: 1) the amount of DNA left by touching objects and 2) the suitability of recovery and extraction techniques [47]. Daly (2011) concludes that wood surfaces will have a greater chance of recovering a DNA than fabric surfaces or glass surfaces [48]. The article also stated that any low level DNA quantification result of less than .03ng/µL should not be amplified. This means that any sample that has an amount of less than 30 pg of DNA should not be amplified [48]. An uncommon surface to swab for and obtain a sufficient DNA profile is from firearms and fired cartridge cases [49].

Raymond (2004) used five surfaces for their research: aluminum foil, polyethylene bags, paper, clear glass, and adhesive tape [50]. The fingerprint processes they used were UV light, DFO, ninhydrin, ninhydrin with a zinc metal salt treatment, white light, white powder, black powder, magnetic powder, cyanoacrylate (alone and with rhodamine 6G and vacuum metal deposition), stickyside powder, amido black, luminol, and diamino-benzidine (DAB). Each

surface was washed with ethanol before a print was applied. The subject placed a fingerprint on the surface for 30 seconds and each surface was left untouched for two days. Amplification used was the AMPF*l*STR Profiler Plus running on 34 cycles. They found that DNA profiles were obtained on microscope slides, plastic bags and tape before and after fingerprint processing, but found that paper and foil had less success [50]. They also state three factors that could have affected a significantly low success rate:

- "The amount of liquid left in the microcon after the extraction process was much greater for the paper samples than for any other surface…resulting in the template DNA being more dilute." [50]
- "A large surface area of the paper was covered, which then needed to be fit in the incubation tube. Given these cramped conditions, the chelex may not have come into contact with all of the skin debris."[50]
- "Bleach and other whitening agents used in the manufacture of the paper may have interacted with the DNA during incubation."[50]

It is stated by Kanable (2005) that "when fingerprints are subjected to chemical fingerprint processing before DNA profiling, Ramotowski says the amount of DNA is diminished further" [51]. Even though this is true, DNA analysis after fingerprint process application is still possible. She also states that ninhydrin, DFO, black powder and white powder do not interfere with DNA typing [51]. This is confirmed from several researches in the past [40, 41, 52]. Raymond states numerous fingerprint processes and their effect on DNA analysis [53]. This research is focused on ninhydrin and DFO, so these two reagents will only be discussed from the article. Both ninhydrin and DFO will reduce the amount of DNA quantitated, but it will not significantly affect the recovery of DNA profiles [53].

Other researchers have focused on determining DNA profiles after the application of ninhydrin and DFO on fingerprints. Schulz (2004) placed DNA probes on cellulose, dried the probes, and then applied ninhydrin spray [52]. The group also tested for DNA profiles by using ninhydrin-dyed fingerprints found on wallpaper. The DNA probes were extracted and quantitated using a TaqMan® probe (Applied Biosystems) during real-time PCR while the ninhydrin-dyed prints were amplified. Even though the DNA samples were smaller in amount than normal, it was found that the ninhydrin did not interfere with DNA analysis in either the probes or the ninhydrin-dyed prints, [52]. In a study, 285 swabs were taken from crime scenes and stained with ninhydrin [54]. The swabs were then analyzed for DNA profiles. It was found that out of 285 samples treated with ninhydrin, 158 developed a purple color. Out of those swabs, 120 (76%) yielded a DNA profile suitable for the German national DNA database. It was concluded that ninhydrin could be used as a DNA screening method, but only for less serious crimes [54]. This study confirms that ninhydrin does not completely inhibit DNA analysis, but only reduce the amount of DNA recovered.

The research conducted by Grubwieser, et al. focused on recovering DNA profiles from a number of blood, saliva and finger mark samples [55]. They used various fingerprint detection techniques, including ninhydrin and DFO. They found that ninhydrin had no adverse effects on DNA amplification. For DFO, however, they found the shorter the incubation time, the better the recovery. Their efforts for DFO were to determine if absolute temperature or incubation time led to a reduction of recovery [55]. After testing samples for 60 minutes at 60°C and for 15 minutes at 100°C, they concluded that longer incubation time rather than absolute temperature affected the samples and recovery more [55]. Bhoelai (2011) found that the risk of DNA contamination should be taken into effect when fingerprint reagents are used before analysis

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[56]. The samples were processed for fingerprints, such as cyanoacrylate fuming, ninhydrin and

DFO, and then they were processed for DNA analysis. It was determined that even though

ninhydrin or DFO did not affect DNA analysis, some samples showed contamination and it has

been recommended that fresh solutions are made before immersion [56].

Few researchers have attempted to develop DNA profiles from fingerprints deposited on

paper. Balogh and company (2003) extracted DNA after pieces of paper were handled by

volunteers [57]. After trying four different extraction methods on paper, the samples were

amplified and analyzed. It was found that phenol/chloroform extraction yielded only 10% of

deposited DNA, but DNA profiles are able to be obtained after analysis [57]. Sewell and

company tested a number of various papers for DNA analysis after the application of either

ninhydrin or DFO [58]. Prints were placed down on four types of paper and sealed in plastic

evidence bags at 4°C. Some fingerprints were developed with DFO, ninhydrin, or both. The

samples were extracted with DNeasy® Plant Mini Kit, concentrated and quantitated using

Quantifiler®. The samples with the highest DNA concentration was amplified using

AmpF*l*STR® SGM *plus*™ amplification kit at 28 cycles. They reported that magazine paper

recovered the most DNA while office paper recovered the least. They also found no profiles for

untreated fingerprints deposited on office paper after 28 cycles. However, an increase of profile

presence was noted when the cycles were increased from 28 to 34. They stated that the samples

will be reduced in recovered DNA if treated with ninhydrin or DFO [58]. Lastly, they stated that

the amount of DNA deposited on paper can vary and contribute a significant part in the

development of profiles.

Balogh (2003) deposited fingerprints on small cuttings of white paper during several

various environmental methods: touching periods, delay, time of day, swab experiment, after

enhancement, and mixture experiment [59]. Their results only showed percentages of profile

recovery, but they concluded that the touching periods, delay, and time of day experiments

provided strong partial to full profiles while the after enhancement experiment showed the least

favorable results. They suggested increasing the cycle numbers to 38 to increase sensitivity, but

also introduces more stochastic effects [59].

The majority of the articles are dated because the instrumentation, extraction methods,

quantitation, amplification, or analysis is no longer used in forensic laboratories. Extraction

methods that were once too long or too tedious are now obsolete. Quantitation, such as using a

yield gel or a slot blot, is now being replaced by real time PCR. Older amplification methods,

such as RFLP and HLA-DQα, are no longer used because of the amount of DNA needed for

each process to work.

II.   <u>Materials/Methods</u>

It is important to note that proper protective equipment was worn during all extractions,

quantitations, amplifications and analyses. The bench and pipets were cleaned with 10% bleach

solution and had an ultraviolet light on for fifteen minutes before and after each process.

<u>Phase I- Initial determination of DNA profiles</u>

Extraction

Commercial office paper was bought at a local retail store. The type of paper used was

International Paper Hammermill® Copy Plus Item number 105090. The paper brightness, which

is the degree to which paper reflects light, was 92. For the first phase of the research, two

different processes occurred: (1) both hands were placed on separate papers simultaneously and (2) one finger (right index finger) was placed down for a span of one to two seconds ten consecutive times. Boxes were created on the paper with a black sharpie marker for the purpose of placing fingers down inside them. The boxes for the first process were marked from numbers one to ten, starting with the right thumb as number one and ending with the left little finger as number ten. The boxes for the second process were marked from one to ten and the right index finger was placed consecutively. Once the fingerprints were placed in the boxes, small samples measuring approximately 2cm by 1cm from inside the box were cut out with a clean scalpel. The scalpel was cleaned with 10% bleach solution before each cut. The samples were placed into individually marked 1.5mL microcentrifuge tubes. The two processes were done three times for a total of sixty samples in Phase I. Cheeks were swabbed with clean sterile cotton swabs. These swabs were the positive reagents. Two negative controls were also included in the research. The positive reagents came from the left cheek while there was no sample for the negative control.

Each sample went through a phenol-chloroform extraction underneath a hood. The phenol-chloroform extraction method was performed under the same guidelines of the West Virginia State Police DNA Analysis Laboratory. 500μL of digest buffer and 15μL of Proteinase K was pipetted into each sample. The samples were vortexed and placed into a water bath set at 56°C for digestion. The samples were incubated overnight but never past 24 hours. After digestion, the samples were taken out of solution and placed into a spin basket tube.

The samples were centrifuged for five to ten seconds to extract all possible DNA. The samples and spin baskets were discarded into a biohazard bag. Half of the samples (#21-50) were placed into a refrigerator at that point for an extended period. Once the samples were ready for extraction, 500μL of phenol-chloroform was pipetted into each sample. The samples were vortexed and centrifuged at 15,000 * g for two minutes. New microcentrifuge tubes were marked and the top aqueous layer was pipetted from the old solution to the new tubes. The used phenol-chloroform was placed into a phenol waste container located in the hood. 500μL of phenol-chloroform was pipetted into the samples; the samples were vortexed and centrifuged at the same settings. The same procedure was repeated again for a total of three times.

Amicon Ultra-.5mL centrifugal filter units, or Microcon units, were assembled and labeled for respective samples. 100μL of distilled water was placed into the concentrators. The top aqueous layer from the phenol-chloroform extraction was pipetted from there into the concentrators. The samples were centrifuged at 3,000 * g for five minutes. The filtered waste was discarded into a separate tube. 400μL of hot distilled water was pipetted into each sample. The samples were centrifuged at 4,000 * g for five minutes. The test tubes were discarded and 60μL of distilled water was pipetted into the concentrator. A new tube was added to each concentrator and the samples were briefly vortexed. The samples were inverted and centrifuged at 10,000 * g for three minutes. The concentrators were discarded and the micro test tubes were stored in a labeled box in the freezer.

Quantitation

Samples were retrieved from the freezer and allowed to thaw. For quantitation, the
procedure was the same of the West Virginia State Police DNA Laboratory. Several processes
needed to be completed before the samples could be quantitated. The standard stock solution
was obtained from the Quantifiler™ Human DNA Quantification Kit (Applied Biosystems); the
standards needed to be created and diluted. Table 1 shows how the standards were prepared. A
quantitation worksheet needed to be created. A quantitation master mix was created for every set
of quantitations. Equation 1 determines the amount of master mix needed to be created.

Table 1: Creation of Standard Concentrations for Quantitation

Quant Std. #	Amount/Description	TE buffer amount (µL)	[Standard] (ng/µL)
1	10µL of 200ng/uL stock solution	30	50.0
2	10µL from Std. 1	20	16.7
3	10µL from Std. 2	20	5.56
4	10µL from Std. 3	20	1.85
5	10µL from Std. 4	20	.620
6	10µL from Std. 5	20	.210
7	10µL from Std. 6	20	.0680
8	10µL from Std. 7	20	.0230

Equation 1: Determination of the amount of master mix needed for quantitation

 # samples currently + 2 rows of standards (16) + 2 for error = total samples
PCR Reaction Mix: total # samples x 12.5µL = total amount of Reaction mix
PCR Primer Mix: total # samples x 10.5µL = total amount of Primer mix

23µL of the master mix is pipetted into individual wells. 2µL of each sample was pipetted into
the respective wells. Flat-top lids were placed on the rows which were then centrifuged for
twenty seconds before being placed into the quantitation instrumentation. The instrument used
for quantitation was the ABI Prism 7500 Real Time PCR (Applied Biosystems) and the software

for the instrument was the 7500 SDS Software v.1.2. A SDS document was created for each

quantitation run. The results were observed and recorded.

Amplification

Phase I samples were amplified using the manufacture recommended guidelines.

Samples were taken out of the freezer and allowed to thaw. The target value for amplification

using the AmpF*l*STR® Identifiler® kit is .05ng/µL to .125ng/µL. Most samples were below the

target value of .125ng/µL. Therefore, no dilution was needed. The amount of master mix

needed to be calculated. Equation 2 illustrates how the Identifiler® master mix was prepared.

Equation 2: Calculation of Amplification Master Mix for AMPF*l*STR® Identifiler®

 # samples + positive control + negative control + 1 sample extra for error = total samples
AmpFlSTR PCR Reaction Mix: total samples x 10.5µL = total amount of PCR reaction mix
AmpliTaq Gold DNA Polymerase: total samples x .5µL = total amount of DNA polymerase
AmpFlSTR Identifiler Primer Set: total samples x 5.5µL = total amout of primer

After the master mix was created, it was vortexed. 15µL of the master mix was dispensed into

individually labeled PCR tubes. 10µL of the samples was pipetted into its respective PCR tubes.

Rounded caps were placed on the rows when finished. The positive control was supplied with

the Identifiler ® Amplification Kit labeled the AmpF*l*STR® Control DNA 9947A tube

(.10ng/µL) while the negative control was TE buffer. The samples were placed into the

GeneAmp® ThermoCycler PCR System 9700 (Applied Biosystems) and set to Identifiler® run

setup. The parameters for the Identifiler® are described below in Table 2. After its run, the tubes

were placed in a freezer stored in the amplification laboratory until analysis.

Table 2: Parameters for the Identifiler® LCN Profile Run Setup on the ThermoCycler PCR System 9700

Initial Incubation Setup	Denature	Anneal	Extend	Final Extension	Final Step
HOLD	CYCLE (28 cycles)			HOLD	HOLD
95°C	94° C	59° C	72° C	60° C	4° C
11 min	1 min	1 min	1 min	60 min	(forever)

Analysis

Equation 3: Calculation of the analysis solution (Hi-Di™ Formamide/GeneScan™-500 LIZ™ Size Standard)

 # samples + one AmpFlSTR® Identifiler™ Allelic Ladder for every ten samples
Hi-Di™ Formamide: (number of samples + 2) x 24.5μL = total amount of formamide
GeneScan™-500 LIZ™ Size Standard: (number of samples + 2) x .5μL = total amount of standard

The guidelines for setup are the same from the recommended manufacturer guidelines. Equation 3 illustrates how the Hi-Di™ Formamide/GeneScan™-500 LIZ™ Size Standard solutions is calculated. The solution was vortexed and centrifuged. The samples from amplification were taken out of the amplification laboratory freezer. The number of allelic ladders was determined. 25μL of the standard solution was pipetted into new PCR tubes. 1.5μL of each sample, allelic ladder, control and reagent was pipetted into its respective tubes. Each tube was mixed by pipetting the solution up and down. The tubes were sealed with a septum, vortexed, and centrifuged. The samples were denatured for three minutes at 95°C in the GeneAmp® ThermoCycler PCR System 9700 instrument, and then chilled for three minutes in the amplification laboratory freezer. The samples were placed on a prepared ABI 3130 Genetic Analyzer (Applied Biosystems). Results were observed and recorded.

Phase II- Recovery of DNA Profiles given Time Periods

Time intervals were determined for Phase II. These time intervals are the following: 1hr, 2hr, 3hr, 6hr, 12hr, 24hr, 36hr, 48hr, 72hr, 96hr, and 120hr. Eleven sheets of paper were given certain time intervals. Five boxes were created on the sheets of paper to place the fingerprints on. The numbering is similar to the phase I samples; the first sample is the right thumb and the last sample is the right little finger. Eleven sheets of paper with five samples on each paper totals fifty-five total samples for Phase II. The right hand was placed on each paper, but not consecutively. The hand was rubbed either through hair, with both hands or from the skin. When the time interval was complete, the samples were cut with a clean scalpel. A positive reagent (buccal swab from the right cheek) and a negative control were created. The samples would go through the same process for extraction, quantitation, amplification and analysis. Selected samples were amplified using the AMPF*l*STR® Identifiler® amplification kit and the low copy number (LCN) parameters for the Thermocycler. The difference between the LCN and normal parameters is the addition of 3 cycles for a total of 31 cycles.

Phase III – Recovery of DNA Profiles given the Application of Ninhydrin or DFO given Certain Time Periods

The procedure from Phase II was used in Phase III with two exceptions. First, the paper was cut in half. One half of the paper was dipped in a bath of pre-mixed Ninhydrin solution. The paper was allowed to air dry before observation. The other half of the paper was sprayed with pre-mixed DFO solution and allowed to air dry. DFO treated paper was placed in 100°C dry oven for twenty minutes as recommended by the FBI. Three samples were created from the ninhydrin treated prints. The DFO paper was observed using a 450nm light under an orange

filter. Three samples were created from the DFO treated prints. Second, there is an additional

sample to each time period totaling 6 for each period. Some latent palm prints were needed for

six samples.

Three positive reagents (buccal swabs from both cheeks) and one negative control were

created. The samples would go through the same process for extraction and quantitation.

New Amplification Set-Up

The Power Plex® 16 Amplification Kit from Promega Corporation was used for

amplification. The calculation for the master mix is listed below in equation 4. Each sample

contained 10µL master mix and 15 µL of control, reagent, or sample. The same positive control

standard (9947A) was used here along with a new positive control supplied with the Power

Plex® kit: 2800M. The parameters for the thermocycler are listed below in Table 3.

Equation 4: Calculation of Amplification Master Mix for Power Plex® 16

 # samples + 9947A positive control + 2800M positive control + 4 samples extra for error =
total samples
Water: total samples x 4.2µL = total amount of water
Buffer: total samples x 2.5µL = total amount of buffer
Primer Mix: total samples 2.5µL = total amount of primer mix
AmpliTaq Gold DNA polymerase: total samples x .8µL = total amount of DNA polymerase

Table 3: Parameters for the Power Plex® 16 Run Setup on the ThermoCycler PCR System 9700

Hot Start		Step One (10 cycles)			Step Two (22 cycles)			Final Extension	Soak
HOLD	HOLD	Denature	Anneal	Extend	Denature	Anneal	Extend	HOLD	HOLD
95° C	96° C	94° C	60° C	70° C	90° C	60° C	70° C	60° C	4° C
11.0 min	1.0 min	30 sec	30 sec	45 sec	30 sec	30 sec	45 sec	30 min	(forever)

New Analysis Set-Up

A new analysis procedure was prepared in accordance with the Lake County Crime Laboratory Biology Procedure Manual [60]. The mixture amounts to create the solution for analysis were the same with the exception of adding 1.0µL to each tube. The sample tubes were placed in a 48-well tray and analyzed using an ABI Prism® 310 Genetic Analyzer. Results were observed and recorded.

III.   <u>Results</u>

Quantitation

The results from Phase I quantitation are displayed below in Table 4. The Table shows the sample, the amount of DNA in ng/µL, and the amount of DNA in pg/µL. For the first amplification and analysis, every sample was selected. The samples selected for the second set of amplification and analyses are highlighted. The selected samples for the second set were amplified in triplicate.

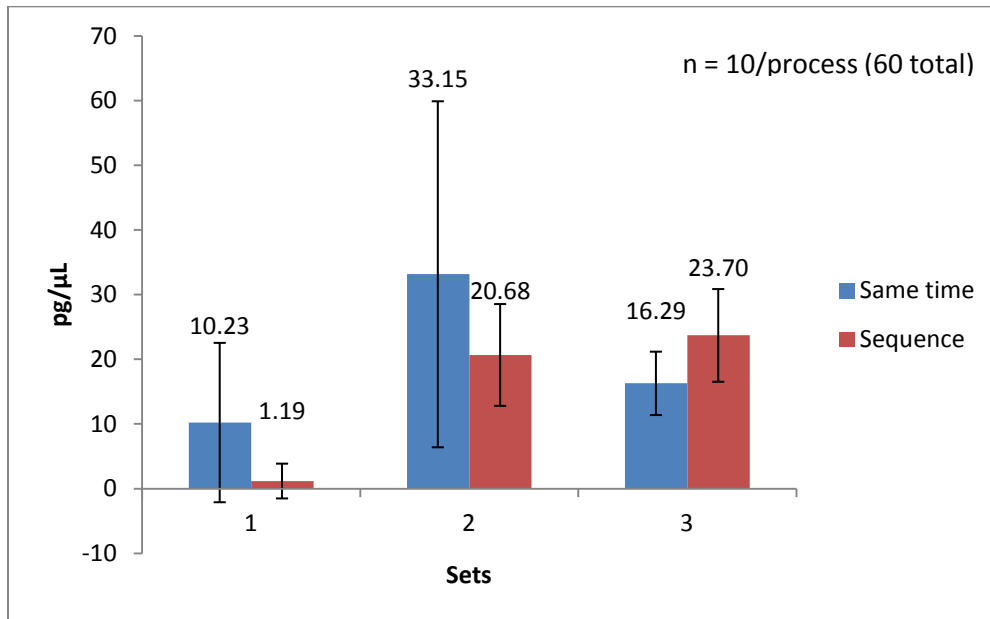### Table 4: Quantitation Results from Phase I in (ng/µL) and in (pg/µL)

Sample	Amount of DNA (ng/µL)	pg/µL	Sample	Amount of DNA (ng/µL)	pg/µL	Sample	Amount of DNA (ng/µL)	pg/µL
1-1	1.51E-03	1.51	1-21	2.22E-02	22.20	1-41	2.12E-02	21.20
1-2	1.00E-02	10.00	1-22	2.21E-02	22.10	1-42	1.92E-02	19.20
1-3	6.68E-04	0.67	1-23	1.13E-01	113.00	1-43	1.41E-02	14.10
1-4	1.40E-02	14.00	1-24	2.30E-02	23.00	1-44	1.77E-02	17.70
1-5	---	0.00	1-25	2.37E-02	23.70	1-45	2.18E-02	21.80
1-6	3.62E-02	36.20	1-26	2.22E-02	22.20	1-46	1.59E-02	15.90
1-7	3.00E-02	30.00	1-27	2.26E-02	22.60	1-47	7.34E-03	7.34
1-8	---	0.00	1-28	3.10E-02	31.00	1-48	1.48E-02	14.80
1-9	3.26E-03	3.26	1-29	2.57E-02	25.70	1-49	8.86E-03	8.86
1-10	6.68E-03	6.68	1-30	2.60E-02	26.00	1-50	2.20E-02	22.00
1-11	---	0.00	1-31	3.48E-02	34.80	1-51	4.29E-02	42.90
1-12	---	0.00	1-32	8.58E-03	8.58	1-52	1.55E-02	15.50
1-13	3.20E-03	3.20	1-33	1.31E-02	13.10	1-53	2.15E-02	21.50
1-14	---	0.00	1-34	2.59E-02	25.90	1-54	2.26E-02	22.60
1-15	---	0.00	1-35	1.03E-02	10.30	1-55	2.28E-02	22.80
1-16	---	0.00	1-36	2.41E-02	24.10	1-56	2.11E-02	21.10
1-17	8.73E-03	8.73	1-37	2.17E-02	21.70	1-57	1.81E-02	18.10
1-18	---	0.00	1-38	1.71E-02	17.10	1-58	2.18E-02	21.80
1-19	---	0.00	1-39	2.37E-02	23.70	1-59	2.19E-02	21.90
1-20	---	0.00	1-40	2.75E-02	27.50	1-60	2.88E-02	28.80

n = 60
Average amount (pg/µL): 21.05
Standard Deviation: 15.85

The average amount of DNA and standard deviation from three sets of the same time process and the sequence process from Phase I quantitation is shown in Table 5 and Figure 2. The highest average amount of DNA from Phase I occurred during the second set of the same time process.

Table 5: Average amount of DNA (pg/µL) and Standard Deviation (pg/µL) per Set from the Same Time Process and the Sequence Process from Phase I Quantitation

Set (n = 10/process)	Average (pg/µL)	Std. Dev. (pg/µL)
Same Time 1	10.23	12.32
Sequence 1	1.19	2.69
Same Time 2	33.15	26.74
Sequence 2	20.68	7.87
Same Time 3	16.29	4.9
Sequence 3	23.7	7.17

Figure 2: Average DNA Concentration (pg/µL) from Phase I Quantitation

The quantitation results from Phase II are described in below in Table 6. The table shows the sample, the amount of DNA in ng/µL, and the amount of DNA in pg/µL. The amount of DNA in Phase II is less than the amount from Phase I. The sample selected for the second set of amplification and analysis is highlighted. The selected sample was amplified in triplicate.

Table 7 and Figure 3 show the average amount of DNA and standard deviation from quantitation per hour period from Phase II. Generally, the average amount of DNA increased from 3 hours (6.05pg/µL) to 48 hours (13.53pg/µL). However, the average amount of DNA from Phase II is still lower than the average amount of DNA from Phase I.
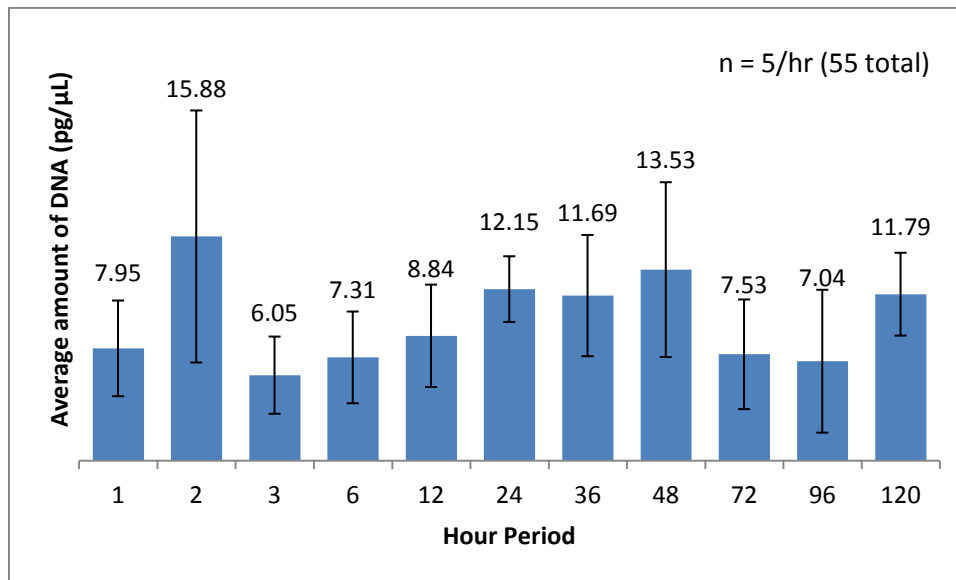
Table 6: Quantitation Results from Phase II in (ng/µL) and in (pg/µL)

Sample	Amount of DNA (ng/µL)	pg/µL	Sample	Amount of DNA (ng/µL)	pg/µL	Sample	Amount of DNA (ng/µL)	pg/µL
1-1	7.39E-03	7.39	**12-1**	7.12E-03	7.12	**72-1**	6.96E-03	6.96
1-2	4.46E-03	4.46	**12-2**	4.61E-03	4.61	**72-2**	6.22E-03	6.22
1-3	4.30E-03	4.30	**12-3**	1.41E-02	14.10	**72-3**	1.83E-03	1.83
1-4	1.08E-02	10.80	**12-4**	6.27E-03	6.27	**72-4**	8.82E-03	8.82
1-5	1.28E-02	12.80	**12-5**	1.21E-02	12.10	**72-5**	1.38E-02	13.80
2-1	3.22E-02	32.20	**24-1**	1.31E-02	13.10	**96-1**	1.56E-02	15.60
2-2	1.06E-02	10.60	**24-2**	1.61E-02	16.10	**96-2**	7.03E-03	7.03
2-3	1.86E-02	18.60	**24-3**	9.94E-03	9.94	**96-3**	8.71E-03	8.71
2-4	1.01E-02	10.10	**24-4**	1.18E-02	11.80	**96-4**	2.07E-03	2.07
2-5	7.89E-03	7.89	**24-5**	9.79E-03	9.79	**96-5**	1.81E-03	1.81
3-1	1.07E-02	10.70	**36-1**	1.10E-02	11.00	**120-1**	1.20E-02	12.00
3-2	7.12E-03	7.12	**36-2**	1.53E-02	15.30	**120-2**	1.66E-02	16.60
3-3	2.62E-03	2.62	**36-3**	5.27E-03	5.27	**120-3**	1.18E-02	11.80
3-4	4.55E-03	4.55	**36-4**	9.48E-03	9.48	**120-4**	1.12E-02	11.20
3-5	5.24E-03	5.24	**36-5**	1.74E-02	17.40	**120-5**	7.37E-03	7.37
6-1	4.69E-03	4.69	**48-1**	2.18E-02	21.80			
6-2	4.19E-03	4.19	**48-2**	6.77E-03	6.77			
6-3	5.17E-03	5.17	**48-3**	9.60E-03	9.60			
6-4	1.18E-02	11.80	**48-4**	9.30E-03	9.30			
6-5	1.07E-02	10.70	**48-5**	2.02E-02	20.20			
n = 55 Average Amount (pg/µL): 9.98 Standard Deviation: 5.52								

Table 7: Average amount of DNA (pg/μL) and Standard Deviation (pg/μL) per Hour Period from Phase II Quantitation

Hour (n = 5/hr)	Average (pg/μL)	Std. Dev. (pg/μL)
1	7.95	3.39
2	15.88	8.93
3	6.05	2.74
6	7.31	3.25
12	8.84	3.63
24	12.15	2.33
36	11.69	4.29
48	13.53	6.20
72	7.53	3.89
96	7.04	5.06
120	11.79	2.93

Figure 3: Average DNA Concentration and Standard Deviation (pg/μL) per Hour Period from Phase II Quantitation

The quantitation results from Phase III are described in below in Table 8. The table shows the sample, the amount of DNA in ng/µL, and the amount of DNA in pg/µL. The amount of DNA in Phase III is the lowest amount quantitated out of the three phases. The largest amount quantitated from Phase III was 96-1 which was 4.39pg. The samples selected for the third set of amplification and analyses are highlighted. These samples were not analyzed in triplicate.

Table 9 and Figure 4 compare the average amount of DNA and standard deviation quantitated from the applications of either ninhydrin or DFO. The amounts varied from no amount detected (ninhydrin-hour two) to 1.93pg/µL (ninhydrin-96 hour). There is no general trend found in the results from Phase III quantitation. It should be stated that no other statistical analysis was performed with the data.

Table 8: Quantitation Results from Phase III in (ng/µL) and in (pg/µL)

Sample	Amount of DNA (ng/µL)	pg/µL	Sample	Amount of DNA (ng/µL)	pg/µL	Sample	Amount of DNA (ng/µL)	pg/µL
1-1	---	0.00	12-1	---	0.00	72-1	4.45E-04	0.45
1-2	5.49E-04	0.55	12-2	2.61E-04	0.26	72-2	6.87E-04	0.69
1-3	---	0.00	12-3	5.46E-04	0.55	72-3	3.37E-04	0.34
1-4	7.22E-04	0.72	12-4	---	0.00	72-4	7.02E-04	0.70
1-5	---	0.00	12-5	---	0.00	72-5	---	0.00
1-6	2.19E-03	2.19	12-6	9.58E-04	0.96	72-6	1.11E-03	1.11
2-1	---	0.00	24-1	---	0.00	96-1	4.39E-03	4.39
2-2	---	0.00	24-2	1.43E-03	1.43	96-2	1.40E-03	1.40
2-3	---	0.00	24-3	1.82E-03	1.82	96-3	---	0.00
2-4	3.21E-04	0.32	24-4	4.61E-04	0.46	96-4	---	0.00
2-5	---	0.00	24-5	4.48E-04	0.45	96-5	---	0.00
2-6	---	0.00	24-6	6.51E-04	0.65	96-6	3.01E-04	0.30
3-1	---	0.00	36-1	---	0.00	120-1	6.89E-04	0.69
3-2	---	0.00	36-2	8.83E-04	0.88	120-2	---	0.00
3-3	3.31E-04	0.33	36-3	2.39E-03	2.39	120-3	8.18E-04	0.82
3-4	3.29E-04	0.33	36-4	---	0.00	120-4	3.95E-04	0.40
3-5	---	0.00	36-5	4.33E-04	0.43	120-5	2.38E-03	2.38
3-6	6.91E-04	0.69	36-6	6.91E-04	0.69	120-6	3.06E-04	0.31
6-1	7.17E-04	0.72	48-1	9.53E-04	0.95	n = 66 Average Amount (pg/µL): .56 Standard Deviation: .77		
6-2	---	0.00	48-2	2.64E-04	0.26			
6-3	---	0.00	48-3	1.61E-03	1.61			
6-4	1.72E-03	1.72	48-4	3.71E-04	0.37			
6-5	4.41E-04	0.44	48-5	4.88E-04	0.49			
6-6	---	0.00	48-6	2.32E-04	0.23			

Table 9: Average Amount of DNA (pg/µL) and Standard Deviation (pg/µL) from the Application of Ninhydrin or DFO from Phase III

Hour (n = 6/hr)	Average Ninhydrin (pg/µL) (n = 3/hr)	Std Dev. (pg/µL)	Average DFO(pg/µL) (n = 3/hr)	Std. Dev. (pg/µL)
1	0.18	0.26	0.97	0.91
2	0	0	0.11	0.15
3	0.11	0.16	0.34	0.28
6	0.24	0.34	0.72	0.73
12	0.27	0.22	0.32	0.45
24	1.08	0.78	0.52	0.09
36	1.09	0.99	0.37	0.29
48	0.94	0.55	0.36	0.1
72	0.49	0.15	0.6	0.46
96	1.93	1.83	0.1	0.14
120	0.5	0.34	1.03	0.96

Figure 4: Comparison of the Average Amount of DNA (pg/µL) and Standard Deviation (pg/µL) from the Application of Ninhydrin or DFO from Phase III
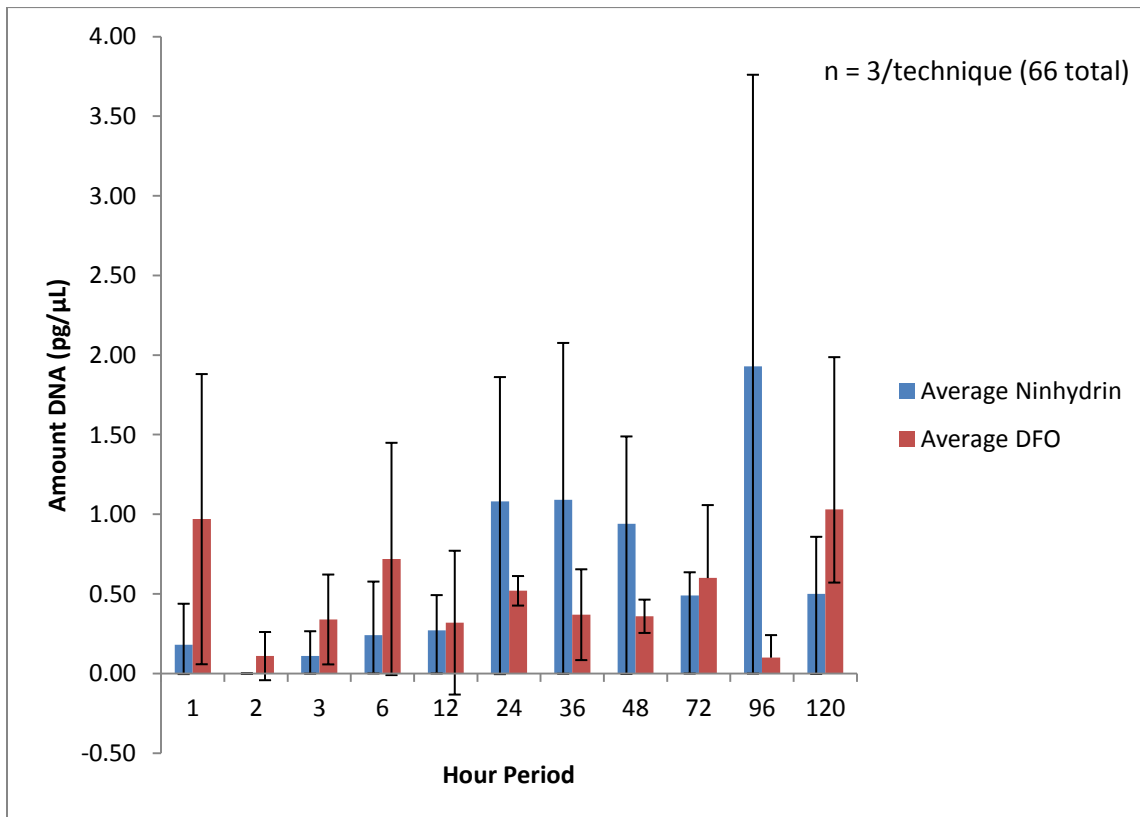
Analysis

Full profiles from the allelic ladder and the positive control (9947A) were created in the first analysis set. A partial profile from one of the positive reagents (PR2) was created from the first set of analysis set. A full profile from the allelic ladder and partial profiles from the two positive controls (9947A and 2800M) were created from the third analysis set. There was no recovery of DNA profiles from any samples analyzed, which included most of the positive reagents (buccal swabs from the cheeks) and all samples from the first and third analysis set. No statistical analysis was performed because there were negative results.

Amplification set #2 was amplified, but not analyzed. Amplification set #2 was amplified with AMPF*l*STR® Identifiler® unknowingly after the United States District Court for the Western District of Wisconsin ruled that AMPF*l*STR® Identifiler® is used for forensic and paternity testing only [61]. The set was held in storage after hearing about the ruling.

IV. **Discussion**

The results from the tables show very low amounts of DNA were found from quantitation for all three phases using these procedures. Results were found to be in low pg/µL and not in the desirable ng/µL range. This is to be expected from fingerprints on paper as previous authors have stated [52, 56-59]. The authors also demonstrated their results by using percentage of the profile recovered. Percentage of profile recovery would have been done if results were more favorable.

It is very difficult to find DNA amounts from fingerprints in the ng/µL range. The paper had debris in the form of dust or dirt before a fingerprint was deposited. The person could have

washed their hands or rubbed their hands together before touching the surface. The person could have touched the surface for a very short amount of time. These possibilities are all reasons why such low levels of DNA amounts occurred in the results. However, according to past research levels of DNA amounts around 30pg/µL were still able to create and recover full profiles, but started recovering stochastic results when the source level was at 10 pg/µL [48, 50]. Amplifying DNA less than 10 pg/µL in a sample is not recommended because amplifying extreme low amounts can lead to negative results or allelic drop-ins. Amplifying low amounts of DNA is very risky if it is used in casework protocol.

The results from Phase III had the lowest amounts of DNA out of all three phases. Phase III research included papers that sat in a typical house setting and dipping into a ninhydrin solution or spraying with DFO. Dipping or spraying the paper with these reagents dislodged some of the epithelial cells from the paper. The results from Phase III confirm what Sewell found when ninhydrin or DFO was applied to paper [58]. The application of ninhydrin or DFO does result in a reduction of recovered DNA profiles. In Sewell's case, it caused a "60% fold reduction", but profiles were still recovered [58]. In this case, no profiles were recovered.

Phase II research showed some favorable results. The amount of DNA quantitated from the samples generally increased from the 3 hour period to the 48 hour period. DNA recovery occurred because the epithelial cells on the paper found microscopic holes in the paper. With more time elapsing, the paper absorbed the fingerprint more and an increased amount of cells could be found there. This occurrence correlates to what Wickenheiser found during the study of touch DNA [22]. Wickenheiser determined that more DNA could be recovered from porous surfaces than nonporous surfaces because the cells could find small areas to hide in. On the other hand, there should be a general increase from the 3 hour period to the 120 hour period then.

This general assumption did not occur. Phase I and Phase III showed random average amounts

of DNA for each set or hour period. This could be an effect from pre-placement activity, such as

rubbing hands together or rubbing hands on the neck or hair. Some of epithelial cells could have

dislodged from the hand or not enough activity occurred for a sufficient amount of DNA to be

quantitated.

As stated in the results, no sample DNA profiles were recovered after amplification and

analysis of Phase I and Phase III. The allelic ladder in the first and third sets of analysis (all of

Phase I) was recovered properly. The positive control (9947A) in the first set recorded a full

profile while both positive controls (9947A and 2800M) in the third set recorded partial profiles.

The second positive reagent in the first set of analysis showed a partial profile, but the peaks

were small and weak. A potential problem in the first set of amplification and analysis was the

parameters of the thermocycler. The thermocycler was set for the normal 28 cycles of

amplification. Previous literature and presentations show that any amplification for LTDNA

needs to be at least 31 cycles [25, 33, 34]. Degradation could have been a factor in Phase III

analysis because the samples were taken out of a freezer and placed in a Styrofoam container

with frozen ice packs. The container could have been slightly warmer than the freezer, but not a

significant factor. There could be not enough amount of DNA to amplify in the first and third set

of analysis. Since there was very low amounts of DNA found in all three phases, the *Taq*

Polymerase probably could not have found the primers and the binding site.

Another factor could be contamination that occurred through extraction. Even though

extraction of DNA from paper occurred in a sterile hood, the extraction method itself could have

caused contamination. The constant changing of tubes and numerous pipetting could have lost

numerous DNA strands that could not be recovered. In the phenol-chloroform extraction, there

were three tube changes in the washing of the sample with chloroform alone. Also, there was the pipetting to a concentrator and constant washing. The DNA strands should have adhered to the concentrator and stayed on during the three washings with distilled water.

The second set of analysis could have given the most optimal results. Selected samples above 25pg/µL from Phase I and Phase II were amplified in triplicate, according to authors [21, 24, 25, 33]. The intention of one sample producing three amplified products was to create replication. With LTDNA, replication has the potential to create a combined full profile using all three samples. Even if the samples from Phase I and Phase II did not have the application of ninhydrin or DFO, it would have significantly resulted in profiles that could be recovered from fingerprints. Unfortunately, the samples were amplified with AMPF*l*STR® Identifiler® after the ruling of the United States District Court for the Western District of Wisconsin [61]. Research with AMPF*l*STR® Identifiler® amplification had to cease once it was known about this ruling.

It is difficult to recover DNA profiles from fingerprints. Research has shown that even though the epithelial cells from a fingerprint is enough for a full profile, there had to be a modification in the DNA analysis procedure that optimized LTDNA, such as increased cycles or increased amounts of reagents used [23, 52, 56-59]. One of the most important things needed for a DNA laboratory is the status of sterilization. A DNA lab needs to be void of contamination. Otherwise, results will be faulty and lose their credibility. When analyzing for LTDNA, the lab and the examiner must make sterilization a top priority because of the small amount of DNA that is potentially present.

This research was attempted to recover profiles under a typical house setting; dust and dirt can collect on things before being analyzed. Dust and dirt present contamination and degraded

DNA to the analysis, especially for LTDNA. Previous authors have stated that ninhydrin and DFO can reduce the recovery of profiles, but not completely inhibit them [52-54, 58]. It was seen that ninhydrin and DFO, coupled with paper found in house settings, will inhibit recovery of profiles and lead to negative results.

LTDNA from fingerprints is a very risky source of DNA. The application of ninhydrin and DFO reduces the probability of recovering any DNA profiles. It would be highly recommended that the forensic scientist or examiner exhaust all other options of analysis before attempting to recover profiles from fingerprints on paper after the application of ninhydrin or DFO. This includes if the paper has been sitting for a significant time or not.

V.   <u>Future Research</u>

Even though the current research did not develop the results as expected, it did generate suggestions and recommendations for future research. Since the phenol chloroform extraction method yielded low amounts of DNA, it is suggested to switch the extraction method to a method that does not use numerous microcentrifuge tube changes. One possible method is the Chelex® 100 Extraction Method. This method does not require as numerous microcentrifuge tube changes and is less of a health hazard than the phenol chloroform extraction method. Another suggestion is to handle the paper mimicking the turning of a page. This introduces more contact between the finger and the paper. This allows cells a more likely chance of adhering to the paper.

It is recommended to have a more sterile laboratory. Since fingerprints contain no to little amount of DNA, any contamination introduced to the process will inhibit results. The last recommendation is to utilize an amplification kit that optimizes low template DNA. The commercial kits used for this research were not sensitive enough to amplify DNA in the samples leading to negative results. A more sensitive kit that is aimed to amplify low amounts of DNA can help create profiles.

VI.   <u>Acknowledgements</u>

VII. <u>References</u>

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