Effects of Temperature and Time since Deposition on Recovery of DNA from Touch Samples

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SUMMARY

Touch DNA analysis is an advanced form of DNA analysis where DNA can be extracted from trace residues deposited on touching an object. It sometimes yields minute quantities of DNA, that vary between donors, conditions of deposition and environmental factors post deposition. Effects of external factors like temperature and time on DNA recovered from blood and other body fluids have been studied previously. The goal of this project was to observe the quality and quantity of DNA recovered after subjecting touch DNA samples to the aforementioned factors. The hypothesis established was that higher temperatures and longer time since deposition will negatively affect DNA yield in terms of quality and quantity.

This research project employed agarose gel electrophoresis to evaluate the quality of DNA, and real-time PCR to get an estimate of the quantity of DNA successfully extracted from touch samples subjected to the various conditions. High temperatures have been known to affect the DNA yield extracted from blood stains over time. Observing the trends in quality and quantity of touch DNA yield after exposure to different temperatures and time since deposition would provide better knowledge on how to collect and analyze these samples. Understanding the effect of time since deposition on DNA yield from touched samples will also help forensic laboratories prioritize samples for analysis, reducing backlogs and improving results.

The degradation of touch DNA samples caused at different temperatures and time intervals was studied. Mock and true fingerprints were collected and subjected to each temperature condition for eleven different time periods, then extracted and quantified using real-time PCR. Mock fingerprints were used due to their unique quality of known amount of initial DNA deposition, from which percent recovery could be calculated. Average percent recoveries after

each treatment were calculated and statistical analysis was conducted to determine if the differences in recovery were statistically significant. Degradation of the extracted DNA was qualitatively analyzed using agarose gel electrophoresis, where the DNA bands were stained with 1X SYBR Gold and viewed under ultraviolet light.

The results indicate that temperature played an important role in the amount of DNA recovered after 2 weeks of ageing, before which recoveries were consistent among different temperatures. At the same temperature, there was maximum difference in amount recovered between time points under a week and those longer than 1 month, indicating that even at 4°C, quantity of DNA recovered declines with prolonged time since deposition and samples should be collected before that to preserve quantity. Interestingly, agarose gels run with true fingerprint samples reveal no substantial decline in quality of DNA extracted, even at 30°C for up to 3 months.

Previous literature indicated significant changes in composition of a fingerprint residue over time. Thus, new artificial fingerprint solutions were made to better represent a true latent print residue, one with eccrine only and one with eccrine and sebaceous secretions both. These new solutions were validated by extraction of different cell counts in 20 replicates and the loss of DNA through the collection/extraction process was monitored. The results support the validity of these fingerprint solutions to prepare mock fingerprints for future research purposes.

Chapter 1: Literature Review

1.1 Introduction

DNA analysis has progressed greatly in the past 66 years: discovery of the structure of the DNA helix by Francis Crick and James Watson in 1953[1], understanding the complementary specificity of the DNA bases and ultimately mapping of the human genome[2]. These developments, along with Sir Alec Jeffreys' discovery in 1984[3] that certain repetitive fragments of a person's DNA, called "mini satellites", are unique to that person, catapulted the use of DNA analysis in the field of forensic science. The individuality of a person's DNA profile provides scientific evidence for the Court. Forensic DNA analysis has surpassed the use of Sir Alec Jeffreys' "mini satellites"[4] to generate DNA profiles. Increased sensitivity of instruments and the techniques employed in DNA analysis has improved the quality and quantity of DNA recovered from forensic samples[5].

One such advancement is the field of touch DNA. Although the terms are often used interchangeably, touch DNA should not be confused with trace DNA, which is the DNA extracted from sources other than body fluids. Touch DNA is a type of trace DNA sample deposited on touching an object, eliminating the restriction of body tissue or body fluid as the required sample for DNA analysis. However, touch DNA analysis is very new, leaving various aspects of it unexplored. The source of DNA from touched samples is not entirely the same as the source of DNA in blood, semen, or other samples such as hair [6]. Touch DNA is believed to be obtained from epithelial cells[7], trace amounts of body fluids, and extracellular DNA associated with skin cells[8]. This implies that physical and chemical properties of the cells providing DNA in case of touched samples is probably not the same as the DNA obtained from traditionally biological sources.

1.2 DNA in Forensic Science

Deoxyribonucleic acid (DNA) is a biomolecule that forms the genetic material which we inherit from our parents. Primarily, this double-stranded molecule contains specific sequences encoding the instructions for all cells in our body. Forensic analysis of DNA exploits the uniqueness of short stretches of repetitive genetic code between individuals. A sequence of the unique stretches of code within a person's DNA forms his genetic profile[9], giving DNA an immense power of discrimination. Analysis of DNA associated with a crime scene, a victim or a suspect provides scientific, physical evidence which is useful for presentation in Court.

Common DNA samples are body fluids, such as blood, semen, saliva, or cellular tissue, hair and more recently, skin cells and residues left upon touching an object. The process of forensic analysis of DNA roughly entails the following steps: extraction, quantification, amplification, and STR (Short Tandem Repeats) profiling. The main idea behind an extraction is to release the DNA from the cell and separate it from the rest of the cell materials such as proteins and lipids[10]. The next step is quantitation, which involves measuring the total amount of human DNA present in the sample. Since microbes are omnipresent, it is quite common to encounter microbial DNA during extraction. It is thus helpful to quantitate the amount of actual human DNA present in the extracted sample by using human-specific probes during quantification. Amplification refers to an exponential replication of the sample using a polymerase chain reaction (PCR). Human-specific primers are used to amplify desired loci to eventually be used in downstream genetic analysis. The amplified PCR product is then profiled based on the STRs present in the DNA sequence to determine the alleles at each locus[11].

1.3 Touch DNA

The field of touch DNA is a specialized branch of forensic DNA analysis which deals with trace amounts of DNA extracted from shed skin cells and residues left behind on touching an object. Its development was possible partially due to the increased sensitivity of extraction methods and amplification of trace quantities of DNA, and partially due to the already developed field of fingerprinting, since extensive research into the components of a latent print residue has been conducted to develop better techniques to visualize them[12,13]. Touch DNA analysis is more informative than regular DNA analysis when it becomes important to answer the question, "Who held this object?". With the progress of touch DNA analysis, we can now extract DNA from non-biological substrates[14], i.e. any object that contains trace quantities of human nucleated cells deposited through touch or other indirect contact can serve as a source for DNA analysis. Although still in research stages, this point of contact can now be visualized with Diamond Dye, a nucleic acid staining dye which specifically binds to human DNA on the external groove, with no effect on downstream analyses.

The advancement of touch DNA has benefited the forensic community by allowing genetic profiles to be generated from objects which previously would not have yielded any genetic information[14]. Trace DNA has been extracted and successful profiles generated from eyeglasses, T-shirts, earphones and watches[15]. Source of origin of touch DNA is a much debated topic in the field, with extracellular DNA considered a major contribution to the DNA found on our skin. Minute traces of saliva and epithelial cell associated cell-free DNA have been found to add to the presence of DNA on our fingertips. The effect of environmental conditions on such samples will help us understand their source and contribute to the literature to better methods of their extraction and analysis.

The amount of DNA recovered from touch samples varies widely and depends on numerous factors: the donor's shedder status[10,16], conditions of deposition, and environmental conditions after deposition[17]. Although the pre-deposition conditions cannot be controlled with factors such as age, sex, medical conditions, prior activities affecting amount of DNA left behind by a donor, the post-deposition conditions such as environmental conditions and time since deposition can be controlled and their effect on amount recovered can be evaluated. The collection and extraction protocol for touch DNA analysis needs to be optimized to maximize DNA yields from samples left at crime scenes[18].

1.4 Latent Print Residue

Fingerprints are a common type of physical evidence found at crime scenes, their evidentiary value being the uniqueness of the pattern deposited. A fingerprint in which the pattern cannot be seen readily by the naked eye is called a latent print by the forensic community and numerous methods exist to develop these prints for better visualization. The composition of a true fingerprint has been studied extensively by the fingerprint community as knowledge of the chemistry involved in a latent print residue helps develop newer, better techniques for its visualization.

Published literature defines a latent print residue, which forms on the tips of our fingers, as a mixture of sweat, sebaceous secretions from touching our face/hair and trace amounts of body fluids i.e. blood, saliva. This implies multiple sources of DNA in a latent print residue, mainly the trace amounts of body fluids, shed non-keratinized epithelial cells, and cell-free DNA carried through pore ducts[8]. True fingerprints have been analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and it has been found to comprise of two types of secretions. One, the eccrine secretion, or sweat, contains 98% water, minute quantities of inorganic salts, amino acids and trace amounts of other water-soluble chemicals. Serine was the most abundant amino acid

found in a latent print reside, followed by glycine, aspartic acid and alanine[19]. The second, sebaceous secretion, accumulates on our fingertips through touching our face or hair, surfaces which contain oily secretions from our body. This component mainly consists of fatty acids, triglycerides and other fat-soluble components. The components detected through the GC-MS analysis showed concentrations of these components and interestingly, no statistical correlation could be found between GC-MS graphs and donor traits, revealing the high intra-donor variability between fingerprint residues[20].

The composition of a latent print residue changes with time[21], influenced by numerous factors both prior to and after deposition. There is gap in knowledge regarding the interaction of the components of a fingerprint with various environmental conditions and changes in their physical aspects with time[22]. In general, work done by Archer et al. proves there is significant reduction in quantity of certain lipid components of a latent print residue stored at room temperature over the period of one month[23]. This analysis was done through GC-MS and peak heights were compared at multiple time points.

A more intensive study on assessing the quality of latent prints developed after storage in various laboratory-controlled conditions attempted to estimate a rate of degradation of fingerprint residue over time[24]. It has been found that over a period of 2 weeks, a latent print residue loses 85% of its mass, attributed to loss of water and volatile components such as cholesterol and free fatty acids. After this time, the fingerprint residue becomes more viscous and prone to changes in physical disturbance of the environment due to increased brittleness of the dried residue[25]. This was corroborated by a study analyzing fingerprints using atomic force microscopy over the period of one month[26]. They found changes in surface adhesion and topography of fingerprints over time, suggesting changes in chemical composition of the residue. Concentration of cholesterol and

unsaturated lipids decreased proportionally with time, with amino acids remaining relatively stable. These internal changes in the chemical environment of a fingerprint over time cause changes in its interaction with the external environment it is subjected to.

1.5 DNA Degradation

An important aspect of genetic material that affects forensic analyses is DNA degradation. Although DNA is a relatively stable molecule in vivo, it has limited chemical stability once outside the body[27]. DNA degrades when exposed to environmental conditions such as temperature[28] or ultraviolet light[29]. Degraded DNA can yield incomplete profiles with loss in number of detectable loci[30], thereby reducing the power of discrimination of DNA profiles. Modified protocols of quantitation by qPCR with TaqMan technology have shown to be indicative of degradation in DNA samples and serve as an important research tool for the post-extraction processing of DNA[31]. It is essential to understand if, and the rate at which, genetic material degrades in quality as well as quantity, to better understand its nature and determine the best practices for its collection, storage and analysis for maximum yields[32].

Degradation of DNA can be brought about in numerous ways, leading to changes in its organic chemistry[33], however, the most important aspect of DNA degradation relevant to forensic science is the introduction of double stranded breaks in the DNA molecule. This structural modification affects amplification of specified loci for downstream analysis, including generation of profiles[34]. A study conducted massively parallel sequencing of DNA extracted from old semen and blood stains and found STR failures and fragmentation of DNA due to degradation occurs uniformly throughout the genome, with no specific sequences or regions more prone to degradation than others[35]. This effect of degradation of DNA emphasizes the need for further research into

patterns of degradation seen in the DNA extracted from touch samples to generate satisfactory profiles for forensic comparison.

1.6 Temperature

Analysis of biological material, especially those needing long-term storage, requires careful consideration of the effects of environmental factors, such as temperature, on the integrity of the material. Particularly, for forensic casework samples, storage protocols, that provide maximum preservation for successful testing and/or retesting in the future, are essential. Previously published literature has shed light on the effects of various environmental factors, such as temperature and humidity, on the extraction efficiency of body fluid samples such as blood, semen, and saliva. Long term storage of DNA is affected by environmental conditions of the storage space[36]. Extracted DNA has been shown to retain maximum integrity at -20°C, with reduced recoveries at 4°C and room temperature[37]. Methods have been developed to promote effective storage of extracted DNA samples at room temperature following the principles of anhydrobiosis, where biological systems are dehydrated to better preserve them for long term storage without affecting their integrity[38,39].

Considering the backlog in forensic laboratories, however, analysts are often tasked with preserving DNA samples before any extraction can be carried out. Forensic casework encounters different sources of DNA i.e. blood, body fluid, semen, tissue, or trace deposits. The nature of the molecule, however, is the same in all samples, with a double helix, complementarity of strands, with the same biochemistry of the genetic material inside the nuclei. Therefore, studies conducted on any source of DNA offers some insight into the behavior of DNA from all sources.

It has been established that DNA analysis from dried bloodstains remains relatively unaffected by harsh environmental conditions, with little to no difference in amplification efficiency when exposed to 93% relative humidity and high temperatures of 45°C for 12 months. However, scientists observed a decline in DNA stability at temperatures of 45°C and 55°C[40]. Additionally, a more recent study determined that DNA extracted from blood/soil mixtures stored at room temperature and 4°C for 12 weeks yielded partial STR profiles[41]. These results are corroborated by another study which stored bloodstains for 20 years at various temperatures. It was shown that, although the DNA yield from samples stored at room temperature and 4°C was significantly degraded in quantity when compared to that obtained from samples stored at -20°C and -80°C, the 4°C and room temperature storage of dried bloodstains did generate partial STR profiles, even after 20 years[42]. These studies reiterate the robustness of DNA analysis from blood, in tubes or as bloodstains, however, they do introduce the possibility of peak loss as a result of room temperature/4°C storage. Touch DNA, already prone to less than optimal yields, requires maximum preservation once deposited. The role of temperature in its degradation process needs to be determined.

Climatic conditions such as temperature and humidity play an important role considering the trace and contamination-prone nature of DNA samples submitted for forensic testing. It becomes crucial to know how an environmental factor such as temperature could affect a certain sample, if we are to maximize DNA recovery from that sample. Before STR profiling became possible, DNA analysis was conducted using a technique called Restriction Fragment Length Polymorphism (RFLP). This required digestion of the extracted DNA by restriction enzymes and analysis of the bands created, usually on a gel which separated them out by size. A pattern of DNA bands was thus called a person's DNA fingerprint.

McNally et al. conducted a research study to evaluate the RFLP pattern generated from bloodstain samples subjected to temperature, relative humidity (RH), UV (light), drying time and soil contamination over a five-day period[43]. They observed no difference in DNA integrity with temperature of 37°C and RH of 0, 33 and 66% RH. The bands in the RFLP profile became weaker with time however, no bands were missing, or new bands created. These results again indicate, that although moderately high temperatures reduce quantity of DNA recovered, the integrity of downstream analyses remains relatively unaffected. The double-stranded helical structure of the DNA molecule, along with loss of the OH group on the 3rd carbon in the DNA sugars provide better stability to this molecule when compared to its single-stranded sister molecule, ribonucleic acid (RNA)[44].

All the literature presented so far has proven that DNA from body fluid samples reduces in quantity at temperatures higher than 25°C, however its integrity is maintained, leading to partial/full STR profiles after several months. While the structural components of DNA from touch samples remains the same as that derived from body fluids, the nature of a trace deposit might afford some interesting pattern to the degradation of DNA in terms of quality and quantity when stored at different temperatures.

1.7 Time since Deposition

Crime scenes do not contain samples with the most ideal quality of DNA for profiling. Even if evidence is collected as soon as the crime is detected, the backlog in the crime labs, especially for DNA analysis of evidence, prevents the collected samples from being analyzed immediately. Samples are stored in evidence locker rooms for weeks or sometimes, months before being examined. This is affordable for body fluid samples such as blood, buccal swabs, or semen

because of the high amount of DNA present in those samples. DNA from blood stained on glass slides has been shown to survive at room temperature with no difference in quality of quantity over 21 days[44]. Touch DNA, on the other hand, with its source of genetic material still relatively unknown, has DNA in such minute quantities, that any loss in the quality or quantity will affect the strength of profiles generated from such samples.

In addition, studies on time since deposition and time since collection focusing on fingerprints as source of DNA have been carried out recently. L Ostojic and E Wurmbach compared STR profiles generated from fingerprint samples left on glass slides over a period of 40 days[45]. They detected full profiles (>70% complete) from more than three-fourths of all samples after 40 days of storage at room temperature. Statistically significant differences were observed between the median of complete profiles at time periods of day 1, day 10, day 20 and day 40, revealing degradation to some extent.

A Russian group of researchers investigated the relationship between reference genetic profiles and profiles generated from fingerprints left on metal surfaces[46]. They concluded no profiles could be generated one month after prints were deposited. They attributed this reduction in quality of profile to degradation of DNA in touched samples when stored at room temperature over time. This provides interesting insight into effect of surface on DNA recovery, with fingerprint samples deposited on non-porous, smooth surfaces such as glass yielded full profiles after 40 days at room temperature, and those deposited on metal surfaces yielding no profiles after a month. The chemical reactivity of the substrate on which samples are deposited, with metal being more reactive than glass, seems to dictate how the fingerprint residue behaves over time. G. E. Meakin et al. carried out a study attempting to simulate real-life scenarios, using knives as the surface of touch DNA deposition and storing the handled knives at room temperature up to a week[47].

Their results showed that DNA of the handler was present in detectable quantities up to a week, with some reduction with time. The collection method here was mini-tape and touch DNA was collected from knife handles.

All previous work detailed above has dealt only with true fingerprints, therefore concrete conclusions, about whether the loss was due to time since deposition or just difference in amount initially deposited, cannot be made. These studies provide a foundation for a more in-depth look into significant trends in DNA loss from touch samples over various times since deposition.

With true fingerprints varying so widely in the amount initially deposited and no way of controlling or predicting how much DNA would have been deposited by a donor, conclusions regarding effect of certain post-deposition conditions on recovery amount seems relative at best. To quantitatively evaluate the trends in recoveries after certain treatments, the amount initially deposited must be known. Mock fingerprints are a step towards that direction, with simulating a true fingerprint in terms of chemicals involved, the difference being, amount of DNA is calculated and known prior to deposition.

1.8 Real-Time PCR

Real-time PCR, also known as quantitative PCR (qPCR), is a technique commonly used to quantify samples of forensic interest for downstream STR or mitochondrial DNA profiling[48,49]. It follows the same principles of a polymerase chain reaction, which is used to exponentially amplify target sequences in a sample, the difference between regular PCR and qPCR being, the ability to measure the quantity of nucleic acid initially present in the sample with real-time PCR (qPCR)[50]. Some real-time PCR techniques use fluorescent dyes such as SYBR Green, to bind non-specifically to double-stranded DNA in the exponential phase of amplification[51,52]. It measures

the amount of time a sample takes to reach a certain cycle threshold (Ct value) of detectable fluorescence. A sample with higher nucleic acid concentration reaches the threshold sooner (lower Ct value). A standard curve run with fluorescence measured at known concentrations allows the concentration of unknown samples to be determined.

Using human sequence specific probes for qPCR assays has made it possible to quantify the amount of human DNA in a sample[53], which is now a standard requirement by most forensic DNA quality assurance and quality control guidelines. ALU sequences are short interspersed repetitive nuclear elements (SINEs), approximately 300bp in size, found abundantly in the human genome[54].The presence of ALU sequences in high copy numbers at fixed intervals throughout the human genome makes them good targets for specifically quantifying human DNA in a PCR assay[55]. Real-time PCR using ALU primers and measuring fluorescence with a SYBR Green dye is now a common procedure in research laboratories and its wide range of detection is especially beneficial while quantifying touch DNA during forensic analysis[56,57].

Real-time PCR assays using TaqMan probes have been modified recently to detect degradation in forensic samples, allowing the analyst to appropriately modify downstream processing methods[58,59]. TaqMan technology uses specially designed probes to detect amplification of specific loci, contrary to SYBR Green dyes which bind non-specifically to double-stranded DNA. Detection of the extent of degradation in artificially degraded samples using TaqMan probes has been linked to loss of peaks in STR profiles[30,60]. Given the trace amount of DNA extracted from touch samples, identifying trends in DNA loss after being exposed to sources of degradation will add to the present literature on this topic.

1.9 Agarose Gel Electrophoresis

Electrophoresis is the separation of the components of a macromolecule, through an electric field, based on their relative charge/mass ratios. All variations of this technique involve loading the samples onto a solid matrix and placing the matrix in a mobile phase, usually a buffer, through which an electric current is applied[61]. Separation of nucleic acids has radically improved with the development of this technique, given that their fragments can be visualized post-separation. Agarose and polyacrylamide gels are routinely used as the solid phase for nucleic acid separation[62], and these gels can be stained using one of many available dyes which bind to nucleic acids and fluoresce under ultraviolet light[63].

DNA fragments move through the pores of the gel at different rates, with the shorter fragments traveling faster than the larger, bulkier fragments. The concentration of the gel determines its pore size and consequently affects the rate of mobility of the samples[64]. A molecular weight ladder, with DNA fragments of known sizes, is commonly run along with the DNA samples to comparatively determine the size of the fragments in the sample, with high molecular weight bands being indicative of high quality DNA and smaller sized fragments, produced by double-stranded breaks in the DNA molecule, indicating decline in DNA quality. Ethidium bromide (EtBr) is commonly used to stain DNA strands in a gel, binding non-specifically to double- stranded DNA and fluorescing under ultraviolet light[65]. However, other, less harmful, staining methods have been developed, which stain DNA fragments with greater sensitivity[66]. SYBR Gold has outperformed numerous dyes, in terms of staining fragments as small as 50bp, detecting DNA in minute amounts of 20 picograms, without affecting molecular techniques downstream[67].

In the field of forensic science, agarose gel electrophoresis serves as a research tool to visualize the quality of nucleic acids present in a sample. Degradation of DNA produces an array of smaller sized fragments, which appear as smears on a gel, as compared to tight linear bands of high-quality DNA containing larger sized fragments.[68]

Chapter 2: Effects of Temperature and Time since Deposition on DNA Recovery from Touch Samples

2.1 Introduction

2.1.1 Background

Forensic analysis of DNA sometimes requires analysis of DNA extracted from samples exposed to adverse environmental factors or deposited over unknown time periods. Touch DNA is a novel field in forensic DNA analysis where genetic material can be extracted from fingerprints, or residues left behind on touching an object. This genetic material, however, is present in trace quantities and numerous intrinsic and extrinsic factors affect the amount of DNA deposited and the amount of DNA collected from these samples. The intrinsic factors affecting the amount deposited mainly include the donor's shedder status, the pressure applied when touching a surface and activities performed prior to deposition[69]. Environmental and temporal aspects are part of the extrinsic factors that affect the amount of DNA available for collection and analysis.

There is little that can be done in terms of controlling the intrinsic factors when considering a crime scene. However, understanding how the extrinsic factors affect the amount of DNA available from these samples will assist in the development of better collection techniques to minimize loss after deposition, and provide insight into the source of DNA in touch samples. This research focused intensively on temperature and time since deposition and their effects on the amount of DNA recovered after analysis.

To standardize the amount of DNA initially deposited, "mock fingerprints" were used. Mock fingerprints have been created and validated to replicate a true fingerprint residue with a known number of buccal cells, resulting in a known quantity of DNA deposited [manuscript has been submitted]. Mock fingerprints have been used to track the loss of DNA in the process of collection and extraction [manuscript has been submitted] and have the potential to be an extremely useful resource in future research involving touch DNA, with the ability to calculate percent recoveries after the samples have been exposed to different treatments.

The mock fingerprint cell counts chosen were representative of the amount of DNA commonly recovered from touch samples, i.e. between 0-9 ng of DNA. Each cell count was deposited in triplicate to derive a good average of the amounts recovered, leading to generation of a standard curve with the 5 cell counts. In order to get a good average of recovery from true fingerprints, them being more variable, 20 of the true fingerprints were collected to be averaged and quantitatively compared, and 4 of the fingerprints were collected to be later combined to serve as concentrated samples for quality assessment using agarose gel electrophoresis.

The three replicates of 5 chosen mock fingerprint cell counts, along with 24 true fingerprints were deposited on clean and sterile glass slides and placed in each temperature setting for the different time points. Once the samples had aged for a given time period, they were collected using DNA-free cotton-tipped swabs moistened with 2% SDS. The standard laboratory protocol for organic extraction was then followed for each sample and the DNA was eluted in 50 µl TE^{-4} buffer. Real-time PCR was carried out to quantify the samples and an average recovery was calculated from the mock fingerprints as a percentage of the amount of DNA deposited. The formula used was:

$$\textbf{\textit{Percent Recovery}} = \frac{\textbf{\textit{Amount Recovered}}\ (\textbf{\textit{ng}})}{\textbf{\textit{Amount Deposited}}\ (\textbf{\textit{ng}})}\ \textbf{\textit{x 100\%}}$$

However, since this research aimed to study how these external variables affected degradation rate of DNA, representing this attribute in terms of percent loss was considered more appropriate.

$$\textbf{\textit{Percent Loss = 100 - Percent Recovery}}$$

The quantities of the three replicates of each mock fingerprint cell count, along with the true fingerprints, were averaged and this data was used to statistically compare the difference between DNA recovery after each treatment.

2.1.2 Sample Treatments

When it comes to storing trace DNA evidence, every little determinant that can better preserve the quality and quantity of DNA available for downstream processing is critical for an analyst. For any biological sample, temperature is a common factor which affects the stability and characteristics of that sample. Theoretically, samples stored at colder temperatures retain their integrity longer since the mechanisms that aid their degradation cease to occur.

Three different temperature settings were arranged to observe the degradation of touch DNA when stored in each. One set of samples was stored at 4°C which is considered the ideal temperature to store biological samples prior to analysis. Another set of samples was kept at room temperature which was recorded daily throughout this experiment. This setting was chosen to see how long DNA, a relatively stable biomolecule, could retain its integrity when stored at room temperature instead of at 4°C. Lastly, one set of samples was stored in an oven maintained at 30°C, to see the effect of moderately high temperatures, commonly experienced during summers in the United States, on the quality and quantity of DNA extracted.

The availability of highly advanced equipment has tremendously increased the sensitivity of extraction and profiling technologies. DNA can now be recovered from decade- and century-old specimens[71], depending on the sample type and its preservation condition. The possibility of successful DNA analysis from such old samples brings into question how long touch samples would retain their DNA for future analysis.

To study the rate of degradation intensively, multiple time points were selected, with a good range of distance between them. The three replicates of the 5 cell counts' mock fingerprints and the 24 true fingerprints were subjected to each temperature setting for 1 hour, 24 hours, 2 days, 3 days, 5 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months and 3 months. This spacing of time points also allowed us to note after what time point precisely, the temperature played more of a role in DNA loss.

2.2 Materials and Methods

2.2.1 Experimental Setup Summary

The effects of two variables i.e. temperature and time-since-deposition on the quality and quantity of DNA recovered from touched samples formed the first two aims of this thesis. An experimental design to combine these two variables was formulated. Three temperature settings and eleven time points were considered, forming a total of 33 independent treatments. Samples were placed in each of the three temperature environments for the different time points and the DNA extracted from these samples was compared in quantity and quality.

2.2.2 Substrate Surface

Glass slides were used as the substrate on which the mock and true fingerprints were deposited. The slides were soaked, 50 at a time, in a beaker containing 100 ml of 1% bleach (The Clorox Company, Oakland, CA) for 10 minutes. The bleach was discarded, and the slides were then soaked in 100 ml distilled water for an additional 10 minutes. Each slide was then wiped clean with 70% cleaning ethanol, wrapped individually in aluminum foil and autoclaved using the dry cycle: Sterilization for 35-40 minutes and Drying for 10-15 minutes. These sterile glass slides were stored at room temperature.

2.2.3 Cell Counting

Mock fingerprints contain artificial fingerprint residue, along with a known quantity of DNA. Buccal cells were de-clumped, stained and counted using a Hemocytometer (Bright-Line, Hausser Scientific, Horsham, PA). A fresh buccal swab was cut off the swab and placed in 1 ml of Accumax™, a cell dissociation solution, containing collagenase and proteolytic enzymes, for 15 minutes, with careful pipetting every 5 minutes to disturb the cells. After 15 minutes, the buccal swab was removed from the solution and discarded. The Accumax™ solution containing the buccal cells was incubated at room temperature for another 15 minutes, with regular pipetting, to further aid cell dissociation.

The de-clumped buccal cell suspension prepared with the help of Accumax™ formed the source of DNA used to prepare mock fingerprints. Ten microliters of the suspension was taken in a sterile 0.6 ml tube and the cells were stained using 10 microliters of Trypan Blue dye. This suspension was again pipetted to ensure appropriate staining and 10 microliters of the solution was added to each side of the Hemocytometer slide. The hemocytometer slide had provision for two 10 microliter suspensions to be counted, with each block further divided in 9 squares to make counting easy. The cells were viewed under 10X magnification and each side counted separately, with a total of 9 readings on each side. Since the cell suspension viewed under the microscope had an equal volume of dye included, the sum from each side was multiplied by the dilution factor, i.e. 2, to calculate thetotal cells counted.

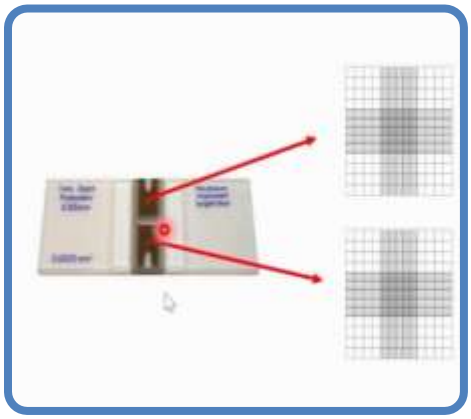


Figure 1: Hemocytometer slide used for cell counting

In order to convert the cells counted under the microscope to cells/µl, the following formula was used:

$$\textit{Cells/ul} = \frac{\textit{Total number of cells counted on each side}}{9} \textit{ x 10}$$

The value derived from the formula from both sides was averaged to obtain the final cells/µl to be used in further calculations. To determine the amount of suspension needed for each mock fingerprint solution, the cell counts were divided by the cells/µl calculated for the cell suspension. For example, if the formula yielded an average of 200 cells/µl in the suspension, the amount of cell solution to be added for the 275 cell count would be 275/200=1.375, for the 475 cell count as 475/200=2.375, and so on.

2.2.4 Preparing the Mock Fingerprints

The laboratory protocol for preparing mock fingerprints was followed, with each mock fingerprint solution having a final volume of 20 µl: 2 µl of fingerprint solution, and a combined 18 µl of cell suspension and 1X PBS, depending on the cell count. Each cell count's mock fingerprint solutions were made for 10 mock fingerprints at a time, since the 3 replicates in each temperature setting [3*3=9 prints in total for each cell count] had to be deposited in one setting. All amounts making up each cell count's mock fingerprint was thus multiplied by 10. The final solutions were mixed well by pipetting and 9 replicates of each cell count solution were pipetted onto clean, sterile glass slides under the hood and left undisturbed until completely dry. Three replicates of each cell count's mock fingerprints were placed in each of the three temperature settings i.e. 4°C, room temperature and 30°C, for the different time periods.

2.2.5 Collection of True Fingerprints

True fingerprints were collected from four subjects who were instructed to go about their daily activities without washing their hands an hour prior to deposition. Each subject deposited 6 fingerprints, one each from the index, middle and ring fingers of both hands, on sterile glass slides, amounting to 6 prints from each subject. The subjects were instructed to roll their fingers on the slides with moderate pressure for 10-15 seconds. A total of 24 fingerprints was thus collected from 4 subjects for each independent treatment.

2.2.6 Extraction

Once the mock and true fingerprints, placed in 4°C, room temperature and 30°C, had aged for a set time period, all the samples were extracted using the laboratory protocol for organic extraction: each print was collected off the slide with a sterile cotton swab (Puritan Medical Products Company LLC, Guilford, Maine) moistened with 20 µl of 2% SDS. Each swab was cut and placed in a sterile 2 ml extraction tube (Corning Incorporated, Salt Lake City, UT), to which 400 µl of stock extraction buffer and 13 µl of 20 mg/ml proteinase K were added and incubated at 56°C overnight. Each time-point extraction also included a positive control i.e. a fresh buccal swab; a blank swab control; and a negative control i.e. reagent blank, which included only the extraction buffer and proteinase K. The next day, the swabs were removed from each tube and placed in spin baskets (Corning Incorporated, Salt Lake City, UT) placed over the 2 ml tubes and centrifuged at 19000 x g for 5 minutes (Hermle Labortechnik, Germany) to remove the excess liquid into the 2 ml tubes. The dried swabs and the spin baskets were then discarded and 400 µl of 25:24:1 phenol-chloroform-isoamyl alcohol (Acros Organics, New Jersey) was added to the extraction tubes. These tubes were inverted vigorously to ensure sufficient interaction between the two phases, after which they were centrifuged at 19000 x g for 5 minutes to allow the two phases to separate, with the heavier, phenol-chloroform layer containing cell debris in the bottom, isolating the DNA in the upper aqueous layer.

The upper, DNA-containing layer was transferred to sterile 1.5 ml tubes (Brinkmann Instruments Inc., Blue Bell, PA) and 1 ml of cold 100% absolute ethanol (Janssen Pharmaceuticalaan, Fair Lawn, NJ) was added to precipitate the DNA out of solution. The samples, at this stage, were placed in -20°C for an hour for maximum precipitation. After an hour in -20°C, the samples were centrifuged at 19000 x g for 15 minutes, allowing the DNA to pellet at the bottom of each tube. Supernatant ethanol was discarded, and the pellets were washed with 1 ml of 70% room temperature ethanol and centrifuged at the same speed for 5 minutes. The wash was repeated

a second time, the ethanol supernatant discarded, and the pellets were allowed to dry at 56℃ for approximately 2 hours. Each sample was re-solubilized in 50 µl of TE^{-4} buffer and incubated at 56°C overnight.

2.2.7 Quantification

All the extracted samples were quantified using Applied Biosystems™ 7500 Real-Time PCR with SYBR® Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA). Forward and reverse ALU primers (forward: GTCAGGAGATCGAGACCATCCC reverse: TCCTGCCTCAGCCTCCCAAG) were used to amplify human-specific double-stranded DNA fragments and quantify the amount of template DNA that was present in the sample.

The laboratory protocol for quantification using real-time PCR with a SYBR Green target was followed. The first step was preparation of master mix, which included, for each sample quantified: 5 µl of Bio-Rad's 2X SYBR Green Supermix, 2.6 µl of sterile water, and 0.2 µl each of the forward and reverse ALU primers (2.5 pmol/µl). Each well contained 8 µl of master mix and 2 µl of the sample to be quantified. Eight standards were run with each plate to create a standard curve of serial dilutions of the following concentrations, in ng/µl: 16.7, 5.56, 1.85, 0.62, 0.21, 0.068, 0.023, 0.0077. Each PCR plate was prepared with the 8 standard dilutions, a positive amplification control (control genomic DNA) and a negative amplification control or No Template Control (i.e. NTC).

The conditions for each run were as follows: Holding at 95°C for 2 minutes followed by 35 cycles of denaturation at 95°C for 15 seconds and annealing/extending at 68°C for 1 minute. The melt curve stage was set to default: 95°C for 15 seconds, 60°C for 1 minute, 95°C for 30 seconds and 60°C for 15 seconds. All the extracted mock fingerprint and true fingerprint samples for each treatment were quantified using the same protocol and the data was compiled to compare the average loss of DNA after each treatment.

2.2.8 Combining True Fingerprints

To test the process of combining fingerprints at the swabbing step, multiple true fingerprints were deposited on sterile glass slides and using the same swab moistened with 20µl of 2% SDS, one, two and three fingerprints were collected. These swabs were extracted using standard organic extraction protocol and quantified using the real-time PCR protocol described above. To test the process of combining fingerprints at the ethanol precipitation step, multiple true fingerprints were collected off slides following standard fingerprint collection protocol i.e. with a swab moistened with 20 µl of 2% SDS, and the organic extraction process was started. However, instead of transferring the 400 µl of the aqueous layer into independent 1.5 ml tubes, they were transferred into a single 1.5 ml tube, either two or three at a time. The rest of the original protocol was followed, and the DNA eluted from both mini experiments was quantified using real-time PCR.

The results showed little difference between the quantity of DNA extracted using either protocol, especially when 20 µl of 2% SDS was added before each fingerprint was swabbed using the same swab, as outlined in the first case. Keeping these preliminary results in mind, four fingerprints from each treatment were combined at the swabbing step and run on agarose gels to assess for quality of the DNA recovered.

2.2.9 Agarose Gel Electrophoresis

All gel electrophoresis experiments using ethidium bromide (EtBr) as the staining method were run following standard laboratory procedure: 1000 ml of 1X Tris Acetate EDTA (TAE) buffer was prepared by diluting the 50X stock (recipe on page 28), to which 100 µl of ethidium bromide (Fisher Scientific, Fair Lawn, NJ) was added, 100 ml of this TAE/EtBr buffer was taken in a conical flask and 1 g of agarose (Fisher Scientific, Fair Lawn, NJ) was measured and added to it. The solution was heated in the microwave for 30 second intervals until the agarose was completely dissolved.

The dissolved solution was cooled to about 56°C and poured into a gel tray secured on both open ends by tape and the well combs were inserted. The gel was allowed to set until it hardened completely, and then the combs and tapes were removed. The gel was then placed in the electrophoretic chamber which was filled with the remaining 900 ml of 1X TAE/EtBr buffer. The preliminary gels were run at 120 V for 30 minutes, after which the gel was viewed under 302 nm UV light using Azure Biosystems c400 gel viewer (Azure Biosystems, Dublin, CA, USA). The addition of ethidium bromide to the gel solution and in the running buffer allowed any DNA to be seen under the UV light without any post-run processing.

Experiments were run to test the better staining method between ethidium bromide and SYBR Gold (Life Technologies, Eugene, OR). Gels stained with SYBR Gold were run following the same process described above, except the addition of ethidium bromide to the gel and running buffer was omitted. The SYBR Gold gels were prepared with 1X TAE buffer, run at 120 V for 30 minutes and then post stained with 1X SYBR Gold, which was prepared by diluting the 10,000X stock in 500 ml of TAE buffer. The staining times were tested at 30 minutes, 1 hour and overnight, viewing the gel under 300 nm UV light to determine the optimum staining time.

To determine the sensitivity of an agarose gel using the two staining methods described above, serial dilutions of a standard DNA stock (BioChain, Newark, CA) were quantified on real-time PCR following the lab protocol described previously on page 24, run on native 1% agarose gels, one stained with 1% ethidium bromide and another post- stained with 1X SYBR Gold following the procedures described above. 1X SYBR Gold was chosen as the staining method for future gels because of its relatively higher sensitivity for samples of lower DNA concentration as seen under the UV light.

The above sensitivity experiments revealed that 10 ng of total DNA would be sufficient to be visualized using agarose gels stained by 1X SYBR Gold. The combined fingerprint samples were quantified using real-time PCR to check if they were concentrated enough, any samples lower

than the limit of detection for visualization were discarded and that temperature/time point extraction was repeated.

The final gel runs were designed in a way to allow comparison between the different time point samples in a similar temperature setting. Therefore, three 1% agarose gels were prepared, one for each temperature setting. The gel was double combed, allowing all eleven time point samples, a control DNA sample of known concentration and 4 Lambda/Hind III ladder samples (Promega, Madison, WI) to be loaded in a single run.

The agarose gels were each made by mixing 1 gram of agarose in 100 ml of 1X TAE buffer. The solution was heated in the microwave for 30 second intervals until the agarose was completely dissolved. Once the agarose solution had cooled till about 56°C, it was poured on the gel tray, covered tightly on both ends by tape. The combs were inserted in the gel solution and the gel was allowed to set for approximately 20 minutes until it hardened, after which the tapes from both sides were removed and the gel was placed in an electrophoretic chamber filled with 1 liter of 1X TAE running buffer, ensuring the wells were completely submerged. The combined fingerprint samples were mixed with a 6X loading dye in the ratio of 5:1 sample:dye i.e., 40 µl of the sample was mixed with 8 µl of the loading dye. As for the molecular weight ladder, 1 µl of the Lambda/Hind III ladder was mixed with 159 µl of sterile water and 32 µl of 6X loading dye. The ladder sample was inserted in 4 wells, on both ends of the double-combed gel. A control sample was prepared by mixing 10 µl of the known, standard DNA sample (BioChain, Newark, CA) with 30 µl of sterile water and 8 µl of the 6X loading dye. The final volume for each sample was 48 µl, inserted in the wells carefully. Once all the samples were loaded, the gels were run for 1 hour at 120 V and then stained using 1X SYBR Gold solution prepared in a plastic tray and placed on a shaker. After 1 hour of staining, the gels were viewed under 302 nm UV light at different exposure times to determine any visible degradation at higher temperatures and longer time since deposition.

2.2.10 Prepared Stock Reagents

Extraction buffer used for all organic extraction protocols included: 5.84 g NaCl, 0.5 M EDTA, 1M Tris-HCl (pH 8.0), mixed in 900 ml deionized water and 100 ml 20% SDS added after autoclave. The buffer used to elute the DNA pellet after extraction, i.e. TE^{-4} buffer included: 1M Tris and 0.5 M EDTA. Agarose gels were prepared and run in 1 X TAE buffer, which was diluted from a 50X stock containing: 242 g Tris base, glacial acetic acid, 0.5 M EDTA (pH 8.0). The artificial fingerprint solution used to create mock fingerprints for these aims included: 0.19 M urea, 0.195 M NaCl, 0.086 M KCl, 0.0678 M lactic acid.

2.3 Results and Discussion

2.3.1 Optimization of Real-Time PCR

Studying the rate of degradation meant quantifying the amount of DNA extracted from touch samples exposed to each treatment. The protocol for real-time PCR was optimized to include known standards of lower quantity for accurate touch DNA quantification. The anneal/extend temperature was also experimented with, to generate sharp, narrow melt curves. A higher anneal/extend temperature of 68°C yielded better melt curve peaks, indicating higher specificity of DNA-dye binding. The real-time PCR protocol was therefore modified to increase the anneal/extend temperature from 60°C to 68°C and implemented as such for all further quantifications.

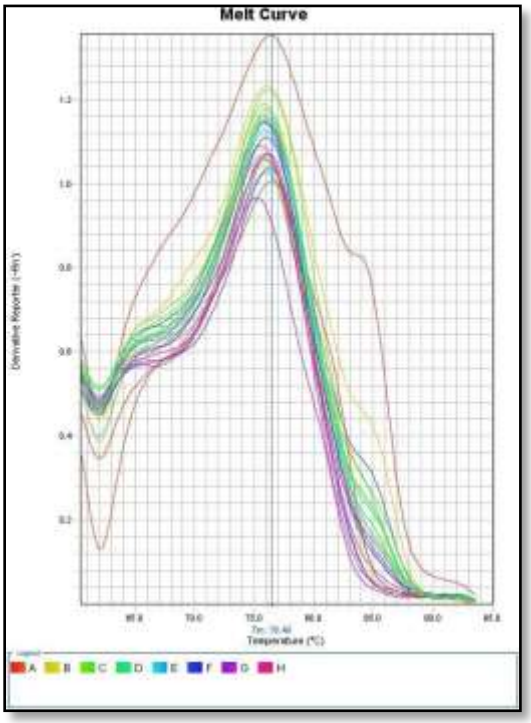


Figure 2: Melt Curve at 60°C

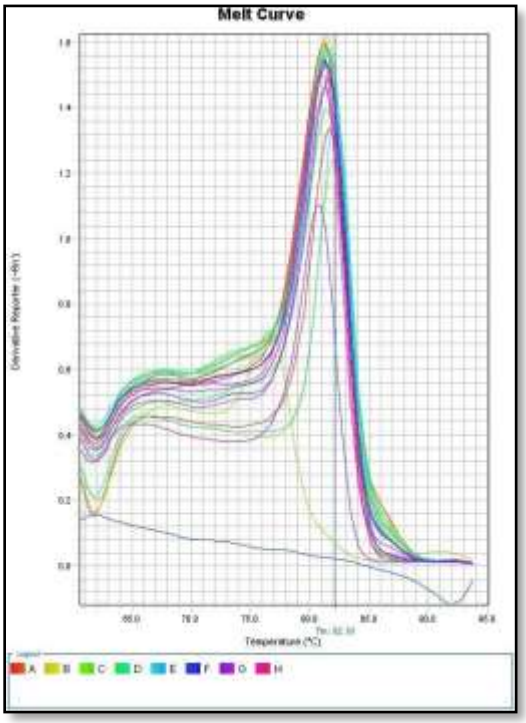


Figure 3: Melt Curve at 68°C

2.3.2 Optimization of Agarose Gel Electrophoresis

As DNA degrades, it fragments into smaller strands and bases oxidize leading to denaturation. The quality of DNA can be assessed through gel electrophoresis, where high-quality DNA is visualized as tight bands, and degraded DNA is seen as a smear[34]. Agarose gel electrophoresis was thus chosen to visualize the extent of degradation caused due to temperature and time-since-deposition. The maximum volume that could be added to a well was decided to be 40 µl, as the extracted DNA samples were eluted in 50 µl of TE^{-4} buffer. Since the sensitivity of an agarose gel is a lot lower than real-time PCR, DNA extracted from single true fingerprints, although quantified by real-time PCR, would not be visible on the agarose gel. Preliminary experiments were run with samples of standard DNA stock dilutions and DNA extracted from true fingerprints on a 1% agarose gel stained with ethidium bromide according to the protocol described in the previous section. The image below *(Figure 4)* shows the double-combed agarose gel as viewed under 302 nm ultraviolet light. The stock dilutions are visible as bands gradually decreasing in intensity away from the DNA molecular weight ladder. The right side of the gel contains the wells in which the true fingerprints were added. No visible bands are seen from the true fingerprints' samples.

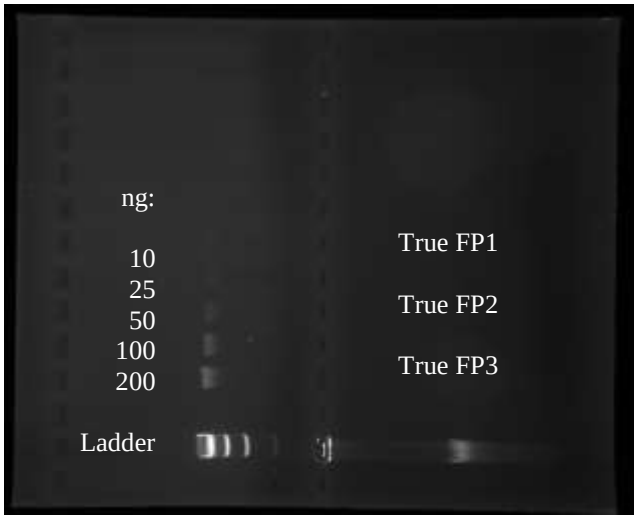


Figure 4: Gel run with DNA stock dilutions and single true fingerprints

Keeping these results in mind, further experiments were conducted to, first, determine the sensitivity of the agarose-ethidium bromide gel, with regard to the minimum amount of DNA needed for bands to be visible on the gel, and second, how true fingerprints could be combined, to provide a more concentrated sample for visualizing the quality of DNA.

Concentration of the Gel

Two agarose gels were run with the same DNA dilutions and a Lambda/Hind III ladder, with one gel containing 1% agarose in 1X TAE/EtBr buffer *(Figure 5)* and the other containing 3% agarose in 1X TAE/EtBr buffer *(Figure 6)*, to determine if the concentration of the gel affected the sensitivity as visualized by ethidium bromide. However, the 3% gel only yielded tighter bands, with the limit of detection still 25 ng in both the 1% and the 3% gels. As the pore size of the 3% gel was comparatively smaller than that of the 1% gel, DNA travelled through the gel at a slower rate, accumulating into tighter bands.

Figure 5: DNA stock dilutions on 1% agarose gel (A-DNA ladder, B-blank, C-200ng, D-100ng, E-50ng, F-25ng, G-10ng, H-5ng)

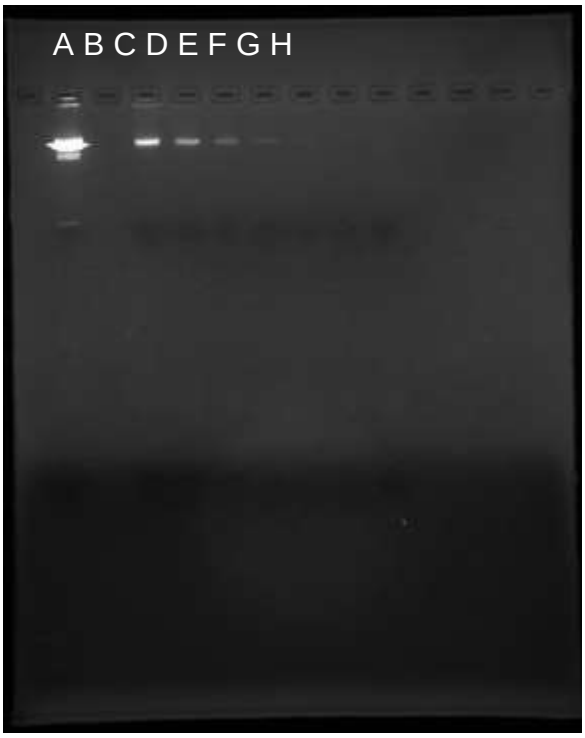


Figure 6: DNA stock dilutions on a 3% agarose gel (A-DNA ladder, B-blank, C-200ng, D-100ng, E-50ng, F-25ng, G- 10ng, H-5ng)

It was thus concluded that the concentration of the gel did not affect the sensitivity of the gel electrophoresis process and for all further experiments, 1% agarose gels in 1X TAE buffer were used.

Staining Method

The next step of the optimization process was testing different staining methods to establish the more sensitive dye between the two, ethidium bromide and SYBR Gold. To test the sensitivity of SYBR Gold, a 1% agarose gel was prepared in 1X TAE buffer and the standard DNA stock dilutions were run on it at 120 V for 40 minutes *(Figure 8).* The gel was stained post-run with 1X SYBR Gold stain for an hour and viewed under UV light. The bands on the SYBR Gold-stained gel were compared to the bands seen on the 1% agarose gel stained with ethidium bromide *(Figure 7).*

Figure 7: DNA stock dilutions on gel stained with ethidium bromide (A-DNA ladder, B-blank, C-200ng, D-100ng, E- 50ng, F-25ng, G-10ng, H-5ng)

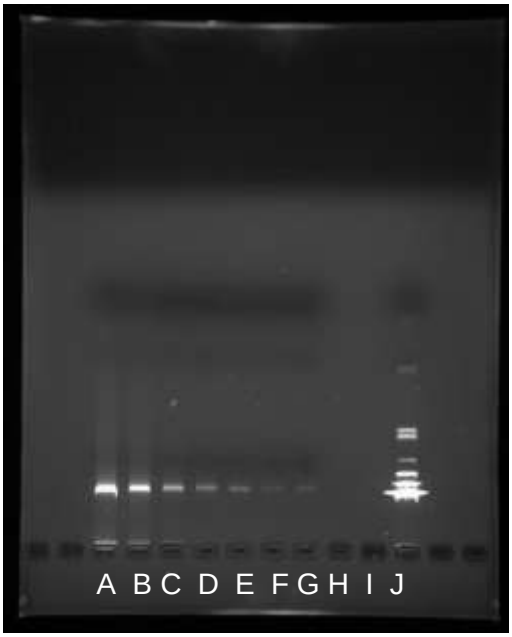


Figure 8: DNA stock dilutions on gel stained with 1X SYBR Gold (A-200ng, B-100ng, C-50ng, D-25ng, E-10ng, F-5ng G-2.5 H-Blank, I-Blank, J-DNA ladder)

This experiment successfully helped ascertain that 1X SYBR Gold was 2.5 times more sensitive than ethidium bromide, and that the lowest amount of DNA needed for any bands or any smearing of bands to be easily visualized on a 1% agarose gel stained with 1X SYBR Gold was 10 ng.

2.3.3 Combining True Fingerprints

To visualize any damage caused to the DNA recovered, agarose gels were run, with the tightness of the bands being indicative of the quality of DNA in the sample. However, touch samples i.e. true fingerprints, contain trace amounts of DNA which escapes detection in regular agarose gels. A more concentrated sample was required to visualize degradation from true fingerprints exposed to the different treatments. The concept of combining fingerprints at different stages of the extraction protocol was discussed and test extractions were conducted (protocol described in materials/methods section), combining mock fingerprints either at the swabbing step or at the ethanol precipitation step.

Sample	Quantity (ng/µl)	Total DNA (ng)	% Recovery	% Loss
MockFP_250cells	0.02	0.89	59.66	40.34
MockFP_250cellscombined	0.04	2.17	72.17	27.83
MockFP_250cellsswabbed	0.04	2.02	67.37	32.63
MockFP_1500cells	0.15	7.69	85.44	14.56
MockFP_1500cellscombined	0.18	9.03	50.15	49.85
MockFP_1500cellsswabbed	0.17	8.71	48.36	51.64
Buccalpos_1:100	2.49	124.73		
Extneg	0.00	0.00		
NTC	0.00	0.00		

Table I: Real-time PCR Data for Mock FP Combination Experiments

The above preliminary testing summarized in *Table I* showed no significant difference in the amount of DNA recovered when two mock fingerprints were swabbed together using the same swab and when the individually swabbed mock fingerprints were combined at the ethanol precipitation step of the organic extraction protocol. Both techniques yielded roughly double the amount of DNA compared to a single mock fingerprint sample, regardless of the cell count.

Therefore, to assess the quality of DNA recovered, four true fingerprints from each treatment were combined at the swabbing step, as described in the materials and methods section, quantified using the real-time PCR and any sample below the threshold for visualization in gel i.e. 10 ng total DNA, was discarded and more fingerprints were collected and the extraction for that treatment was repeated.

2.3.4 Cell Counts and Calculations for Mock Fingerprint Preparation

Mock fingerprint preparation includes counting epithelial cells under a microscope and computing the amount of cell suspension needed for depositing a known amount of DNA. Five cell counts were used to prepare mock fingerprints i.e. 0, 275, 475, 825 and 1500 cells. The cells were dissociated using a cell dissociation solution containing collagenase and proteolytic enzymes and stained as described in the material and methods section. The cells counted under the microscope using a hemocytometer slide. All samples for a given time point were prepared in the same day and left at the three temperature settings. The cell counts and calculations for each time point are given in *Table II* and *Table III*:

	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	11	8	12	4	10	6	5	5	15	17	9
2	9	11	14	5	11	7	4	5	15	12	8
3	12	9	10	6	10	7	3	13	17	6	17
4	15	12	11	2	7	4	8	4	19	19	16
5	8	10	9	4	12	5	5	9	17	12	13
6	8	10	13	5	7	4	6	6	15	14	12
7	10	8	10	1	6	10	7	8	14	14	6
8	11	9	12	4	11	4	5	9	23	11	14
9	14	10	10	5	8	11	5	10	13	18	15
10	10	7	11	5	9	5	5	8	16	9	18
11	11	10	10	2	7	6	9	13	8	13	13
12	10	9	11	5	9	13	6	12	10	14	17
13	11	9	11	5	11	8	10	5	14	18	12
14	16	7	8	7	10	8	8	9	9	19	12
15	6	8	12	4	5	7	3	9	15	13	19
16	8	13	12	4	8	10	2	4	21	12	16
17	15	12	9	3	6	12	4	13	17	13	14
18	12	8	10	5	12	5	10	12	20	14	12
Mean	10.94	9.44	10.83	4.22	8.83	7.33	5.83	8.56	15.44	13.78	13.50
SD	2.66	1.67	1.46	1.44	2.11	2.75	2.32	3.10	3.85	3.36	3.39

Table II: Cell Count Readings for Mock Fingerprints for each Time Point

For each time point, the total number of cells counted on each side using the hemocytometer were converted to cells/µl using the formula mentioned in the materials and methods section and averaged.

$$\textbf{\textit{Cells/µl}} = \frac{\textbf{\textit{Total number of cells counted on each side}}}{9} \textbf{\textit{ x 10}}$$

Since 9 replicates of each cell count mock fingerprints were needed for each time (3 replicates in 3 temperatures), the calculations for the preparation of mock fingerprints were scaled up for 10 prints. Therefore, at each time point, a cell count's mock fingerprint solution included 1X fingerprint solution (FS-1), a combined 180 µl of the cell suspension and 1X PBS solution, depending on the cell count.

	1 hour		1 day		2 days		3 days		5 days		1 week		2 weeks		3 weeks		1 month		2 months		3 months	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
0	0.0	180.0	0.0	180.0	0.0	180.0	0.0	180.0	0.0	180.0	0.0	180.0	0.0	180.0	0.0	180.0	0.0	180.0	0.0	180.0	0.0	180.0
275	12.6	167.4	14.6	165.4	12.7	167.3	32.6	147.4	15.6	164.4	18.8	161.3	23.6	156.4	19.0	161.0	17.8	162.2	20.0	160.0	20.4	159.6
475	21.7	158.3	25.1	154.9	21.9	158.1	56.3	123.8	26.9	153.1	32.4	147.6	40.7	139.3	32.9	147.1	30.8	149.2	34.5	145.5	35.2	144.8
825	37.7	142.3	43.7	136.3	38.1	141.9	97.7	82.3	46.7	133.3	56.3	123.8	70.7	109.3	57.1	122.9	53.4	126.6	59.9	120.1	61.1	118.9
1500	68.5	111.5	79.4	100.6	69.2	110.8	177.6	2.4	84.9	95.1	102.3	77.7	128.6	51.4	103.8	76.2	97.1	82.9	108.9	71.1	111.1	68.9

Table III: Calculations for Mock Fingerprint Preparation (A-Amount in µl of cell suspension added, B-Amount in µl of 1X PBS added)

Table III shows the calculations used to prepare the mock fingerprints solution for each cell count following the formula:

$$\textbf{\textit{Amount of cell suspension (µl)}} = \frac{\textbf{\textit{Cell Count}}}{\textbf{\textit{Cells/µl}}} \text{ x } 10$$

$$\textbf{\textit{Amount of 1X PBS (µl) = 180 - Amount of cell suspension (µl)}}$$

2.3.5 Quantification of DNA Recovered by Real-Time PCR

Average Percent Loss

All mock fingerprints and true fingerprints for a given time point were extracted together following the organic extraction protocol described earlier and quantified using real-time PCR. The amounts recovered from the three replicates of each mock fingerprint cell count and twenty replicates of true fingerprints placed in each treatment were averaged and % loss was calculated using formula described previously. The following are the averaged quantities of the extracted samples along with the averaged % loss for each mock fingerprint cell count.

1-hour samples:

Sample	Averaged Quantity (ng/µl)	Averaged % Loss
4°C_MockFP_0cells	0	
4°C_MockFP_275cells	0.012	64.83
4°C_MockFP_475cells	0.026	54.80
4°C_MockFP_825cells	0.049	50.88
4°C_MockFP_1500cells	0.113	37.23
4°C_TrueFP	0.007	
4°C _4FPcombined	0.015	
RT_MockFP_0cells	0	
RT_MockFP_275cells	0.008	76.83
RT_MockFP_475cells	0.019	66.72
RT_MockFP_825cells	0.068	31.58
RT_MockFP_1500cells	0.064	64.28
RT_TrueFP	0.016	
RT_4FPcombined	0.114	
30°C _MockFP_0cells	0	
30°C _MockFP_275cells	0.008	74.32
30°C _MockFP_475cells	0.030	48.15
30°C _MockFP_825cells	0.045	54.05
30°C _MockFP_1500cells	0.048	73.18
30°C _TrueFP	0.062	
30°C _4FPcombined	0.162	

1-day samples:

Sample	Averaged Quantity (ng/µl)	Averaged % Loss
4°C _MockFP_0cells	0	
4°C _MockFP_275cells	0.015	55.54
4°C _MockFP_475cells	0.025	56.12
4°C _MockFP_825cells	0.034	65.21
4°C _MockFP_1500cells	0.060	66.76
4°C _TrueFP	0.063	
4°C _4FPcombined	0.096	
RT_MockFP_0cells	0	
RT_MockFP_275cells	0.024	25.80
RT_MockFP_475cells	0.039	31.06
RT_MockFP_825cells	0.066	33.05
RT_MockFP_1500cells	0.157	12.59
RT_TrueFP	0.127	
RT_4FPcombined	0.324	
30°C _MockFP_0cells	0	
30°C _MockFP_275cells	0.009	72.37
30°C _MockFP_475cells	0.010	82.66
30°C _MockFP_825cells	0.015	84.85
30°C _MockFP_1500cells	0.022	88.00
30°C _TrueFP	0.007	
30°C _4FPcombined	0.030	

2-day samples:

Sample	Averaged Quantity (ng/µl)	Averaged % Loss
4°C _MockFP_0cells	0	
4°C _MockFP_275cells	0.020	38.86
4°C _MockFP_475cells	0.038	33.83
4°C _MockFP_825cells	0.059	40.49
4°C _MockFP_1500cells	0.130	27.69
4°C _TrueFP	0.236	
4°C _4FPcombined	0.035	
RT_MockFP_0cells	0	
RT_MockFP_275cells	0.022	33.52
RT_MockFP_475cells	0.044	22.17
RT_MockFP_825cells	0.075	23.90
RT_MockFP_1500cells	0.121	27.75
RT_TrueFP	0.075	
RT_4FPcombined	0.759	
30°C _MockFP_0cells	0	
30°C _MockFP_275cells	0.025	24.93
30°C _MockFP_475cells	0.048	16.24
30°C _MockFP_825cells	0.040	25.97
30°C _MockFP_1500cells	0.104	42.41
30°C _TrueFP	0.041	
30°C _4FPcombined	0.759	

3-day samples:

Sample	Averaged Quantity (ng/µl)	Averaged % Loss
4°C _MockFP_0cells	0	
4°C _MockFP_275cells	0.032	35.91
4°C _MockFP_475cells	0.034	40.98
4°C _MockFP_825cells	0.062	37.52
4°C _MockFP_1500cells	0.103	61.75
4°C _TrueFP	0.115	
4°C _4FPcombined	0.231	
RT_MockFP_0cells	0	
RT_MockFP_275cells	0.016	51.23
RT_MockFP_475cells	0.038	32.47
RT_MockFP_825cells	0.066	33.74
RT_MockFP_1500cells	0.129	28.42
RT_TrueFP	0.030	
RT_4FPcombined	0.115	
30°C _MockFP_0cells	0	
30°C _MockFP_275cells	0.030	9.26
30°C _MockFP_475cells	0.041	27.69
30°C _MockFP_825cells	0.093	6.01
30°C _MockFP_1500cells	0.166	7.55
30°C _TrueFP	0.015	
30°C _4FPcombined	0.101	

5-day samples:

Sample	Averaged Quantity (ng/µl)	Averaged % Loss
4°C _MockFP_0cells	0	
4°C _MockFP_275cells	0.012	64.83
4°C _MockFP_475cells	0.026	54.80
4°C _MockFP_825cells	0.049	50.88
4°C _MockFP_1500cells	0.113	37.23
4°C _TrueFP	0.098	
4°C _4FPcombined	0.844	
RT_MockFP_0cells	0	
RT_MockFP_275cells	0.008	76.83
RT_MockFP_475cells	0.019	66.72
RT_MockFP_825cells	0.068	31.58
RT_MockFP_1500cells	0.064	64.28
RT_TrueFP	0.208	
RT_4FPcombined	0.658	
30°C _MockFP_0cells	0	
30°C _MockFP_275cells	0.008	74.32
30°C _MockFP_475cells	0.030	48.15
30°C _MockFP_825cells	0.045	54.05
30°C _MockFP_1500cells	0.048	73.18
30°C _TrueFP	0.223	
30°C _4FPcombined	0.623	

1-week samples:

Sample	Averaged Quantity (ng/µl)	Averaged % Loss
4°C _MockFP_0cells	0	
4°C _MockFP_275cells	0.024	33.31
4°C _MockFP_475cells	0.042	26.42
4°C _MockFP_825cells	0.091	7.90
4°C _MockFP_1500cells	0.094	47.77
4°C _TrueFP	0.051	
4°C _4FPcombined	0.022	
RT_MockFP_0cells	0	
RT_MockFP_275cells	0.017	48.42
RT_MockFP_475cells	0.046	56.82
RT_MockFP_825cells	0.029	71.06
RT_MockFP_1500cells	0.017	90.43
RT_TrueFP	0.023	
RT_4FPcombined	0.000	
30°C _MockFP_0cells	0	
30°C _MockFP_275cells	0.021	35.47
30°C _MockFP_475cells	0.027	52.07
30°C _MockFP_825cells	0.038	61.94
30°C _MockFP_1500cells	0.042	76.82
30°C _TrueFP	0.055	
30°C _4FPcombined	0.000	

2-week samples:

Sample	Averaged Quantity (ng/µl)	Averaged % Loss
4°C_MockFP_0cells	0	
4°C _MockFP_275cells	0.022	32.33
4°C _MockFP_475cells	0.044	22.67
4°C _MockFP_825cells	0.048	51.44
4°C _MockFP_1500cells	0.111	38.10
4°C _TrueFP	0.076	
4°C _4FPcombined	0.615	
RT_MockFP_0cells	0	
RT_MockFP_275cells	0.007	79.26
RT_MockFP_475cells	0.012	78.26
RT_MockFP_825cells	0.021	78.84
RT_MockFP_1500cells	0.059	67.02
RT_TrueFP	0.026	
RT_4FPcombined	0.023	
30°C _MockFP_0cells	0	
30°C _MockFP_275cells	0.006	80.83
30°C _MockFP_475cells	0.010	82.72
30°C _MockFP_825cells	0.001	98.63
30°C _MockFP_1500cells	0.004	97.33
30°C _TrueFP	0.016	
30°C _4FPcombined	0.130	

3-week samples:

Sample	Averaged Quantity (ng/µl)	Averaged % Loss
4°C _MockFP_0cells		
4°C _MockFP_275cells	0.022	34.80
4°C _MockFP_475cells	0.028	51.29
4°C _MockFP_825cells	0.050	49.44
4°C _MockFP_1500cells	0.086	71.00
4°C _TrueFP	0.053	
4°C _4FPcombined	0.451	
RT_MockFP_0cells		
RT_MockFP_275cells	0.009	73.38
RT_MockFP_475cells	0.018	68.22
RT_MockFP_825cells	0.076	23.39
RT_MockFP_1500cells	0.037	79.63
RT_TrueFP	0.037	
RT_4FPcombined	0.049	
30°C _MockFP_0cells		
30°C _MockFP_275cells	0.028	15.93
30°C _MockFP_475cells	0.021	63.62
30°C _MockFP_825cells	0.051	48.72
30°C _MockFP_1500cells	0.007	95.91
30°C _TrueFP	0.114	
30°C _4FPcombined	0.435	

1-month samples:

Sample	Averaged Quantity	Averaged % Loss
4°C _MockFP_0cells	0	
4°C _MockFP_275cells	0.010	70.33
4°C _MockFP_475cells	0.014	75.64
4°C _MockFP_825cells	0.019	80.83
4°C _MockFP_1500cells	0.073	59.62
4°C _TrueFP	0.173	
4°C _4FPcombined	0.185	
RT_MockFP_0cells	0	
RT_MockFP_275cells	0.012	62.35
RT_MockFP_475cells	0.016	72.48
RT_MockFP_825cells	0.019	80.67
RT_MockFP_1500cells	0.106	40.99
RT_TrueFP	0.050	
RT_4FPcombined	0.125	
30°C _MockFP_0cells	0	
30°C _MockFP_275cells	0.011	66.61
30°C _MockFP_475cells	0.025	55.35
30°C _MockFP_825cells	0.013	86.93
30°C _MockFP_1500cells	0.033	81.48
30°C _TrueFP	0.006	
30°C _4FPcombined	0.020	

2-month samples:

Sample	Averaged Quantity (ng/µl)	Averaged % Loss
4°C _MockFP_0cells	0	
4°C _MockFP_275cells	0.024	28.59
4°C _MockFP_475cells	0.045	21.80
4°C _MockFP_825cells	0.086	12.75
4°C _MockFP_1500cells	0.123	31.91
4°C _TrueFP	0.024	
4°C _4FPcombined	0.109	
RT_MockFP_0cells	0	
RT_MockFP_275cells	0.021	36.49
RT_MockFP_475cells	0.023	59.14
RT_MockFP_825cells	0.022	66.17
RT_MockFP_1500cells	0.054	70.02
RT_TrueFP	0.008	
RT_4FPcombined	0.014	
30°C _MockFP_0cells	0	
30°C _MockFP_275cells	0.008	74.28
30°C _MockFP_475cells	0.006	89.46
30°C _MockFP_825cells	0.002	98.05
30°C _MockFP_1500cells	0.002	98.66
30°C _TrueFP	0.002	
30°C _4FPcombined	0.003	

3-month samples:

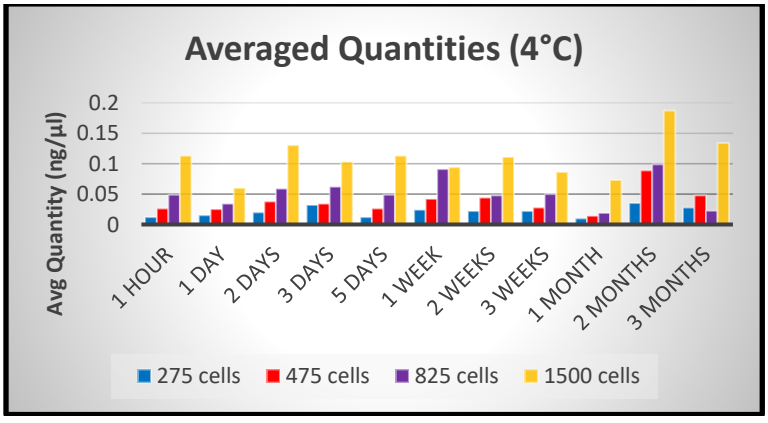
Sample	Averaged Quantity (ng/µl)	Averaged % Loss
4°C _MockFP_0cells	0	
4°C _MockFP_275cells	0.013	61.45
4°C _MockFP_475cells	0.035	38.51
4°C _MockFP_825cells	0.023	77.25
4°C _MockFP_1500cells	0.134	25.65
4°C _TrueFP	0.017	
4°C _4FPcombined		
RT_MockFP_0cells	0	
RT_MockFP_275cells	0.021	37.67
RT_MockFP_475cells	0.013	78.06
RT_MockFP_825cells	0.038	61.64
RT_MockFP_1500cells	0.037	79.68
RT_TrueFP	0.002	
RT_4FPcombined	0.002	
30°C _MockFP_0cells	0	
30°C _MockFP_275cells	0.005	83.38
30°C _MockFP_475cells	0.010	81.87
30°C _MockFP_825cells	0.016	84.25
30°C _MockFP_1500cells	0.004	98.03
30°C _TrueFP	0.008	
30°C _4FPcombined	0.032	

The tables above reveal a higher percent loss for samples placed in the 30°C setting for longer time periods, with little difference in percent loss between samples of different temperature settings for shorter time periods. Samples stored for up to 3 weeks showed 40-60% loss at 4°C and 60-70% loss at room temperature (averaged to 20°C/70°F). Samples stored for 1 month or longer, showed significantly higher difference in percent losses between the temperature setting, indicating for long term storage of mock fingerprints (made with FS-1) and true fingerprints, lower temperatures of 4°C might better preserve DNA quantity. This has corroborated previous literature where it was concluded that blood samples be stored at 4°C for preserving quantity of DNA after 10 days. We are observing DNA recovery from touch samples follow the same general trend in degradation as DNA recovered from body fluid samples.

However, mock fingerprints placed in 30°C for 2 and 3 weeks, showed unusually high percent losses, exceeding 80-90% in most cell counts. This was further researched, and previous literature was found which explained a change in latent print chemistry, involving changes in internal forces between the components. This change was due to the drying process which led to dehydration of a print and shuffling of the components topographically[26]. These results were insightful in explaining the difference in the process of "drying" that occur when a fingerprint has been subjected to different temperature environments, with loss of water and other volatile components occurring faster and more drastically at 30°C and more gradually at 4°C. This trend should be further studied in a manner where this drying process can be microscopically visualized and the changes conclusively quantified.

Graphical Representation of Quantities over Time

The average recoveries of each sample type were plotted on graphs to better visualize the trend of DNA recovered at different temperatures over the 11 time periods. The trend of reduced recovery of DNA with time is seen in samples placed in the 30°C setting, whereas the samples in the fridge remained visibly constant over time.



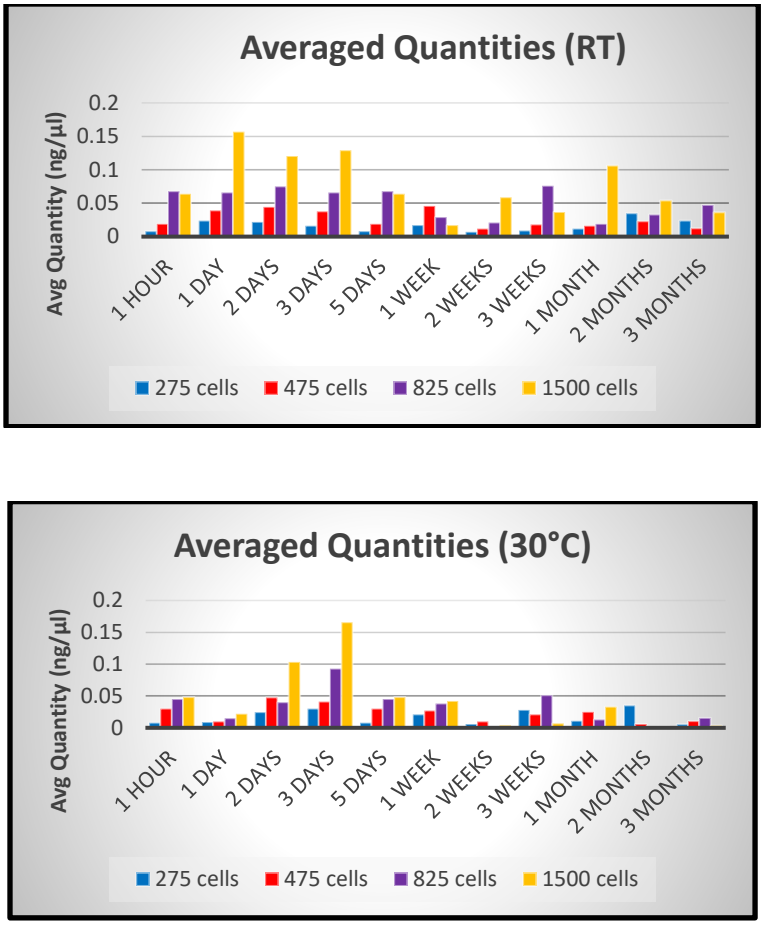


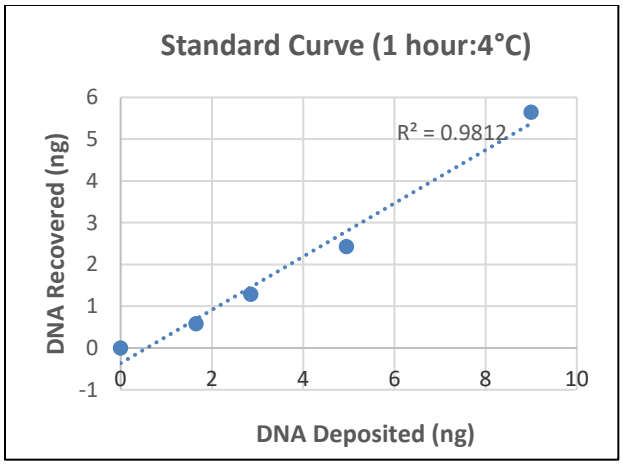
Figure 9: Graphical visualization of trend in DNA Recovery over Time at Different Temperatures

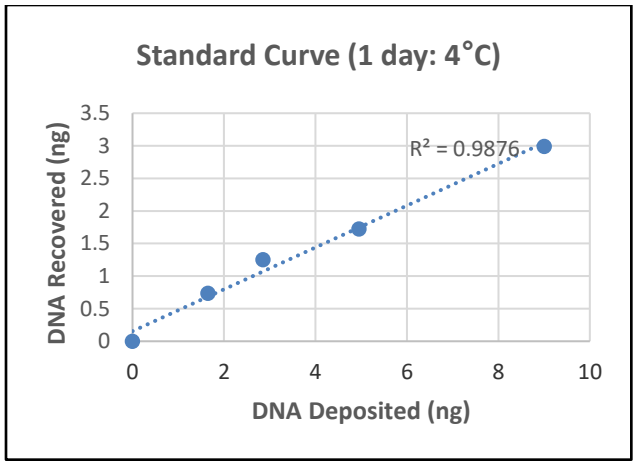
The graphs in *Figure 9* display the trends in average DNA recovery from touch samples stored at different temperature settings over a period of 3 months, with the 4 colored bars corresponding to averaged quantities of the three replicates for each cell count. The quantities recovered from samples stored at 4°C remain relatively constant over time, with no different in height of bars, while those at 30°C decline significantly after 2 weeks. The overall height of bars at 30°C is also significantly smaller than those at 4°C, indicating reduction in amounts recovered between these temperature settings.

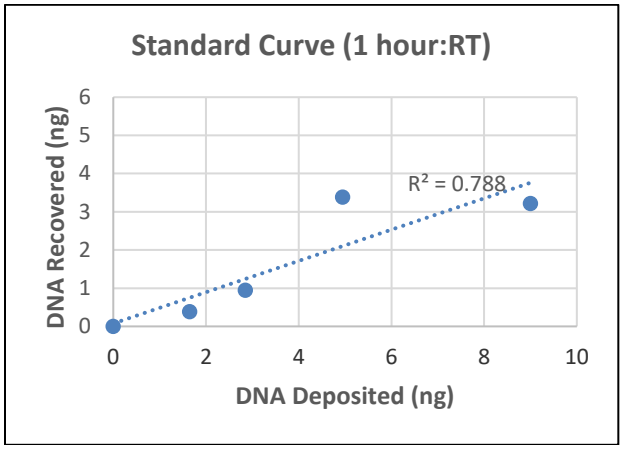
2.3.6 Standard Curves

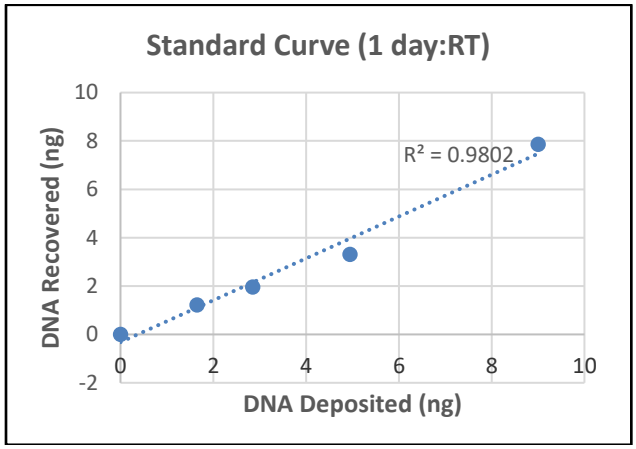
Mock fingerprints formed a crucial set of samples for these temperature and time point studies since they allowed the amount of DNA initially deposited to be known, which cannot be known with true fingerprints. Mock fingerprints of 5 different cell counts were made and deposited, in triplicate, so amounts recovered could be averaged and all 5 cell counts plotted on a standard curve of DNA Recovered (ng) versus DNA Deposited (ng). A scatter plot was thus generated for mock fingerprint recovery for each time point/temperature treatment and a trend line was added. Ideally, a tight correlation was expected between DNA Deposited (ng) and DNA Recovered (ng), leading to high R^2 values for the trend line.

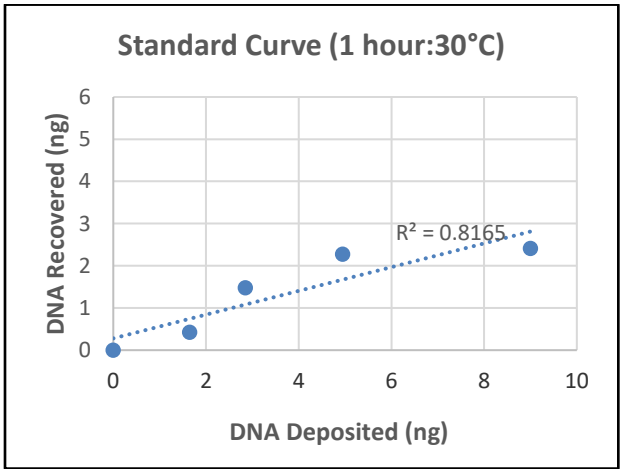
However, as seen by the standard curves generated for longer times since deposition in the higher temperature settings (room temperature and 30°C), less than ideal R^2 values were obtained, indicating a low correlation between DNA Recovered (ng) and DNA Deposited (ng). A few anomalies were seen, with standard curves of mock fingerprints left at room temperature for a week generating a trend line with R^2 of 0.2241, lower than the R^2 of samples placed in the oven (30°C) for the same time. Two- and three-week samples in the oven (30°C) also generated standard curve with unusually low R^2, indicating little to no linear fit of their data in this model.

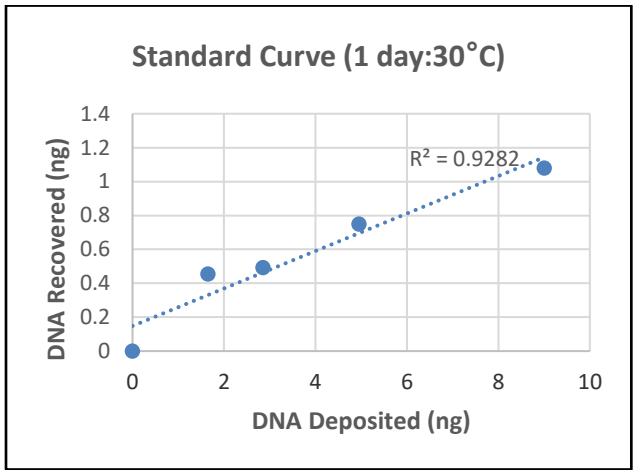

Standard Curve (1 hour:4°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.9812

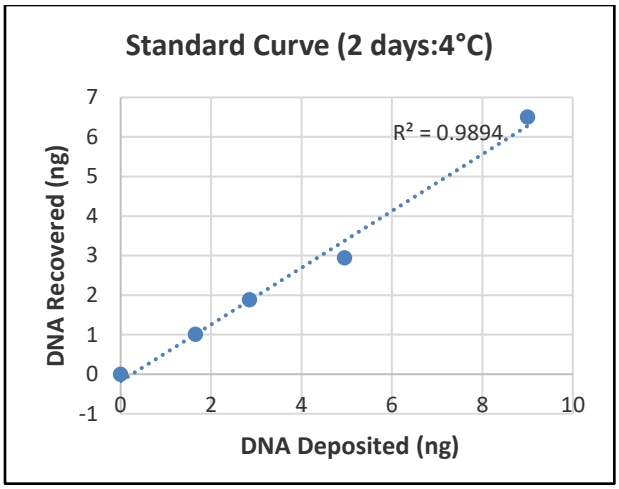

Standard Curve (1 day: 4°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.9876

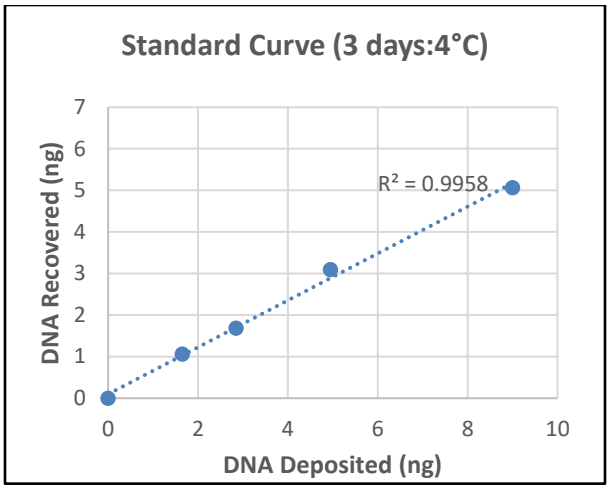

Standard Curve (1 hour:RT)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.788

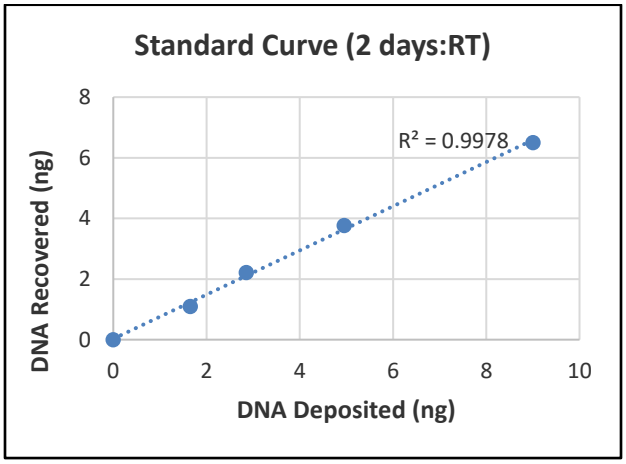

Standard Curve (1 day:RT)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.9802

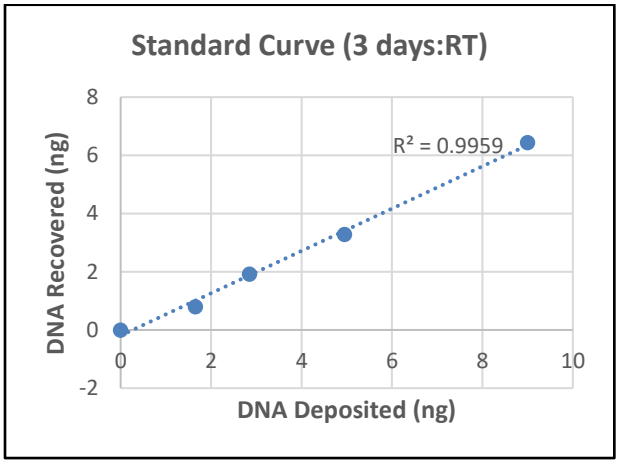

Standard Curve (1 hour:30°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.8165

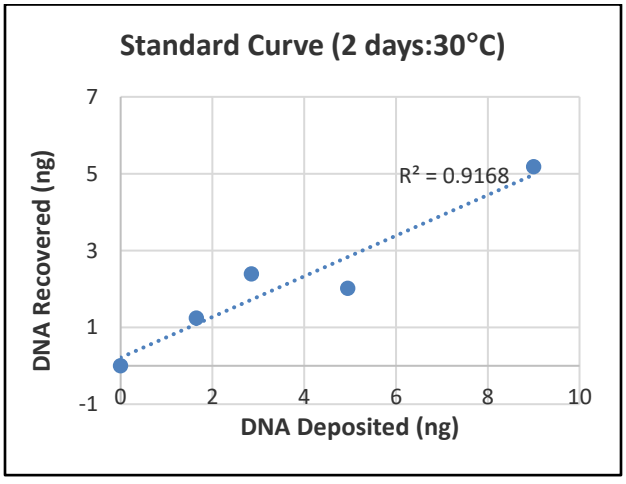

Standard Curve (1 day:30°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.9282

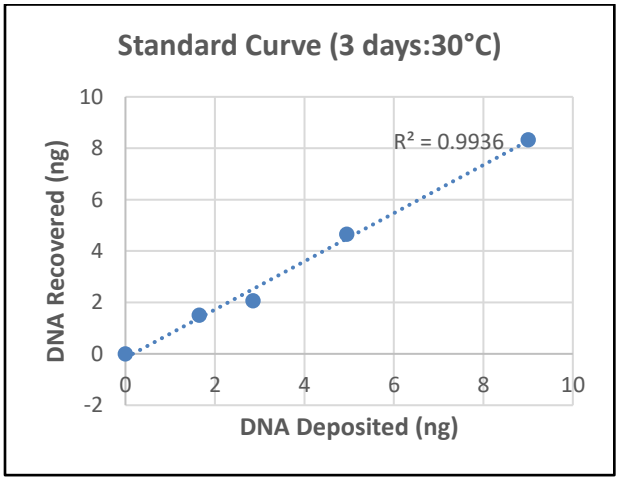

Standard Curve (2 days:4°C)
R² = 0.9894
DNA Recovered (ng)
DNA Deposited (ng)
7
6
5
4
3
2
1
0
-1
0
2
4
6
8
10

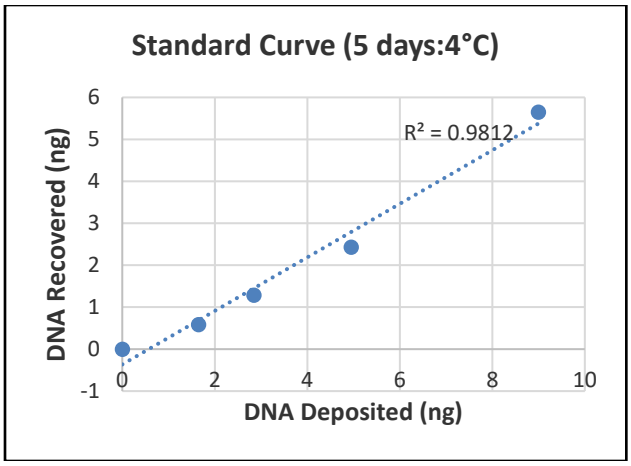

Standard Curve (3 days:4°C)
R² = 0.9958
DNA Recovered (ng)
DNA Deposited (ng)
7
6
5
4
3
2
1
0
0
2
4
6
8
10

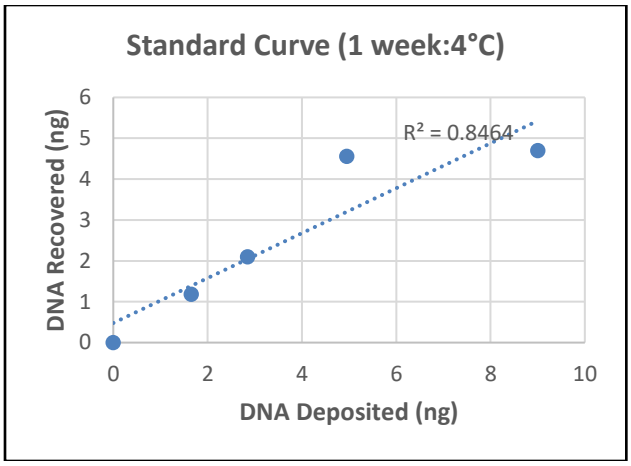

Standard Curve (2 days:RT)
R² = 0.9978
DNA Recovered (ng)
DNA Deposited (ng)
8
6
4
2
0
0
2
4
6
8
10

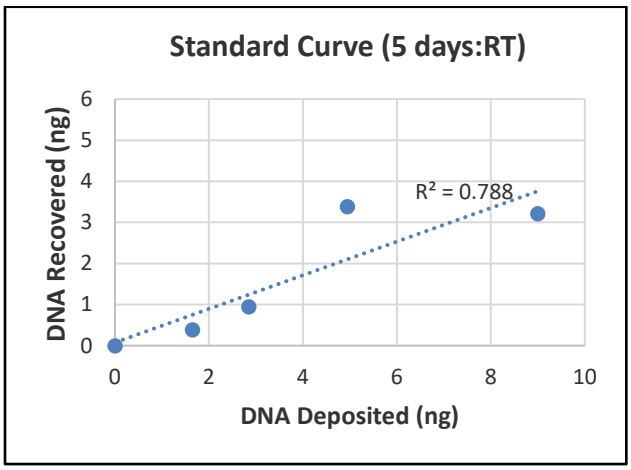

Standard Curve (3 days:RT)
R² = 0.9959
DNA Recovered (ng)
DNA Deposited (ng)
8
6
4
2
0
-2
0
2
4
6
8
10

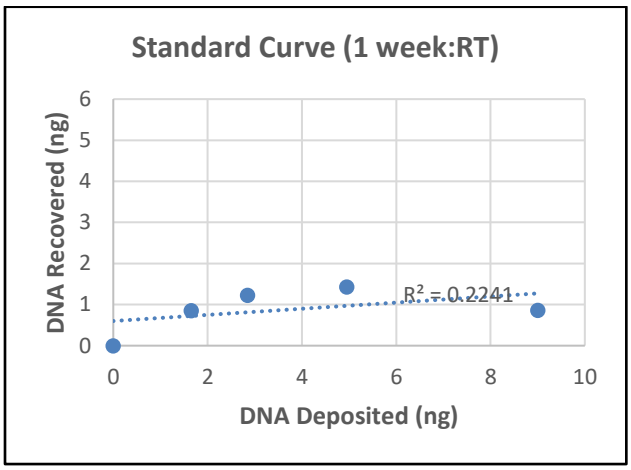

Standard Curve (2 days:30°C)
R² = 0.9168
DNA Recovered (ng)
DNA Deposited (ng)
7
5
3
1
-1
0
2
4
6
8
10

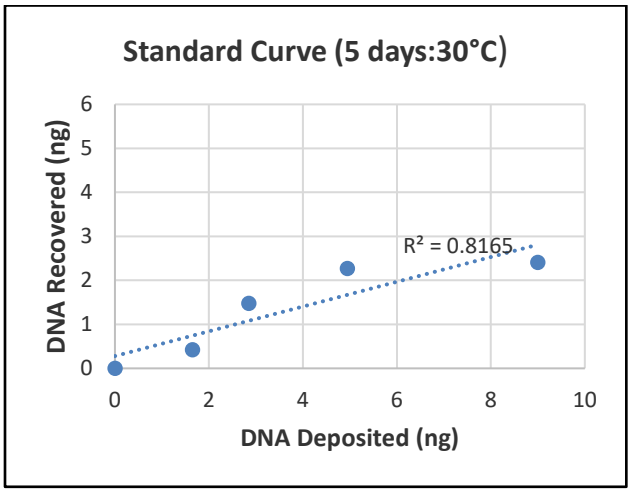

Standard Curve (3 days:30°C)
R² = 0.9936
DNA Recovered (ng)
DNA Deposited (ng)
10
8
6
4
2
0
-2
0
2
4
6
8
10

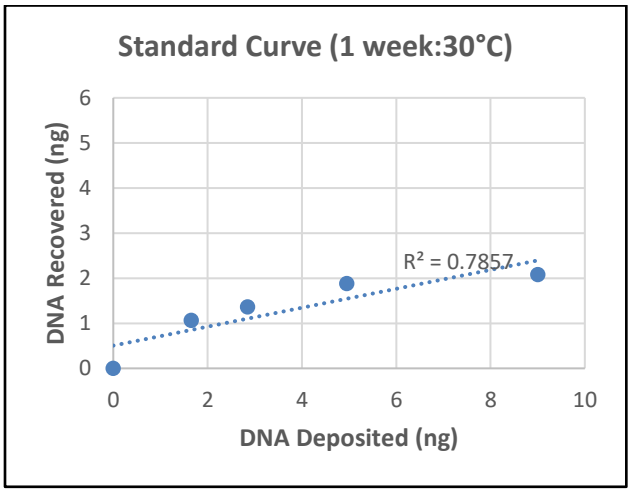

Standard Curve (5 days:4°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.9812

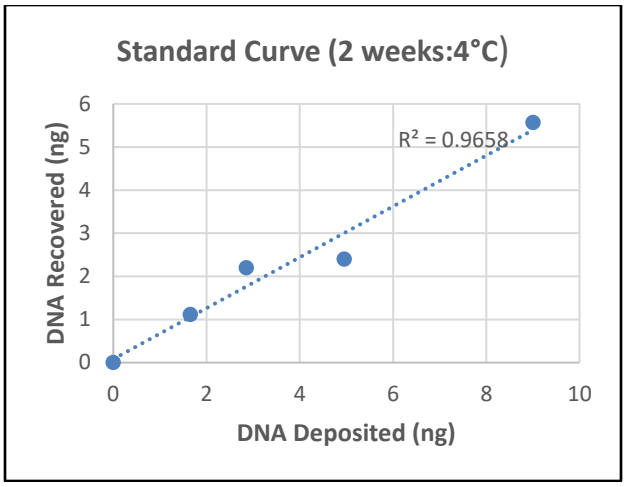

Standard Curve (1 week:4°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.8464

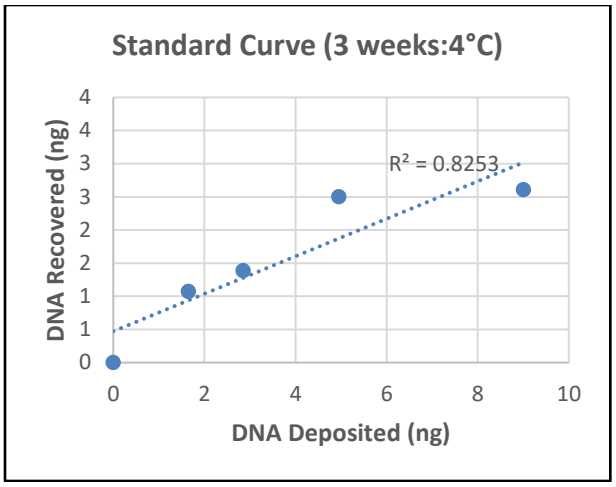

Standard Curve (5 days:RT)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.788

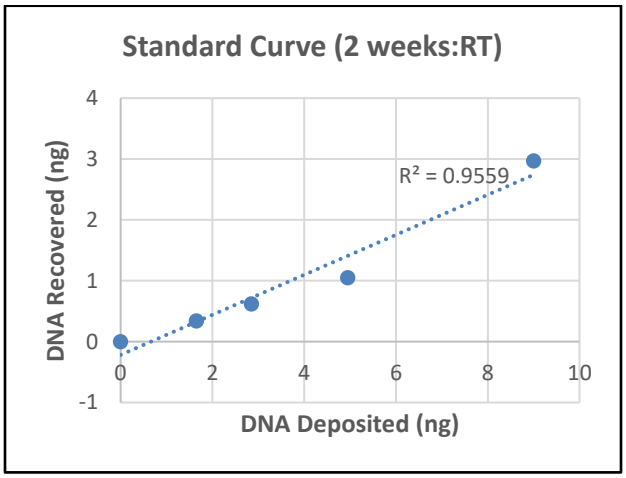

Standard Curve (1 week:RT)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.2241

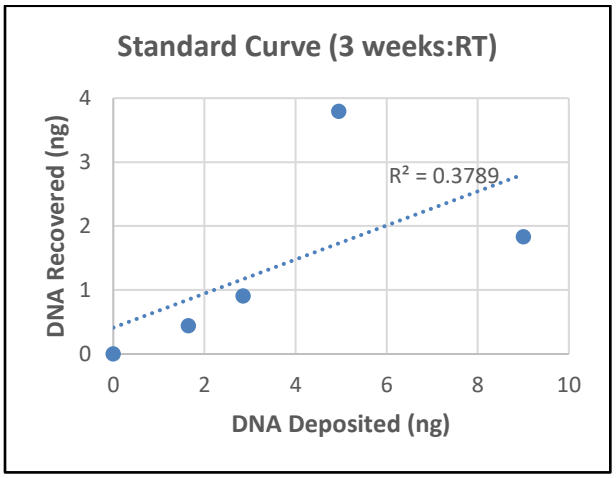

Standard Curve (5 days:30°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.8165

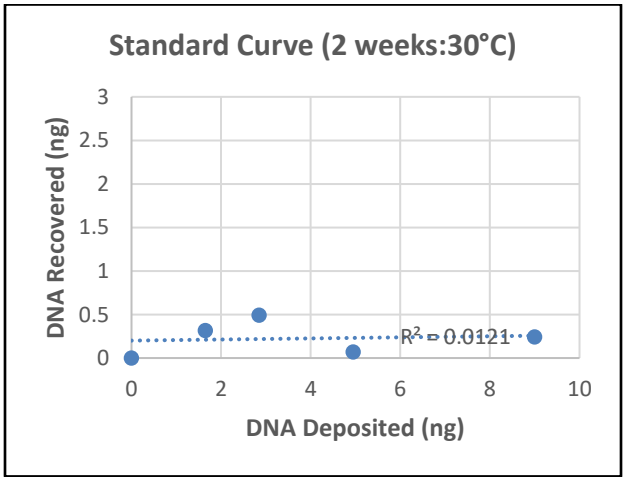

Standard Curve (1 week:30°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.7857

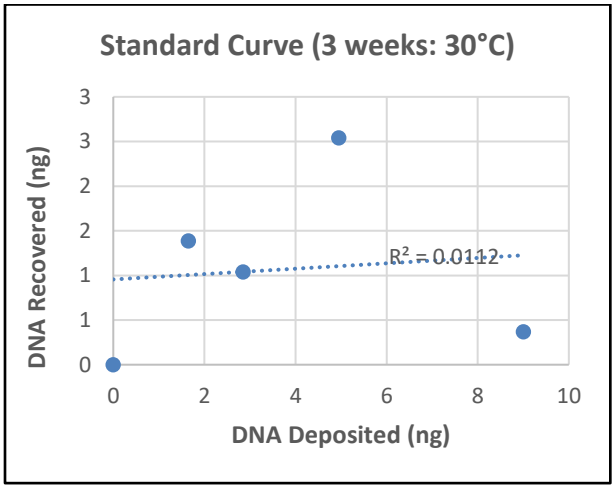

Standard Curve (2 weeks:4°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.9658

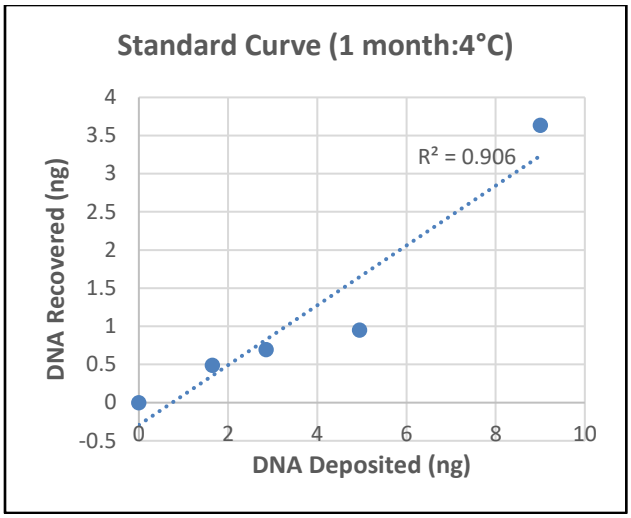

Standard Curve (3 weeks:4°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.8253

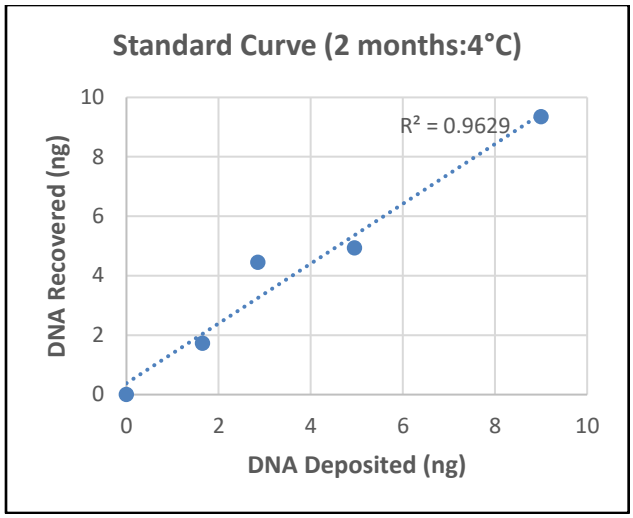

Standard Curve (2 weeks:RT)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.9559

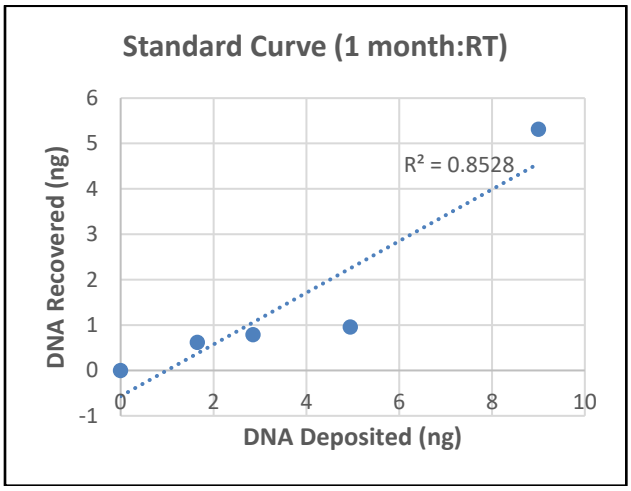

Standard Curve (3 weeks:RT)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.3789

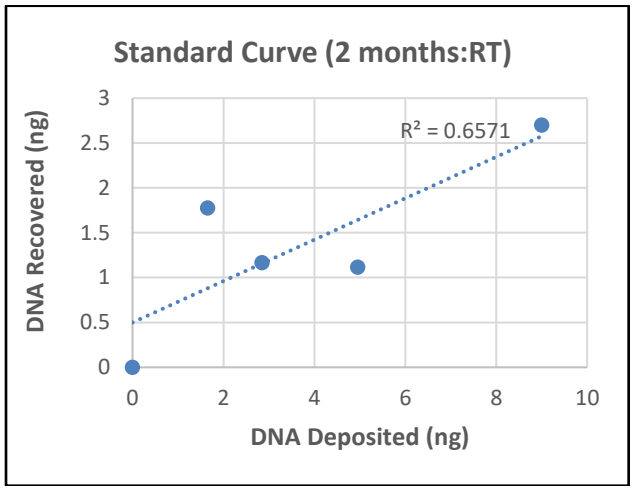

Standard Curve (2 weeks:30°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.0121

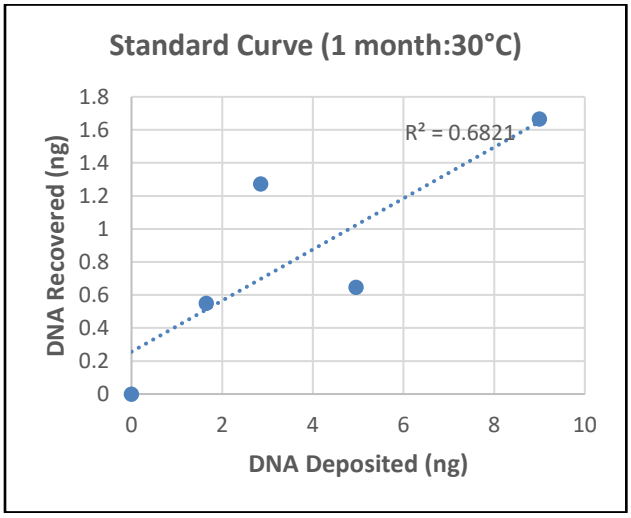

Standard Curve (3 weeks: 30°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.0112

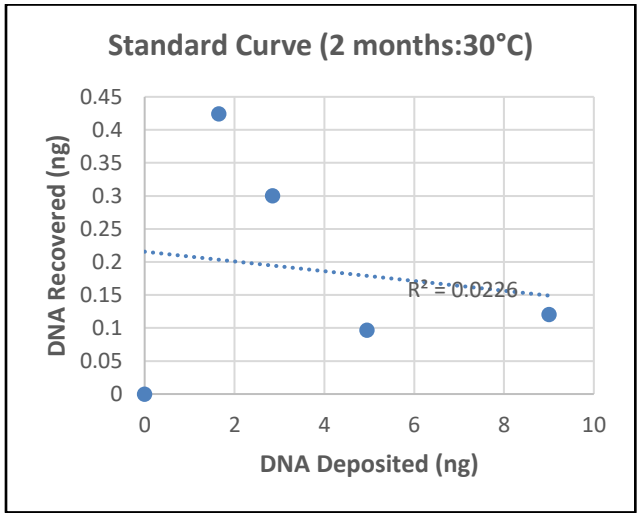

Standard Curve (1 month:4°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.906
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
0
2
4
6
8
10

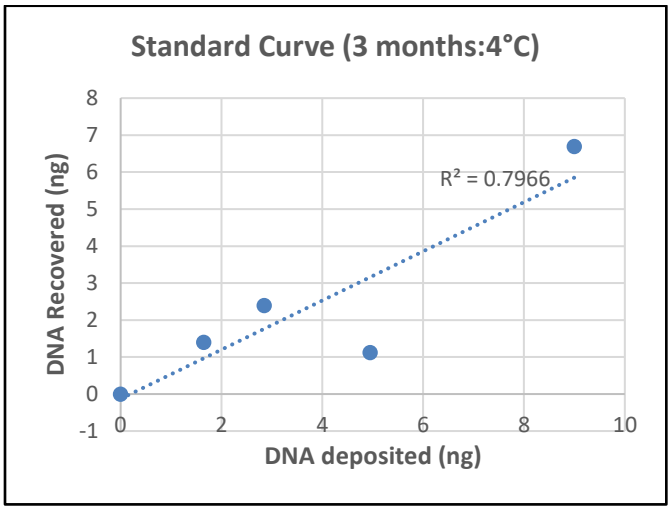

Standard Curve (2 months:4°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.9629
10
8
6
4
2
0
0
2
4
6
8
10

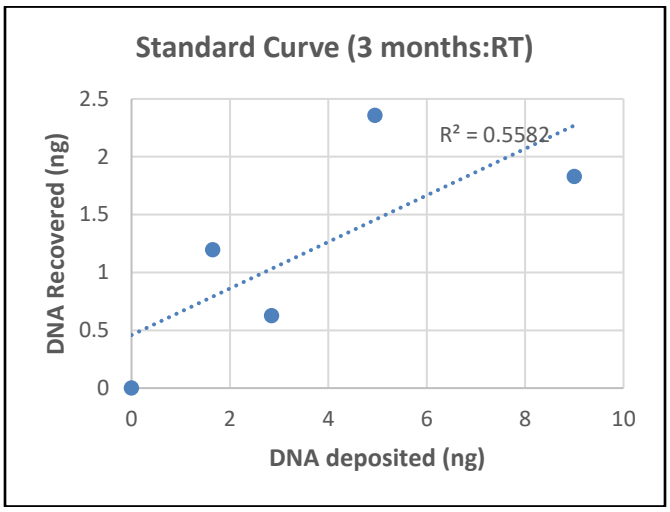

Standard Curve (1 month:RT)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.8528
6
5
4
3
2
1
0
-1
0
2
4
6
8
10

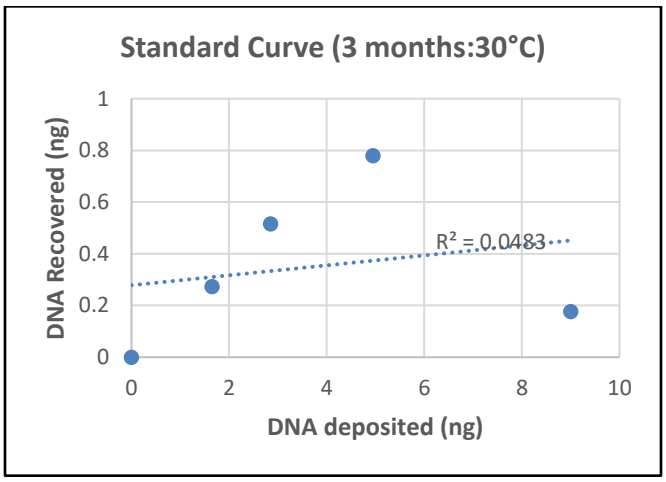

Standard Curve (2 months:RT)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.6571
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10


Standard Curve (1 month:30°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.6821
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10


Standard Curve (2 months:30°C)
DNA Recovered (ng)
DNA Deposited (ng)
R² = 0.0226
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
2
4
6
8
10


Standard Curve (3 months:4°C)
DNA Recovered (ng)
DNA deposited (ng)
R² = 0.7966
8
7
6
5
4
3
2
1
0
-1
0
2
4
6
8
10


Standard Curve (3 months:RT)
DNA Recovered (ng)
DNA deposited (ng)
R² = 0.5582
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10


Standard Curve (3 months:30°C)
DNA Recovered (ng)
DNA deposited (ng)
R² = 0.0483
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10

2.3.7 Eliminating Operator Error

To confirm that the low R^2 values were a result of the two variables' effect on the quantity of DNA recovered, it was decided that operator error needed to be eliminated. To achieve this, 20 replicates of the 5 cell counts were deposited on sterile glass slides and collected immediately. These samples were extracted using standard organic extraction protocol described previously (on page 23) and quantified using real-time PCR. The average DNA recovered from the samples is presented below *(Table IV)*, along with a standard curve generated with those values (*Figure 10).* An R^2 value of 0.9881 suggests strong correlation between the samples and validates the analytical skills of the operator.

Sample	DNA Deposited (ng)	DNA Recovered (ng)
0	0	0
275	1.65	0.49
475	2.85	0.67
825	4.95	1.51
1500	9	3.09

Table IV: Average DNA Recovered (ng) from Immediate Mock Fingerprints

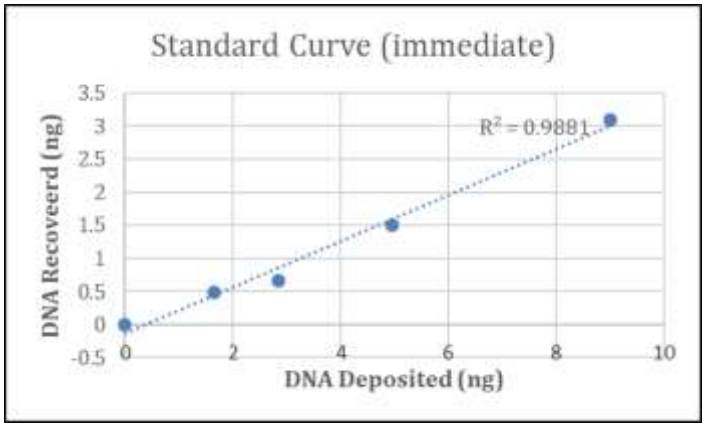


Figure 10: Standard Curve of Mock Fingerprints Analyzed Immediately as Control

This experiment established the mock fingerprint cell counts as good controls, when immediately collected, and validated the skills of the operator performing the extractions. The standard curves presented in the previous section are thus, a result of the treatments the samples were subjected to and demand further research into the role of temperature and time since deposition on patterns of DNA recovery with other artificial fingerprint solutions.

The R^2 values of the standard curve obtained from samples stored at 4°C remains high consistently with time, with the lowest R^2 being 0.796 at 3 months. This indicates that the mock fingerprints made from FS-1 could be used as good controls when stored at 4°C for up to 2 months. However, with room temperature and 30°C storage, we see a decline in the R^2 values with time, bringing to light the inability of these mock fingerprints cell counts to be used as standard controls after 3 weeks of room temperature (R^2 = 0.3789) and 1 week of 30°C storage (R^2 = 0.7857). These results, combined with previous literature regarding role of time and temperature on the quality of latent print residue, sparked interest in the development of additional artificial fingerprint solutions to create mock fingerprints more similar to a true fingerprint and observe how they would affect DNA recovery through the collection/extraction process.

2.3.8 Statistical Analysis of Real-Time Data

Analysis of Variance (ANOVA) was used to test for statistically significant differences in the amount of DNA recovered after the temperature and time-since-deposition treatments. This was done by organizing data in two ways: first, data was grouped by time since deposition for each temperature setting, and second, the data was grouped by temperature for each time since deposition. Single/One-way ANOVA was carried out in Microsoft Excel, separately for each mock fingerprint cell count and true fingerprints, and the p-values were compared against α=0.01. p-values lower than 0.01 revealed statistically significant differences in the recovery of DNA between those data groups.

ANOVA for Time-Since-Deposition

Sample	p-values		
	4°C	RT	30°C
275 cells	1.4E-04	1.1E-02	2.6E-06
475 cells	6.5E-04	6.9E-03	4.7E-06
825 cells	2.8E-03	3.5E-03	1.1E-02
1500 cells	3.3E-04	7.4E-08	2.3E-04
True FP	4.5E-05	1.3E-08	8.5E-22

Table V: p-values of ANOVA Testing between Time Periods

Table V displays the p-values of the ANOVA analysis for data grouped by time-since-deposition for each temperature setting and each sample type. All p-values being less than 0.01 indicate statistically significant differences in the quantity of DNA recovered between time points. These results support the hypothesis of the research, that time-since-deposition influences DNA recovered from touch samples quantitatively. It is also interesting to note that differences in recovery amounts, over time, are seen in all three temperature settings, indicating that storage of samples at a lower temperature (4°C) does not completely save DNA from reducing in quantity when stored for time periods exceeding 1-2 months.

Post-hoc Tukey analysis showed maximum difference in recoveries was seen between shorter time periods of up to a week and longer time periods longer than 1 month. Since mock fingerprints were controlled, artificially created solutions, significant differences in DNA recovery conclusively points to negative effects of time-since-deposition on quantity extracted from touch samples, at all three temperature settings. True fingerprints seem to show larger differences in p-values at the different temperatures, indicating that time-since-deposition affected the quantity of DNA recovered from true fingerprints most at 30°C and least at 4°C. These differences in recovery from true fingerprints can also be attributed to the inherent variability in DNA deposited in true fingerprints, with other pre-deposition factors playing a role in amount of DNA initially deposited by donors. The analysis of these time-since-deposition experiments provides information that could be applied practically when storing true fingerprints from forensic casework.

ANOVA for Temperature

Sample	p-values										
	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
275 cells	0.62	0.00	0.79	0.05	0.62	0.73	0.02	7.3E-04	0.26	0.07	1.5E-03
475 cells	0.50	0.00	0.36	0.82	0.50	0.64	3.9E-04	0.52	0.01	5.7E-04	1.2E-03
825 cells	0.13	0.00	0.46	0.71	0.13	0.18	0.13	0.05	0.22	9.3E-04	1.3E-03
1500 cells	0.09	0.00	0.68	0.33	0.09	0.24	0.01	0.31	1.6E-03	2.6E-04	2.3E-04
True FP	5.6E-07	7.1E-02	1.7E-02	2.0E-03	5.7E-02	2.0E-01	5.9E-02	1.3E-02	9.5E-04	2.7E-04	1.1E-03

Table VI: p-values of ANOVA Testing between Temperature Settings

ANOVA testing for when the data was grouped by temperature revealed interesting results *(Table VI)*, with shorter time periods of up to 2 weeks having no statistically significant difference in the amount of DNA recovered from mock fingerprints placed in different temperature settings. Longer time-since-deposition on the other hand, revealed p-values lower than 0.01, inferring that temperature of the environment did affect recovery of DNA when stored for longer periods of time, i.e. over a month. All true fingerprints showed difference in the recovery amounts between temperature settings for all time points. This is crucial to note because it confirms that storage of evidence samples at lower temperatures of 4°C might help preserve more DNA for future analysis, rather than room temperature storage, or at higher temperatures of 30°C, commonly experienced during summers.

However, it also shows that mock fingerprints are relatively more stable at different temperatures when stored for time intervals up to 2 weeks. This could be accounted for by the difference in the chemistry of residue between true and mock fingerprints and calls for an improved artificial fingerprint solution that is more homologous to a true fingerprint. These differences were only in quantity and do not prove any degradation in the quality of DNA recovered after these treatments.

2.3.9 Assessing Quality of DNA Recovered by Agarose Gel Electrophoresis

The effects of temperature and time-since-deposition on DNA recovery included assessing the quality of DNA extracted from touch samples after the different treatments. True fingerprints were combined at the collection step and concentrated samples were obtained from each temperature/time point. These samples were run on 1% agarose gels and stained with 1X SYBR Gold. The gels as viewed under ultraviolet light are shown in *Figures 11-13*.

The eleven time-point samples stored at 4°C appear as tight, straight bands of relatively equal intensity. The samples extracted from room temperature show a little curving of bands, and little to no smearing. The samples stored at 30°C appear as bands of unequal intensity, with some accumulation of small size DNA for the longer time intervals.

These gels suggest there is little to no decline in the quality of DNA extracted after these time intervals even after being placed in an oven set to 30°C. There is no complete loss of the high molecular weight band in any of the samples, indicating DNA samples could be used for downstream profiling. This confirms previous literature where DNA samples subjected to moderately high temperatures yielded complete STR profiles with only a reduction in peak heights, proving no change in the quality of DNA. It was thus concluded that true fingerprint samples stored at temperatures up to 30°C for time periods up to 3 months did not substantially degrade the quality of DNA recovered.

Figure 11: Agarose gel electrophoresis of samples stored at 4°C

Figure 12: Agarose gel electrophoresis of samples stored at RT

Figure 13: Agarose gel electrophoresis of samples stored at 30°C

Gel Legend

A, J, K, T: Hind III ladders B, I, L, S: Blanks
C: No degradation control D: 1 hour
E: 1 day
F: 2 days
G: 3 days
H: 5 days
M: 1 week
N: 2 weeks
O: 3 weeks
P: 1 month
Q: 2 months
R: 3 months

2.4 Conclusion

In forensic analyses, DNA is routinely extracted from fingerprints to place individuals at a crime scene. The advancement of forensic technologies has increased the number of samples sent to the laboratory for DNA analysis, leading to huge backlogs, both in terms of turnover rates and problems relating to storage of samples. Trace DNA evidence, containing minute quantities of DNA available for analysis, is the first sample type to be affected by any improper storage conditions or delays in processing. Research exploring the limits of survival of evidence samples exposed to various external conditions will help forensic laboratories devise a more balanced, scientifically backed system to store their evidence samples.

The results of these temperature and time-point experiments reveal information which could aid in the development of such a storage system in forensic laboratories and lead to better collection and analyses from these samples. Fingerprint evidence can be stored at temperatures up to 30°C for up to 2-3 weeks without any significant reduction in the amount of DNA recovered. Therefore, if any sample is due for processing within this time frame it can be stored at room temperature and storage space in the refrigerator/freezer can be used for other evidence, which may be more dependent on lower temperatures to preserve its integrity. This data also reiterates previous literature advocating the ideal temperature for long-term storage of DNA samples at lower temperatures (4°C), with statistically significant reduction in the quantity of DNA recovered from touch samples at higher temperatures of 30°C. The process of drying of fingerprints gets drastically accelerated when exposed to higher temperatures for prolonged time periods, affecting DNA recovery from both mock and true fingerprints. There was also a difference in the ability of mock fingerprints made from FS-1 to serve as controls at higher temperature as the R^2 values of the standard curves declined with time at room temperature and 30°C, while remaining stable over time at 4°C. This might signify the role of changes in a fingerprint residue with time on DNA recovery and further research into this aspect of touch samples is highly recommended.

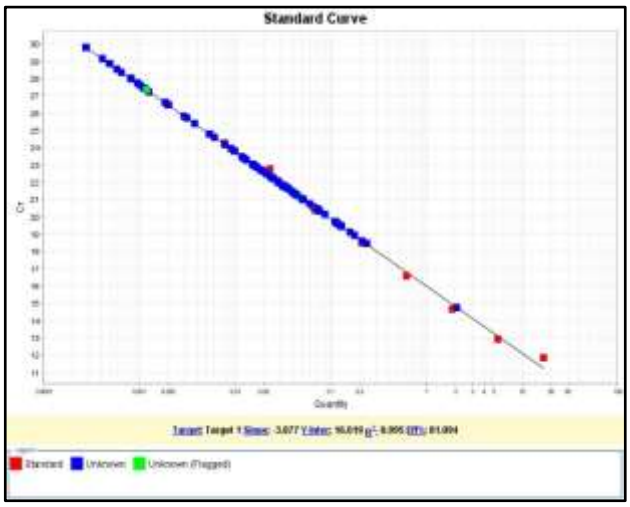
Although a reduction in the quantity of DNA recovered was observed over longer time-since-deposition, the quality of DNA recovered remained largely unchanged, even at higher temperatures. This suggests further downstream processing would yield STR profiles, if enough DNA could be extracted. For the scope of this thesis however, studying the quantity through real-time PCR and quality through agarose gel electrophoresis was considered enough, and profiling was not carried out.

A major set of samples used in these experiments were mock fingerprints of various cell counts. The data suggests mock fingerprints prepared with the current artificial fingerprint solution (FS-1) used in the laboratory degrade in this pattern. It provides strong groundwork on which further research can continue, exploiting the inherent quality of mock fingerprints, i.e. amount of DNA deposited is known. The effect of other external factors such as humidity, surface textures, or time since collection on percent recovery can be investigated. It would be interesting to study the patterns of percent recovery from mock fingerprints containing additional, more complex eccrine and sebaceous elements found in a true fingerprint, in terms of chemistry of the residue.

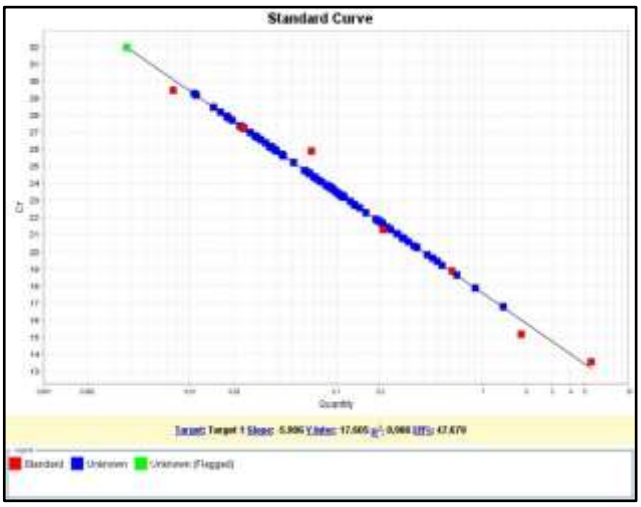
Appendix A

Real-time PCR Standard Curves

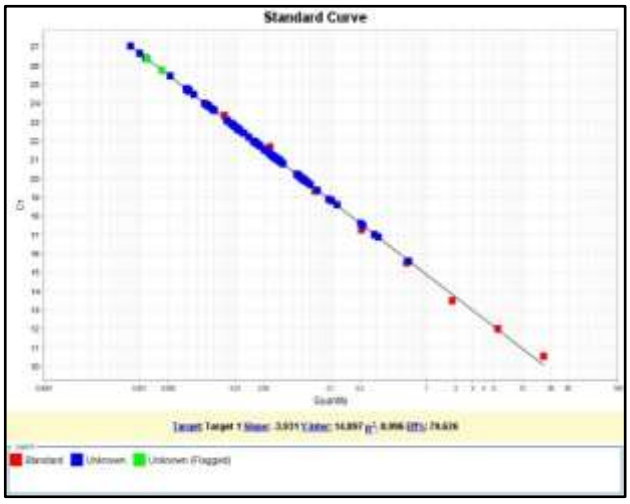
1-hour



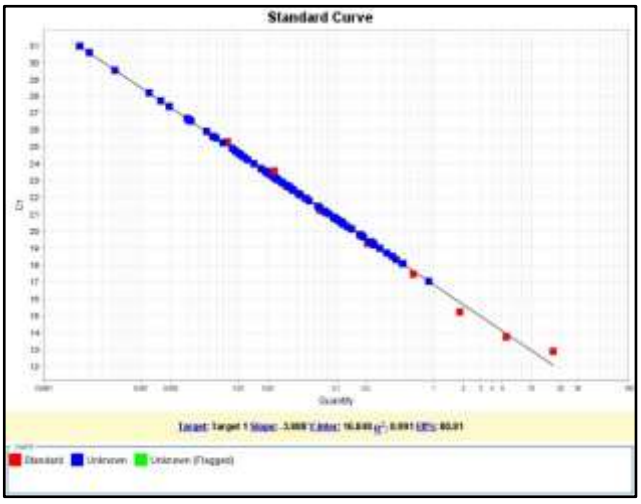
2-day



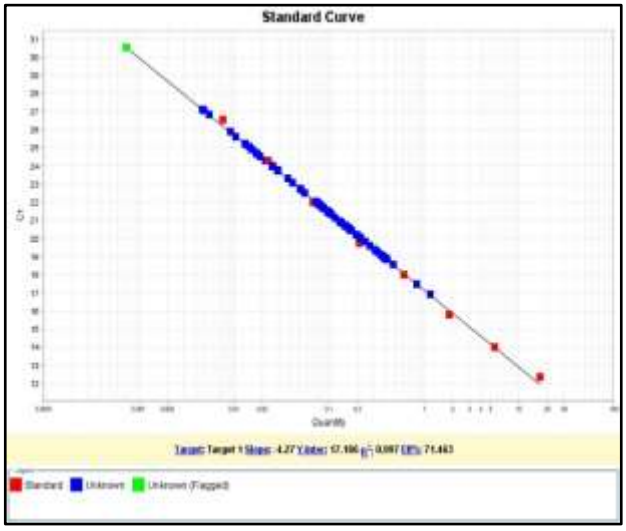
1-day



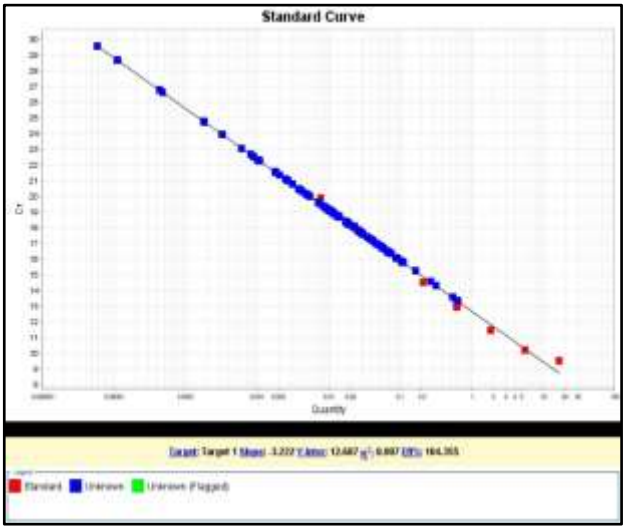
3-day



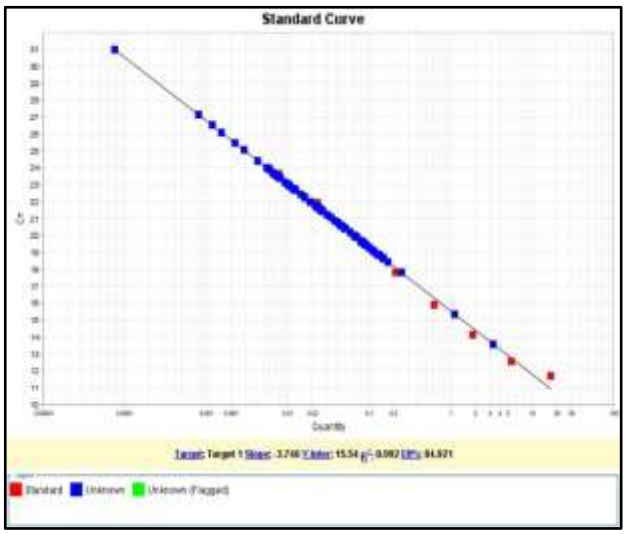
5-day



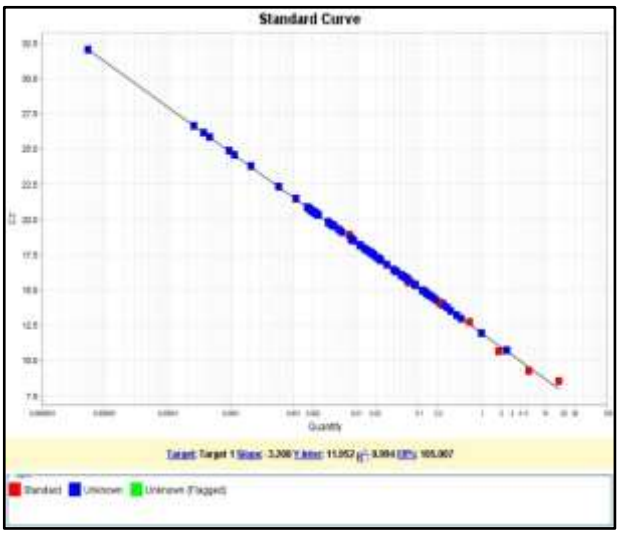
2-week



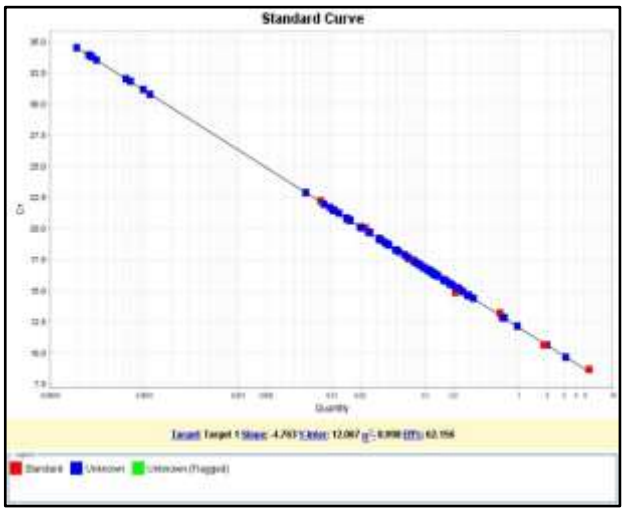
1-week



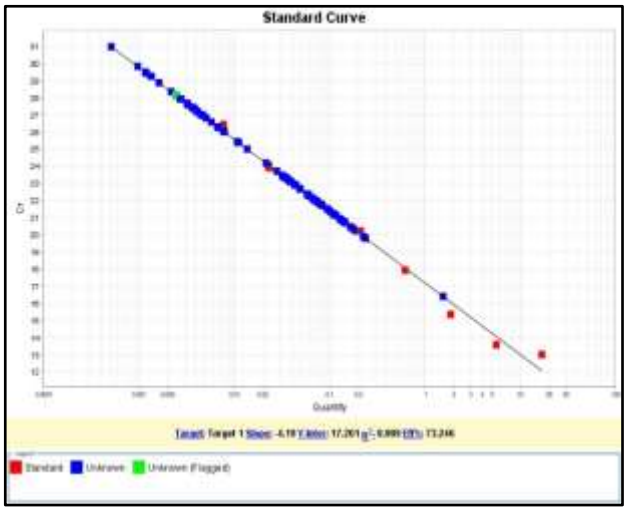
3-week



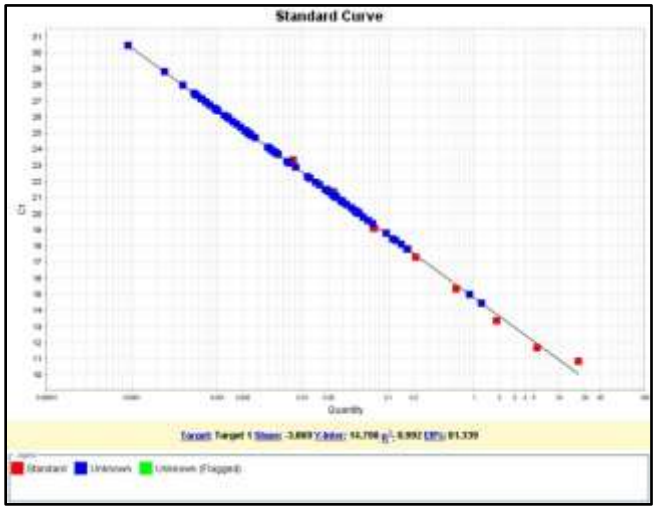
1-month



2-month



3-month



Master Table of all Quantified Samples

DNA recovered (ng/µl) from 4°C samples:

275 cells	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.010	0.011	0.018	0.022	0.010	0.025	0.017	0.022	0.011	0.048	0.022
2	0.010	0.017	0.026	0.028	0.010	0.023	0.021	0.022	0.008	0.023	0.033
3	0.015	0.016	0.017	0.019	0.015	0.024	0.029	0.020	0.010	0.033	0.029
Mean	0.012	0.015	0.020	0.023	0.012	0.024	0.022	0.022	0.010	0.035	0.028
SD	0.002	0.003	0.004	0.004	0.002	0.001	0.005	0.001	0.001	0.010	0.004

475 cells	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.034	0.020	0.028	0.029	0.034	0.027	0.048	0.046	0.016	0.054	0.045
2	0.025	0.023	0.036	0.031	0.025	0.044	0.043	0.018	0.013	0.056	0.051
3	0.018	0.032	0.049	0.041	0.018	0.055	0.041	0.020	0.013	0.042	0.047
Mean	0.026	0.025	0.038	0.034	0.026	0.042	0.044	0.028	0.014	0.051	0.048
SD	0.007	0.005	0.008	0.005	0.007	0.011	0.003	0.013	0.001	0.006	0.003

825 cells	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.058	0.049	0.085	0.068	0.058	0.091	0.087	0.046	0.022	0.093	0.018
2	0.046	0.027	0.075	0.064	0.046	0.065	0.046	0.047	0.016	0.084	0.025
3	0.041	0.027	0.017	0.053	0.041	0.078	0.011	0.057	0.019	0.082	0.024
Mean	0.049	0.034	0.059	0.062	0.049	0.078	0.048	0.050	0.019	0.086	0.023
SD	0.007	0.011	0.030	0.007	0.007	0.011	0.031	0.005	0.002	0.005	0.003

1500 cells	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.116	0.053	0.134	0.101	0.116	0.115	0.068	0.087	0.082	0.190	0.167
2	0.118	0.072	0.147	0.095	0.118	0.109	0.105	0.092	0.053	0.144	0.121
3	0.105	0.054	0.109	0.111	0.105	0.167	0.162	0.065	0.084	0.227	0.113
Mean	0.113	0.060	0.130	0.103	0.113	0.130	0.111	0.081	0.073	0.187	0.134
SD	0.005	0.009	0.016	0.007	0.005	0.026	0.039	0.012	0.014	0.034	0.024

True FP	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.025	0.206	1.653	0.282	0.050	0.030	0.072	0.077	0.098	0.037	0.042
2	0.021	0.102	0.211	0.093	0.029	0.098	0.102	0.053	0.075	0.043	0.040
3	0.012	0.115	0.599	0.482	0.019	0.090	0.266	0.046	0.088	0.058	0.043
4	0.005	0.028	0.224	0.414	0.037	0.050	0.026	0.085	0.084	0.072	0.028
5	0.032	0.057	0.552	0.375	0.025	0.132	0.540	0.051	0.121	0.036	0.019
6	0.017	0.044	0.315	0.037	0.150	0.024	0.318	0.000	0.127	0.060	0.065
7	0.001	0.010	0.026	0.002	0.005	0.001	0.001	0.016	0.026	0.007	0.001
8	0.001	0.001	0.024	0.002	0.009	0.016	0.001	0.024	0.032	0.003	0.003
9	0.001	0.016	0.017	0.000	0.001	0.148	0.005	0.014	0.033	0.005	0.003
10	0.001	0.006	0.111	0.001	0.006	0.023	0.006	0.020	0.015	0.011	0.001
11	0.003	0.010	0.022	0.005	0.005	0.006	0.002	0.010	0.020	0.005	0.001
12	0.001	0.003	0.022	0.000	0.017	0.027	0.002	0.008	0.016	0.008	0.001
13	0.001	0.014	0.027	0.011	0.211	0.012	0.009	0.109	0.692	0.002	0.007
14	0.003	0.217	0.109	0.105	0.152	0.249	0.026	0.046	0.143	0.004	0.007
15	0.002	0.026	0.061	0.032	0.327	0.041	0.010	0.000	0.716	0.001	0.020
16	0.001	0.029	0.396	0.117	0.117	0.016	0.009	0.040	0.184	0.006	0.020
17	0.002	0.025	0.071	0.144	0.306	0.025	0.018	0.060	0.664	0.001	0.008
18	0.004	0.288	0.241	0.078	0.130	0.019	0.000	0.038	0.085	0.003	0.005
19	0.008	0.047	0.021	0.098	0.347	0.010	0.017	0.213	0.128	0.042	0.020
20	0.002	0.017	0.018	0.025	0.015	0.007	0.090	0.148	0.123	0.071	0.012
Mean	0.007	0.063	0.236	0.115	0.098	0.051	0.076	0.053	0.173	0.024	0.017
SD	0.009	0.080	0.369	0.147	0.113	0.061	0.137	0.052	0.222	0.025	0.018

DNA recovered (ng/µl) from room temperature samples:

275 cells	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.013	0.026	0.007	0.013	0.013	0.001	0.004	0.011	0.013	0.052	0.020
2	0.003	0.024	0.027	0.025	0.003	0.026	0.010	0.010	0.013	0.017	0.023
3	0.007	0.024	0.032	0.010	0.007	0.024	0.009	0.006	0.011	0.038	0.028
Mean	0.008	0.024	0.022	0.016	0.008	0.017	0.008	0.009	0.012	0.035	0.024
SD	0.004	0.001	0.011	0.007	0.004	0.012	0.003	0.002	0.001	0.014	0.003

475 cells	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.006	0.035	0.051	0.043	0.006	0.032	0.014	0.017	0.017	0.019	0.001
2	0.016	0.035	0.049	0.024	0.016	0.016	0.010	0.020	0.017	0.019	0.022
3	0.034	0.048	0.034	0.049	0.034	0.057	0.014	0.018	0.014	0.032	0.014
Mean	0.019	0.039	0.044	0.038	0.019	0.035	0.012	0.018	0.016	0.023	0.013
SD	0.012	0.006	0.007	0.011	0.012	0.017	0.002	0.001	0.001	0.006	0.008

825 cells	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.079	0.068	0.077	0.084	0.079	0.003	0.003	0.091	0.023	0.026	0.042
2	0.061	0.074	0.053	0.033	0.061	0.004	0.030	0.080	0.022	0.030	0.042
3	0.063	0.057	0.097	0.079	0.063	0.079	0.030	0.057	0.012	0.041	0.058
Mean	0.068	0.066	0.075	0.066	0.068	0.029	0.021	0.076	0.019	0.032	0.047
SD	0.008	0.007	0.018	0.023	0.008	0.035	0.013	0.014	0.005	0.006	0.007

1500 cells	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.035	0.138	0.116	0.143	0.035	0.006	0.069	0.023	0.101	0.063	0.029
2	0.064	0.175	0.154	0.095	0.064	0.008	0.053	0.030	0.114	0.041	0.037
3	0.094	0.159	0.121	0.148	0.094	0.038	0.056	0.057	0.103	0.058	0.043
Mean	0.064	0.157	0.130	0.129	0.064	0.017	0.059	0.037	0.106	0.054	0.037
SD	0.024	0.015	0.017	0.024	0.024	0.015	0.007	0.015	0.006	0.010	0.006

True FP	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.025	1.202	0.113	0.011	0.051	0.004	0.034	0.000	0.161	0.005	0.002
2	0.023	0.301	0.105	0.003	0.056	0.014	0.058	0.102	0.126	0.006	0.001
3	0.016	0.202	0.042	0.012	0.102	0.010	0.041	0.095	0.263	0.025	0.001
4	0.025	0.048	0.093	0.020	0.042	0.079	0.091	0.020	0.063	0.004	0.001
5	0.013	0.157	0.101	0.006	0.079	0.108	0.108	0.090	0.062	0.013	0.002
6	0.018	0.100	0.000	0.014	0.079	0.147	0.034	0.025	0.148	0.011	0.019
7	0.001	0.005	0.010	0.003	0.051	0.012	0.003	0.048	0.003	0.003	0.000
8	0.000	0.003	0.018	0.003	0.063	0.006	0.004	0.012	0.001	0.004	0.000
9	0.000	0.003	0.021	0.004	0.101	0.011	0.002	0.016	0.012	0.026	0.001
10	0.000	0.003	0.091	0.007	0.118	0.009	0.007	0.012	0.004	0.006	0.001
11	0.001	0.005	0.003	0.004	0.084	0.010	0.019	0.006	0.008	0.005	0.000
12	0.001	0.003	0.010	0.014	0.138	0.010	0.011	0.003	0.003	0.003	0.001
13	0.044	0.034	0.066	0.079	0.899	0.007	0.008	0.006	0.010	0.002	0.001
14	0.040	0.024	0.117	0.127	0.237	0.002	0.005	0.012	0.030	0.003	0.001
15	0.025	0.119	0.152	0.039	0.319	0.012	0.009	0.001	0.006	0.001	0.004
16	0.020	0.060	0.129	0.033	0.183	0.007	0.013	0.055	0.024	0.006	0.002
17	0.009	0.027	0.032	0.035	0.266	0.007	0.004	0.073	0.005	0.004	0.002
18	0.010	0.024	0.080	0.075	0.286	0.008	0.012	0.101	0.005	0.004	0.002
19	0.012	0.148	0.141	0.059	0.581	0.000	0.028	0.046	0.041	0.028	0.001
20	0.034	0.064	0.168	0.049	0.416	0.002	0.026	0.026	0.024	0.009	0.004
Mean	0.016	0.127	0.075	0.030	0.208	0.023	0.026	0.037	0.050	0.008	0.002
SD	0.013	0.259	0.053	0.033	0.211	0.039	0.029	0.035	0.069	0.008	0.004

DNA recovered (ng/µl) from 30°C samples:

275 cells	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.009	0.010	0.026	0.030	0.009	0.010	0.004	0.027	0.013	0.013	0.007
2	0.002	0.008	0.024	0.032	0.002	0.029	0.002	0.032	0.009	0.008	0.005
3	0.015	0.009	0.023	0.028	0.015	0.025	0.013	0.024	0.010	0.005	0.005
Mean	0.008	0.009	0.025	0.030	0.008	0.021	0.006	0.028	0.011	0.008	0.005
SD	0.005	0.001	0.001	0.002	0.005	0.008	0.005	0.003	0.002	0.003	0.001

475 cells	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.009	0.006	0.047	0.060	0.020	0.034	0.018	0.013	0.023	0.006	0.016
2	0.002	0.011	0.049	0.020	0.034	0.044	0.011	0.028	0.030	0.004	0.011
3	0.015	0.012	0.047	0.044	0.034	0.038	0.001	0.022	0.021	0.009	0.004
Mean	0.008	0.010	0.048	0.041	0.030	0.039	0.010	0.021	0.025	0.006	0.010
SD	0.005	0.003	0.001	0.017	0.007	0.004	0.007	0.006	0.004	0.002	0.005

825 cells	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.063	0.014	0.070	0.140	0.063	0.011	0.001	0.044	0.012	0.001	0.015
2	0.030	0.016	0.051	0.001	0.030	0.022	0.000	0.051	0.016	0.002	0.017
3	0.044	0.016	0.066	0.138	0.044	0.080	0.003	0.058	0.009	0.002	0.015
Mean	0.045	0.015	0.062	0.093	0.045	0.038	0.001	0.051	0.013	0.002	0.016
SD	0.014	0.001	0.008	0.065	0.014	0.030	0.001	0.006	0.003	0.000	0.001

1500 cells	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.022	0.018	0.029	0.178	0.022	0.044	0.001	0.010	0.049	0.003	0.004
2	0.097	0.022	0.149	0.237	0.097	0.033	0.002	0.008	0.025	0.002	0.005
3	0.026	0.025	0.132	0.084	0.026	0.048	0.009	0.004	0.026	0.003	0.002
Mean	0.048	0.022	0.104	0.166	0.048	0.042	0.004	0.007	0.033	0.002	0.004
SD	0.034	0.003	0.053	0.063	0.034	0.006	0.004	0.003	0.011	0.001	0.001

True FP	1 hour	1 day	2 days	3 days	5 days	1 week	2 weeks	3 weeks	1 month	2 months	3 months
1	0.102	0.011	0.030	0.004	0.210	0.130	0.037	0.203	0.029	0.001	0.043
2	0.075	0.008	0.052	0.003	0.816	0.239	0.074	0.141	0.002	0.005	0.012
3	0.173	0.003	0.076	0.014	0.236	0.077	0.014	0.155	0.004	0.003	0.006
4	0.136	0.001	0.069	0.002	0.283	0.176	0.012	0.169	0.023	0.005	0.024
5	0.079	0.002	0.058	0.023	0.170	0.119	0.010	0.391	0.002	0.016	0.013
6	0.081	0.006	0.067	0.003	0.225	0.189	0.009	0.387	0.000	0.005	0.004
7	0.013	0.028	0.007	0.014	0.047	0.024	0.002	0.008	0.021	0.000	0.003
8	0.016	0.004	0.011	0.008	0.051	0.011	0.000	0.011	0.002	0.002	0.001
9	0.022	0.003	0.006	0.003	0.078	0.023	0.001	0.013	0.004	0.004	0.001
10	0.018	0.001	0.007	0.022	0.067	0.021	0.002	0.010	0.000	0.001	0.002
11	0.015	0.003	0.006	0.011	0.202	0.017	0.002	0.013	0.002	0.001	0.001
12	0.011	0.001	0.005	0.012	0.183	0.009	0.005	0.017	0.001	0.000	0.001
13	0.073	0.002	0.022	0.001	0.178	0.005	0.000	0.090	0.005	0.004	0.003
14	0.041	0.003	0.181	0.004	0.210	0.003	0.043	0.020	0.004	0.000	0.002
15	0.081	0.005	0.023	0.004	0.125	0.007	0.019	0.052	0.004	0.000	0.000
16	0.029	0.012	0.046	0.001	0.260	0.019	0.019	0.009	0.006	0.001	0.009
17	0.149	0.005	0.032	0.002	0.165	0.019	0.022	0.100	0.006	0.000	0.001
18	0.026	0.005	0.054	0.002	0.071	0.001	0.024	0.253	0.002	0.000	0.002
19	0.013	0.046	0.024	0.043	0.376	0.009	0.012	0.259	0.010	0.002	0.012
20	0.081	0.005	0.053	0.166	0.507	0.003	0.017	0.030	0.002	0.002	0.017
Mean	0.062	0.008	0.041	0.017	0.223	0.055	0.016	0.117	0.006	0.003	0.008
SD	0.048	0.010	0.039	0.036	0.175	0.072	0.018	0.122	0.008	0.003	0.010

Chapter III: Development and Validation of Artificial Fingerprint Solutions

3.1 Introduction

Mock fingerprints serve as impressive controls in touch DNA research, imitating true fingerprints in chemistry and allowing a known amount of DNA to be deposited [manuscript submitted]. Since the methods to visualize latent prints rely on the physical and chemical properties of a latent print residue, its composition has been researched extensively by the forensic fingerprint community. Recent literature provides detailed accounts of the relative amounts of different chemicals encountered in such residues. The residue forming a "fingerprint" is composed of two types of secretion, the eccrine and the sebaceous. The eccrine secretions of the sweat glands constitute the aqueous, water-soluble component of a latent print residue, with mainly inorganic salts and amino acids. The sebaceous secretion forms the oily, water-insoluble component of a latent print residue, making its way on our fingertips through contact with our hair, scalp or other areas of our skin, and it contains mostly fatty acids, triglycerides and other fat-soluble compounds.

The objective of Aim 3 was to prepare artificial fingerprint solutions representative of the components of a latent print residue, so that mock fingerprints, more homologous to a true fingerprint, could be produced. A detailed description of the components of a latent print residue was obtained from an article which attempted to create artificial fingerprints for topography studies[74]. This recipe was used to create two different fingerprint solutions, one with only the eccrine secretions and one with both eccrine and sebaceous secretions. They were named fingerprint solutions 2 and 3 (FS-2 and FS-3), as they were modified versions of the original fingerprint solution (FS-1) used routinely in the laboratory and for the first two aims of the thesis. FS-1 differed from FS-2 with respect to the addition of amino acids and other inorganic salts found in eccrine secretions in FS-2. Fatty acids and other components of sebaceous secretions were added into FS-2 to yield FS-3, making it a more complex, viscous solution, with different physical and chemical properties.

Twenty replicates of each cell count were prepared using both new fingerprint solutions and a standard curve was generated with the averaged amounts recovered. High R^2 values validate the ability of these solutions to serve as artificial fingerprint solutions in mock fingerprint preparation. To extract further information about mock fingerprints made from these solutions, loss of DNA in the extraction process was tracked by dividing samples into three treatments. Mock fingerprints were either deposited on sterile glass slides, pipetted directly on the swab or added directly to the lysis buffer. Twenty replicates of the 5 cell counts' mock fingerprints were extracted from each treatment and the samples were quantified to generate a standard curve for each. The *slide* samples represented the overall loss encountered during the entire collection/extraction process. *Swab* and *direct* samples were designed as controls to estimate the amount of DNA lost on the swab and slide. Percent loss during the extraction process alone could be evaluated from the *direct* samples.

3.2 Materials and Methods

3.2.1 Experimental Setup Summary

The same experimental design used to validate the first fingerprint solution was used to validate the two new fingerprint solutions i.e. by generating a standard curve of DNA recovered vs. DNA deposited. To extract further information about mock fingerprints made from these solutions, loss of DNA in the extraction process was tracked by dividing samples into three treatments. Mock fingerprints were either deposited on sterile glass slides and regular collection protocol was followed, or they were added directly to sterile swabs, or directly in the lysis buffer. Twenty replicates of the 5 cell counts' mock fingerprints, for each new fingerprint solution, in each treatment were extracted and the samples were quantified to generate a standard curve.

3.2.2 Fingerprint Solutions

The recipe for the new fingerprint solutions was found in a peer-reviewed journal article[74]. All the chemicals required for these solutions were ordered through Thermo Fischer, Sigma Aldrich (Milwaukee, WI, US). The quantities of chemicals required were converted to a reduced final volume as shown in the tables VII and VIII. Any chemicals liquid at room temperature (shown by yellow cells) were measured in µl by converting the mass (gm) to volume (µl) using the density formula:

$$\textit{Density} = \textit{Mass}/\textit{Volume}$$

The new artificial aqueous solution was called Fingerprint Solution-2 (FS-2) and the solution containing equal parts new artificial aqueous and artificial sebaceous secretion was called Fingerprint Solution-3 (FS-3).

Table VII: Final measurements for Artificial Eccrine Secretion

Sr. No.	Chemical	Amount (mg) for 1000 ml	Amount (mg) for 100ml	Density (kg/m3)	Volume needed (µl) for 100ml
1.	Potassium chloride	1400	140		
2.	Sodium chloride	1300	130		
3.	Sodium bicarbonate	250	25		
4.	Ammonium hydroxide	175	17.5	823.35	22.15
5.	Magnesium chloride	40	4		
6.	Serine	275	27.5		
7.	Glycine	135	13.5		
8.	Ornithine	110	11		
9.	Alanine	80	8		
10.	Aspartic Acid	40	4		
11.	Threonine	40	4		
12.	Histidine	40	4		
13.	Valine	30	3		
14.	Leucine	30	3		
15.	Lactic acid	1900	190	1206	157.5
16.	Urea	500	50		
17.	Pyruvic acid	20	2	1250	1.6
18.	Acetic acid	5	0.5	1049	0.47
19.	Hexanoic acid	5	0.5	930	0.5

All the 19 chemicals were measured (final measurements in green boxes) and added to a glass bottle containing 90 ml of sterile, autoclaved water. This mixture was sonicated in a water bath for 15 minutes to ensure proper mixing. The pH of the mixture was adjusted to 5.5 (pH of sweat) using 5M NaOH. The volume was adjusted to a 100 ml and the final mixture was sonicated another 15 minutes to ensure homogeneity. This final solution (FS-2) was then autoclaved again and stored in the same closed cap glass bottle at room temperature.

Table VIII: Final Measurements for Artificial Sebaceous Secretion

Sr. No.	Chemical	Amount (mg) for 20 ml	Density (kg/m3)	Amount (ul) for 20ml
1.	Hexanoic acid	50	930	53.76
2.	Heptanoic acid	50	918	54.46
3.	Octanoic acid	50	910	54.94
4.	Nonanoic acid	50	900	55.55
5.	Dodecanoic acid	50		
6.	Tridecanoic acid	50		
7.	Myristic acid	50		
8.	Pentadecanoic acid	50		
9.	Palmitic acid	55		
10.	Stearic acid	55		
11.	Arachidic acid	50		
12.	Linoleic acid	55		
13.	Oleic acid	55		
14.	Triolein	275		
15.	Tricaprylin	20	950	21.05
16.	Tricaprin	20		
17.	Trilaurin	20		
18.	Trimyristin	20		
19.	Tripalmitin	20		
20.	Squalene	120	858	139.86
21.	Cholesterol	30		
22.	Cholesterol n-decanoate	40		
23.	Cetyl palmitate	155		

The chemicals were measured in mg and any chemicals liquid at room temperature (shown in yellow cells) were measured in µl, converted using the density formula. All 23 chemicals (final measurements in green cells) were added to 20 ml of sterile, autoclaved water in an amber bottle. The mixture was sonicated for 15 minutes to ensure homogeneity and stored in the amber bottle at room temperature. To prepare FS-3, equal volume of FS-2 and the artificial sebum was mixed in a separate container and sonicate again. The final solution (FS-3) was autoclaved to ensure decontamination.

3.2.3 Cell Counting

A fresh buccal swab cutting was placed in 1 ml aliquot of Accumax™, a cell dissociation solution for 15 minutes with careful pipetting every 5 minutes to aid dissociation of buccal cells. The swab was then discarded, and the solution was allowed to incubate at room temperature for additional 15 minutes with regular pipetting to prevent the cells settling to the bottom. Ten microliters of this suspension was transferred to a 0.6 ml tube where it was mixed with 10 µl of Trypan Blue dye. This 20 µl stained cell solution was pipetted onto two sides of a hemocytometer slide (Bright-Line, Hausser Scientific, Horsham, PA), with 10 µl of solution on each slide. The slide was viewed under 10X magnification on a compound microscope and the cells on each side were counted as 9 readings corresponding to the 9 squares on each side of the hemocytometer slide. The total counts from both sides were multiplied by the dilution factor i.e. 2 and the following formula was used to convert cells counted to cells/µl.

$$\textit{Cells/µl} = \frac{\textit{Total number of cells on each side}}{9} \textit{ x 10}$$

The cells/µl from each side of the hemocytometer were averaged and this value was used to calculate the amount of cell suspension required by each cell count to get the correct number of cells in the mock fingerprints.

3.2.4 Preparing the Mock Fingerprints

Mock fingerprints were made for 5 cell counts: 0, 250, 475, 825, and 1500; with each diploid cell having 6pg of DNA, the cell counts corresponded to 0, 1.5, 2.85, 4.95 and 9 ng of DNA respectively. Each cell count was divided by the averaged cells/µl to calculate the amount of cell suspension needed for the correct number of cells in each mock fingerprint solution. Mock fingerprint solution for each cell count was prepared in replicate, in a master mix, i.e. for each 20 µl of mock fingerprint solution: 2 µl of the 10X fingerprint solution being tested (FS-2/FS-3), and a combined 18 µl of the cell suspension and 1 X PBS depending on the cell count, were mixed in a tube. This master mix solution for each cell count was deposited on clean sterile glass slides, 20 µl for each deposit, in the hood and allowed to air dry.

3.2.5 Sample Treatments

Twenty replicates of the 5 cell counts' mock fingerprints prepared using both newly prepared fingerprint solutions were deposited on a glass slide, pipetted directly onto a clean swab, or pipetted directly into lysis buffer.

Table IX: Experimental Design for Aim 3

	Slide	**Swab**	**Direct**
FS-2	20 replicates of 5 cell counts' mock fingerprint	20 replicates of 5 cell counts' mock fingerprint	20 replicates of 5 cell counts' mock fingerprint
FS-3	20 replicates of 5 cell counts' mock fingerprint	20 replicates of 5 cell counts' mock fingerprint	20 replicates of 5 cell counts' mock fingerprint

For the *slide* samples, mock fingerprint solutions of the 5 cell counts [20 replicates each: 5 x 20=100] were prepared as described in the protocol above and 20 replicates of each cell count solution was pipetted onto clean, sterile glass slides and allowed to air dry in the hood. The mock fingerprints were collected off the slides using sterile swabs (Puritan Medical Products Company LLC, Guilford, Maine) moistened with 20 µl of 2% SDS and the organic extraction protocol described in the next section was followed.

For the *swab* samples, 20 µl of each of the 5 cell counts' mock fingerprint solutions [20 replicates each: 5 x 20=100] were pipetted onto clean, sterile cotton swabs moistened with 20 µl of 2% SDS and the organic extraction protocol described in the next section was followed.

For the direct samples, 20 µl of each cell counts' mock fingerprint solution [20 replicates each: 5 x 20=100] was pipetted directly into 2 ml extraction tubes following organic extraction protocol (described below).

3.2.6 Extraction

Depending on the treatment, either swabs of the mock fingerprints, or the mock fingerprint solution itself, were placed in sterile 2 ml extraction tubes (Corning Incorporated, Salt Lake City, UT) and 400 µl of stock extraction buffer (recipe on page 28) and 20 mg/ml proteinase K was added to each tube. The samples were then incubated in a water bath maintained at 56°C overnight. The next day, for the slide and swab samples, the swabs were transferred to spin baskets (Corning Incorporated, Salt Lake City, UT) placed on top of each 2 ml tube, and they were centrifuged (Hermle Labortechnik, Germany) at 19000 x g for 5 minutes. The dry swabs were discarded along with the spin baskets and 400 µl of 25:24:1 phenol-chloroform-isoamyl alcohol (Acros Organics, New Jersey) was added to all the tubes (i.e. *slide*, *swab* and *direct* samples). The tubes were inverted vigorously to ensure enough interaction between phases and then centrifuged at 19000 x g for 5 minutes to separate the phases.

The upper aqueous layer of each tube was transferred to a new, sterile 1.5 ml tube (Brinkmann Instruments Inc., Blue Bell, PA) and 1 ml of cold 100% absolute ethanol (Janssen Pharmaceuticalaan, Fair Lawn, NJ) was added to each of the 1.5 ml tubes. The tubes were kept at -20°C for an hour and then centrifuged at 19000 x g for 15 minutes to allow precipitation and pelleting of purified DNA to the bottom of the tubes. The supernatant ethanol from each tube was discarded and the pellets were washed with 1 ml of 70% room temperature ethanol. The tubes were again centrifuged at 19000 x g for 5 minutes and the supernatant ethanol was discarded. This ethanol wash was repeated once more, and the final supernatant discarded. The samples were allowed to dry at 56°C in the oven. Lastly, 50 µl of TE^{-4} buffer (recipe on page 28) was added to each sample and they were allowed to resolubilize overnight in a water bath maintained at 56°C.

3.2.7 Quantification

The samples were quantified following the standard laboratory protocol using Applied Biosystems™ 7500 Real-Time PCR with SYBR® Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA). Human-specific ALU primers (forward: GTCAGGAGATCGAGACCATCCC reverse: TCCTGCCTCAGCCTCCCAAG) were used to amplify the extracted DNA and determine the quantity of template DNA in the sample. A master mix of was prepared for the samples which included, for each sample: 5 µl of the 2X SYBR Green Supermix, 2.6 µl of sterile water, and 0.2 µl each of the 2.5 pmol/µl forward and reverse primers. A total of 8 µl of the master mix was added to each well, along with 2 µl of the sample to be quantified. Each PCR plate was run with 8 standard dilutions, in ng/µl: 16.7, 5.56, 1.85, 0.62, 0.21, 0.068, 0.023, 0.0077; a positive amplification control (1ng/µl control DNA) and a negative amplification control (No template Control i.e. NTC).

The conditions for each run were as follows: Holding at 95℃ for 2 minutes followed by 35 cycles of denaturation at 95℃ for 15 seconds and annealing/extending at 68℃ for 1 minute. The melt curve stage was set to default: 95℃ for 15 seconds, 60℃ for 1 minute, 95℃ for 30 seconds and 60℃ for 15 seconds. All the extracted mock fingerprint samples for each treatment were quantified using the same protocol and the data was compiled to compare the average loss of DNA after each treatment, for each fingerprint solution.

3.3 Results and Discussion

3.3.1 Cell Counts and Calculations for Mock FP Preparation

The 20 replicates of the 5 cell counts' mock fingerprints were broken down into sets of 4, 8 and 8 replicates for both fingerprint solutions. The cell count readings for each set of replicates is given below with their mean and standard deviation. Buccal epithelial cells were counted using the hemocytometer as described in the materials and methods section. The total number of cells was multiplied by the dilution factor and converted to cells/µl using the formula

$$Cells/\mu l = \frac{Total\ number\ of\ cells\ counted}{9}\ x\ 10$$

FS-2 cell counts for each set of replicates:

	4 replicates	8 replicates_1	8 replicates_2
1	13	12	18
2	11	10	15
3	15	8	17
4	13	11	22
5	10	13	18
6	14	14	14
7	12	15	20
8	8	11	16
9	9	12	12
10	8	12	10
11	13	14	12
12	12	13	11
13	15	12	21
14	13	14	17
15	13	14	11
16	12	13	14
17	11	11	10
18	15	13	14
Mean	12.06	12.33	15.11
SD	2.15	1.67	3.65

FS-3 cell counts for each set of replicates:

	4 replicates	8 replicates_1	8 replicates_2
1	13	13	14
2	11	11	12
3	15	12	13
4	13	15	14
5	10	15	10
6	14	12	13
7	12	15	11
8	8	13	13
9	9	10	14
10	8	11	8
11	13	9	12
12	12	11	13
13	15	9	14
14	13	12	10
15	13	10	14
16	12	10	10
17	11	11	13
18	15	8	14
Mean	12.06	11.50	12.33
SD	2.15	2.03	1.76

Following the protocol for preparation of mock fingerprints, in each 20 µl of mock fingerprint solution, 2 µl of artificial fingerprint solution was added, along with a combined 18 µl of cell suspension and 1X PBS depending on the cell count. Depending on the number of replicates being extracted simultaneously, 4 or 8, the calculations were scaled up proportionally to create a single master mix solution for each cell count. For example, the 4 set of replicates needed 12 mock fingerprints for each cell count in each treatment (4 x 3=12), rounding it up to 20, making the calculation for each cell count solution: 40 µl of artificial fingerprint solution, and a combined 360 µl of the cell suspension and 1X PBS, depending on the cell count.

	4 replicates		8 replicates-1		8 replicates-2	
Cell Count	**A**	**B**	**A**	**B**	**A**	**B**
0	0.0	360.0	0.0	540.0	0.0	540.0
250	20.7	339.3	30.4	509.6	26.0	514.0
475	39.4	320.6	57.8	482.2	49.3	490.7
825	68.4	291.6	100.3	439.7	85.7	454.3
1500	124.4	235.6	182.4	357.6	155.8	384.2

Table X: Calculations for FS-2 Mock FP Replicates (A-Amount in µl of cell suspension added, B-Amount in µl of 1X PBS added)

	4 replicates		8 replicates-1		8 replicates-2	
Cell Count	**A**	**B**	**A**	**B**	**A**	**B**
0	0.0	360.0	0.0	540.0	0.0	540.0
250	20.7	339.3	32.6	507.4	30.4	509.6
475	39.4	320.6	62.0	478.0	57.8	482.2
825	68.4	291.6	107.6	432.4	100.3	439.7
1500	124.4	235.6	195.7	344.3	182.4	357.6

Table XI: Calculations for FS-3 Mock FP Replicates (A-Amount in µl of cell suspension added, B-Amount in µl of 1X PBS added)

3.3.2 Quantification by Real-Time PCR

For both fingerprint solutions, all 20 replicates of each cell count in each treatment were quantified using real-time PCR and the total DNA recovered was averaged. For each treatment, percent recovery and percent loss of each cell count was calculated as follows:

$$\textit{Percent Recovery} = \frac{\textit{DNA Recovered (ng)}}{\textit{DNA Deposited (ng)}} \textit{ x 100\%}$$

$$\textit{Percent Loss} = 100 - \textit{Percent Recovery}$$

Tables XII and XIII provide the average DNA (ng) extracted from the twenty replicates of each cell count's mock fingerprints in each of the three treatments, along with the average percent recovery and average percent loss calculated from the percent recovery and percent loss formulas above.

		Avg. DNA Recovered (ng)			Avg % Recovery			Avg. % Loss		
Cell Count	DNA Deposited (ng)	Slide	Swab	Direct	Slide	Swab	Direct	Slide	Swab	Direct
0	0	0	0	0	0	0	0	0	0	0
250	1.5	0.787	0.899	0.900	52.46	59.93	60.01	47.54	40.07	39.99
475	2.85	1.810	1.772	1.836	63.51	62.19	64.42	36.49	37.81	35.58
825	4.95	2.837	3.094	3.236	57.31	62.50	65.36	42.69	37.50	34.64
1500	9	4.395	5.171	5.610	48.84	57.45	62.33	51.16	42.55	37.67

Table XII: Average % Loss for FS-2 Mock Fingerprints

		Avg. DNA Recovered (ng)			Avg % Recovery			Avg.%Loss		
Cell Count	DNADeposited(ng)	Slide	Swab	Direct	Slide	Swab	Direct	Slide	Swab	Direct
0	0	0	0	0	0					
250	1.5	0.63	0.83	0.85	42.00	55.33	56.67	58.00	44.67	43.33
475	2.85	1.48	1.50	1.55	51.93	52.63	54.39	48.07	47.37	45.61
825	4.95	2.86	2.92	3.34	57.78	58.99	67.47	42.22	41.01	32.53
1500	9	4.90	5.17	5.73	54.44	57.44	63.67	45.56	42.56	36.33

Table XIII: Average % Loss for FS-3 Mock Fingerprints

The experimental design, including the use of mock fingerprints, provides an estimate of DNA loss at different points of the collection and extraction process. The *slide* samples represent the total loss throughout the process, whereas the *swab* samples represent amount of DNA left on the swab and lost during extraction. *Direct* samples simply quantify the amount of DNA lost during the organic extraction protocol. Therefore, subtracting the DNA loss in *direct* samples from the DNA loss from *swab* samples, would give approximate DNA left on the swab.

For mock fingerprints made using fingerprint solution FS-2, the average percent DNA loss is highest for samples deposited on slides, and least for samples pipetted directly into the extraction reagents. This data provides an estimated percent loss of DNA through the entire extraction protocol of mock fingerprints deposited on slides. The samples pipetted directly onto the swab also showed more loss than the direct samples, indicating the average amount of DNA left on the swab during centrifugation.

Similar results were seen with mock fingerprints made from fingerprints solution FS-3. Highest percent loss was observed in *slide* samples, followed by *swab* samples and then *direct* samples. However, the difference between the percent loss between treatments was lower for FS-3 mock fingerprints than those made by FS-2. Statistical analysis was conducted to determine if these differences in percent loss between treatments and between fingerprint solutions were significant.

These results were compared to the data obtained from similar experiments done using mock fingerprints prepared with fingerprint solution FS-1. The same three treatments (*slide*, *swab* and *direct*) were used to determine where in the collection/extraction process most amount of DNA was being lost. *Table XIV* shows the average DNA recovered and percent loss for each cell count in the three treatments for mock fingerprints made with FS-1.

The difference in percent loss between treatments is more predominant in mock fingerprints made from FS-1, compared to percent loss between treatments in mock fingerprints made from FS-2 and FS-3. This might be attributed to the fingerprint solution chemistry in these artificial solutions, with FS-1 having only the inorganic components, FS-2 having the inorganic components along with amino acids, and FS-3 having inorganic, amino acids and lipid secretions. The addition of lipids in FS-3 made its consistency more viscous, attributing different physical properties to the solution when compared to FS-1 or FS-2. These results justify the development of the new fingerprint solutions, with chemistry more analogous to a true fingerprint and less effect of treatment on the recovery of DNA.

Table XIV: Average Recovery and Percent Loss for FS-1 Mock Fingerprints

		Mean (ng)	SD	%DNA Loss
250 cell (1.5ng)	slide	0.46	0.11	69
	swab	0.67	0.12	55
	direct	1.09	0.15	27
475 cell (2.85ng)	slide	0.82	0.11	71
	swab	1.14	0.21	60
	direct	1.64	0.39	42
825 cell (4.95ng)	slide	1.14	0.34	77
	swab	1.93	0.59	60
	direct	3.31	0.46	32
1500 cell (9.0ng)	slide	2.19	0.80	76
	swab	3.99	0.71	56
	direct	5.97	0.44	34

3.3.3 Statistical Analysis

ANOVA between Treatments

For each fingerprint solution, the difference between amount of DNA recovered after each treatment was analyzed by ANOVA testing to determine if the results were statistically significant. The p-values of ANOVA testing for each cell count between the three treatments are given below.

	FS-2	FS-3
250 cells	0.417	0.042
475 cells	0.932	0.881
825 cells	0.417	0.249
1500 cells	0.932	0.150

Table XV: p-values of ANOVA Testing between Treatments

ANOVA analysis revealed no statistically significant difference in the amount of DNA recovered from all the cell counts' mock fingerprints prepared by both fingerprint solutions when the treatment methods were compared. The p-value for all cell counts is higher than α=0.01, indicating no substantial difference in DNA loss between the three treatments i.e. slide, swab or direct.

These findings reveal an interesting aspect of the protocol used for the analysis of mock fingerprints, suggesting that the amount of DNA left on the slide or the swab does not significantly affect amount of DNA recovered after the entire collection/extraction process. It also highlights the impact of the physical and chemical properties of a solution on its tendency to be retained on the slide or the swab. The percent loss for both slide and swab mock fingerprints, made using FS-2 and FS-3, was approximately 40-50% in all cell counts, and 30-40% of that was lost due to extraction, indicating only 10-20% of DNA loss on slide and swab. Therefore, to maximize recovery from FS-2 and FS-3 mock fingerprints even further, the extraction process would be the place to start optimization efforts. This data suggests FS-2 and FS-3 mock fingerprints serve as excellent carriers of the DNA source in mock fingerprints and lead to efficient collection and extraction of the prints. These results thus prove the validity of established protocols used to extract DNA from mock fingerprints left on a glass slide.

ANOVA between Fingerprint Solutions

	Slide	Swab	Direct
250 cells	0.0424	0.8782	0.6536
475 cells	0.5110	0.0899	0.0481
825 cells	0.6028	0.5682	0.6483
1500 cells	7.0E-07	0.0326	0.8223

Table XVI: p-values of ANOVA Testing between Fingerprints Solutions

Further ANOVA testing also showed no statistically significant difference (p-values>α=0.01), in the amount of DNA recovered, between the two new fingerprint solutions after any of the three treatments. This suggests that both new fingerprint solutions interact with the DNA analysis process in a similar way, regardless of the difference in their chemistry. Although the addition of the artificial sebum, containing lipids and fatty acids, to FS-2 made it more viscous and complex in chemistry, it did not affect the DNA recovery from these mock fingerprints significantly.

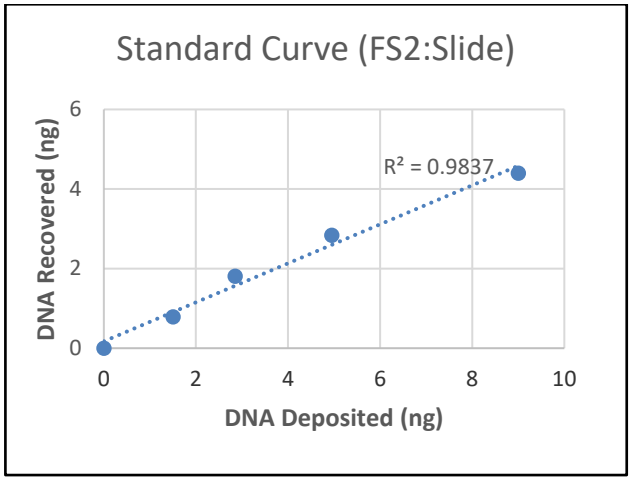
An exception is the 1500 cell count mock fingerprints deposited on the slide. The p-value being 7×10^{-7}, suggests the amount recovered from FS-2 prints and FS-3 prints were significantly different in quantity. This discrepancy in the ANOVA analysis for this sample type was considered an outlier, since the standard deviations between the 20 replicate values for 1500 cell count were greater than 1 ng for both FS-2 and FS-3 mock fingerprints, and the general trend for all other cell counts in all treatments was statistically insignificant. Overall, these ANOVA results indicate no statistically significant difference in DNA recovery between mock fingerprints of the two new fingerprints solutions. Therefore, it can be concluded that the chemistry of the components in both FS-2 and FS-3 solutions affects DNA analysis in similar manners, leading to similar recoveries.

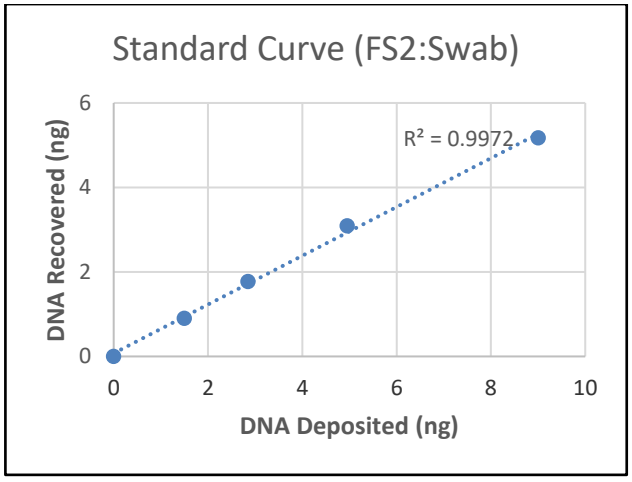
3.3.4 Standard Curves

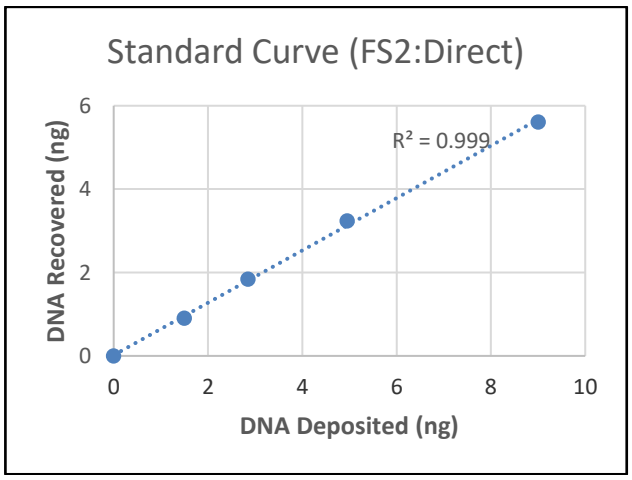
Standard curves were generated for both fingerprint solutions by plotting DNA Recovered (ng) vs DNA Deposited (ng) for the 5 cell counts used to make mock fingerprints. High R^2 values, as seen on the standard curves, of the trend lines suggest a strong positive correlation between the recovery proportions for each cell count. These curves prove that the mock fingerprints prepared using FS-2 and FS-3 solutions serve as good controls to derive DNA deposited from DNA recovery and that DNA loss was proportionate for all cell counts throughout the *slide*, *swab* and *direct* treatments.

The recovery of DNA from the twenty replicates of each cell count were precise and proportionate to the amount of DNA deposited, regardless of the treatment. These standard curves prove that both the new artificial fingerprint solutions FS-2 and FS-3 have good potential to be used in mock fingerprint preparations, providing the touch DNA research community with fingerprints controls that are more representative of a true fingerprint residue.

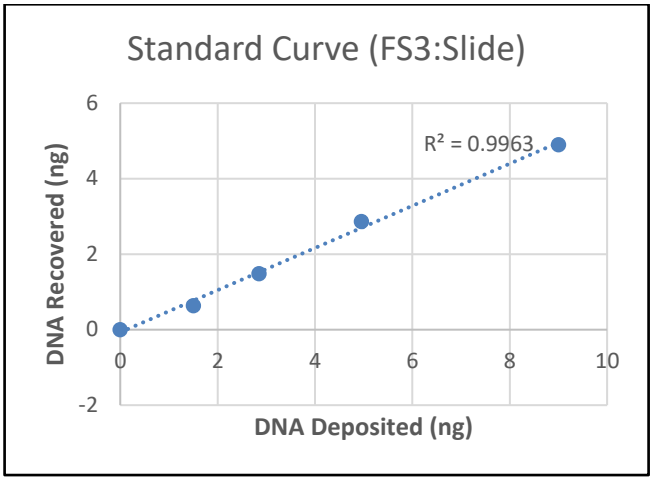
FS-2:

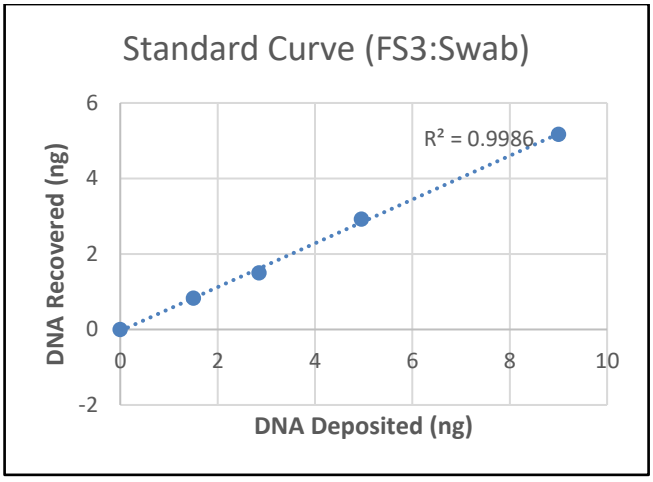

Standard Curve (FS2:Slide)
R² = 0.9837
DNA Recovered (ng)
DNA Deposited (ng)
0
2
4
6
8
10

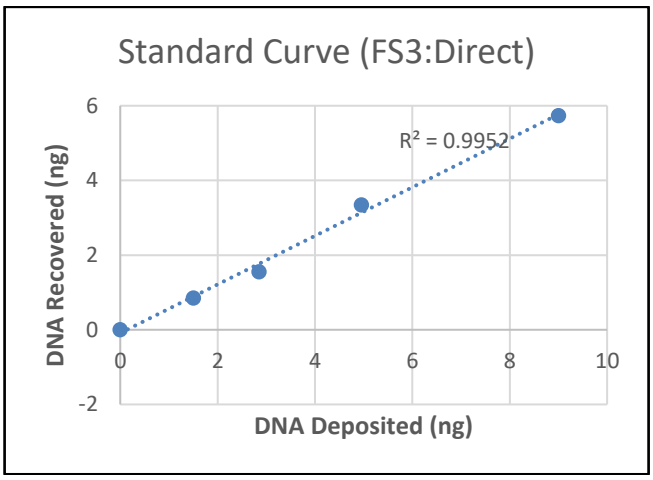

Standard Curve (FS2:Swab)
R² = 0.9972
DNA Recovered (ng)
DNA Deposited (ng)
0
2
4
6
8
10


Standard Curve (FS2:Direct)
R² = 0.999
DNA Recovered (ng)
DNA Deposited (ng)
0
2
4
6
8
10

FS-3:


Standard Curve (FS3:Slide)
R² = 0.9963
DNA Recovered (ng)
DNA Deposited (ng)
-2
0
2
4
6
8
10


Standard Curve (FS3:Swab)
R² = 0.9986
DNA Recovered (ng)
DNA Deposited (ng)
-2
0
2
4
6
8
10


Standard Curve (FS3:Direct)
R² = 0.9952
DNA Recovered (ng)
DNA Deposited (ng)
-2
0
2
4
6
8
10

3.4 Conclusion

Trace DNA analysis has broadened the scope of samples that can be used to extract genetic material. Research in the field of trace DNA has focused extensively on factors affecting DNA transfer[72,73], or analytical practices yielding most DNA recovery, from true fingerprint samples. However, the amount of DNA recovered from true fingerprints falls in a wide range, making it difficult to make any reliable conclusions regarding trends or patterns in recovery of trace DNA. The use of mock fingerprints provides a solution to this problem, with a known amount of DNA initially deposited, loss can be studied against different variables.

To justify using mock fingerprints as substitutes for true fingerprints in research studies, a mock fingerprint should imitate a true fingerprint residue in terms of chemistry of the residue, volume of residue and source of DNA included. Mock fingerprints have been created previously, optimizing the latter two aspects and tracking the loss of DNA from these samples in the collection/extraction process. The chemistry of the residue was optimized in this study, to prepare artificial fingerprint solutions that would better represent a true fingerprint residue.

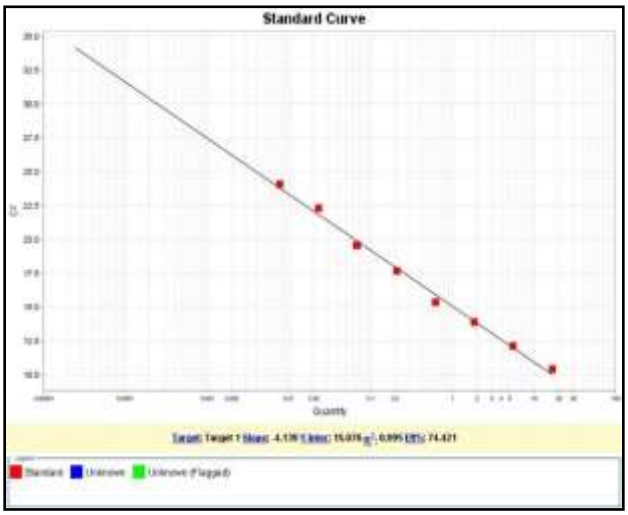
Validation of these fingerprint solutions was done by extracting twenty replicates of various cell counts and creating a standard curve of the amounts recovered. High R^2 of the trend lines confirmed the data fit the general model where DNA recovered was positively correlated to DNA deposited when the mock fingerprint replicates were deposited on slide, swab and when pipetted directly into extraction buffer. Statistical analysis showed depositing these mock fingerprints, made from FS-2 and FS-3, on a glass slide or a cotton swab did not significantly affect overall DNA loss during the collection/extraction process. This further validates the standard protocol used in the laboratory to collect touch DNA samples, with most of the inevitable loss occurring during organic extraction.

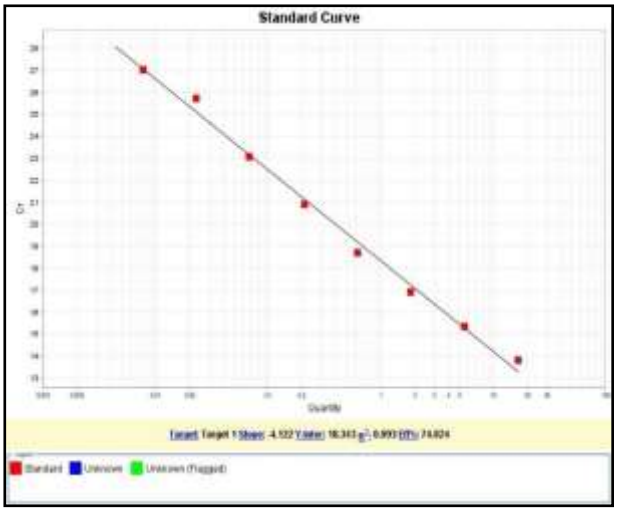
Interestingly, no statistically significant difference was found between the average DNA recovery of the two fingerprint solutions, regardless of the difference in their chemistry. FS-2 contained only the inorganic, “eccrine” secretions, whereas FS-3 contained the “eccrine” and “sebaceous” secretions, forming a more viscous, oily fingerprint solution. This viscous oily solution was relatively harder to work with, in comparison to FS-1 or FS-2. An FS-3 mock fingerprint dried as a visible white stain on the slide (Appendix B), compared to the relatively clear deposits left by FS-1 and FS-2 mock fingerprints.

However, considering the chemistry of a latent print residue, FS-3 is more analogous to a true fingerprint, and serves as a holistic substitute for a true fingerprint residue. These studies provide a strong basis on which additional research can be built. One of the best qualities of a mock fingerprint is the known amount of DNA present in them. This aspect should be exploited, to study how post-deposition factors affect DNA recovery, in a manner similar to the first two aims of this thesis. Experiments to test the recovery of DNA from different substrates will further explain the nature of interaction between fingerprint residue and different substrates.

Appendix B

Standard Curves of Real-time Data





Master Summary of Quantified Samples

FS-2: Total DNA recovered (ng)

	250 cells (Slide)	250 cells (Swab)	250 cells (Direct)
1	0.48	1.29	1.34
2	0.91	1.18	0.87
3	0.90	1.01	0.81
4	1.16	1.33	0.64
5	0.41	0.86	0.91
6	0.87	1.31	0.70
7	0.73	0.87	0.58
8	0.96	0.60	1.08
9	1.25	1.29	0.72
10	0.84	1.27	1.00
11	1.29	1.34	1.44
12	0.65	0.51	0.97
13	0.44	0.09	0.58
14	0.94	0.60	1.08
15	0.77	0.79	0.52
16	0.73	0.77	0.45
17	0.44	0.84	1.44
18	0.94	0.89	0.97
19	0.27	0.70	1.05
20	0.73	0.45	0.85
Mean	0.79	0.90	0.90
SD	0.28	0.35	0.29

	475 cells (Slide)	475 cells (Swab)	475 cells (Direct)
1	1.47	1.96	0.97
2	2.46	2.39	1.52
3	2.15	1.43	2.77
4	2.33	2.43	2.35
5	2.34	1.36	2.21
6	2.31	1.76	2.58
7	2.33	1.98	1.35
8	1.01	2.32	1.37
9	2.32	1.79	2.18
10	1.25	1.94	2.06
11	0.60	2.33	2.45
12	0.55	0.94	1.18
13	1.90	1.98	0.85
14	2.03	1.53	1.37
15	1.68	1.98	2.18
16	1.94	1.78	2.06
17	1.90	0.83	1.95
18	2.03	0.94	1.36
19	1.68	2.10	1.65
20	1.94	1.70	2.30
Mean	1.81	1.77	1.84
SD	0.57	0.48	0.56

	825 cells (Slide)	825 cells (Swab)	825 cells (Direct)
1	3.35	2.96	2.39
2	3.02	3.91	3.06
3	1.07	3.45	3.93
4	4.94	2.54	2.56
5	4.83	2.10	2.25
6	3.55	4.06	2.59
7	1.10	2.22	3.10
8	1.99	3.74	4.14
9	2.24	2.85	3.06
10	1.62	3.07	3.49
11	2.44	3.96	2.89
12	3.76	2.41	3.53
13	3.29	2.22	3.10
14	3.96	3.74	4.14
15	1.87	2.85	3.06
16	1.39	3.07	3.49
17	3.29	3.58	2.89
18	3.96	2.41	3.53
19	3.66	3.15	3.90
20	1.39	3.60	3.60
Mean	2.84	3.09	3.24
SD	1.21	0.64	0.56

	1500 cells (Slide)	1500 cells (Swab)	1500 cells (Direct)
1	4.63	7.88	7.90
2	6.05	5.79	7.45
3	6.01	8.01	8.45
4	3.43	3.49	6.67
5	4.45	2.78	6.48
6	3.85	3.97	4.67
7	1.60	4.17	6.43
8	3.32	6.52	6.27
9	1.59	2.83	4.67
10	4.90	7.70	0.61
11	5.07	5.76	6.86
12	6.21	4.57	6.42
13	2.50	4.17	4.29
14	3.12	6.52	6.27
15	5.89	2.28	4.67
16	6.88	7.70	0.61
17	4.00	5.76	5.90
18	3.12	4.57	6.42
19	5.89	4.35	6.25
20	5.38	4.60	4.90
Mean	4.40	5.17	5.61
SD	1.56	1.78	2.03

FS-3: Total DNA recovered (ng)

	250 cells (Slide)	250 cells (Swab)	250 cells (Direct)
1	0.55	0.28	1.18
2	0.65	0.26	1.05
3	0.55	0.65	1.42
4	0.40	0.19	1.38
5	0.15	0.77	0.30
6	0.40	0.46	0.25
7	0.85	1.00	0.37
8	0.70	0.78	0.67
9	0.75	1.27	0.62
10	0.65	0.97	0.35
11	0.65	0.85	0.62
12	0.65	0.91	1.00
13	0.80	0.69	1.10
14	0.65	1.16	0.95
15	0.75	1.20	0.98
16	0.65	1.08	1.25
17	0.70	0.69	0.89
18	0.95	1.16	1.05
19	0.55	1.20	0.85
20	0.65	1.08	0.80
Mean	0.63	0.83	0.85
SD	0.17	0.33	0.35

	475 cells (Slide)	475 cells (Swab)	475 cells (Direct)
1	1.15	0.95	1.64
2	1.45	0.86	2.27
3	1.15	1.01	1.84
4	1.05	0.95	1.22
5	1.20	0.88	1.18
6	0.85	0.97	1.41
7	1.75	1.35	1.51
8	1.80	2.16	1.59
9	1.95	0.29	1.34
10	1.85	1.05	1.44
11	1.50	0.54	1.80
12	1.80	2.49	1.61
13	1.30	2.34	1.34
14	1.75	2.17	1.22
15	1.95	1.92	1.76
16	1.50	1.82	1.59
17	1.65	2.34	1.34
18	0.85	2.17	1.44
19	1.70	1.92	1.66
20	1.30	1.82	1.77
Mean	1.48	1.50	1.55
SD	0.35	0.68	0.26

	825 cells (Slide)	825 cells (Swab)	825 cells (Direct)
1	1.10	1.49	2.58
2	0.80	1.90	3.19
3	1.30	0.91	3.10
4	2.65	1.54	2.34
5	3.55	1.71	3.16
6	3.70	1.04	4.06
7	2.45	4.08	2.67
8	3.35	3.74	4.60
9	2.70	3.32	3.42
10	3.55	3.41	3.82
11	3.90	3.64	2.11
12	3.80	4.58	3.94
13	1.95	2.51	2.34
14	3.45	4.32	3.16
15	3.60	3.73	3.42
16	3.20	2.94	3.82
17	1.95	2.51	4.56
18	3.45	4.32	2.67
19	3.60	3.73	4.60
20	3.20	2.94	3.19
Mean	2.86	2.92	3.34
SD	0.96	1.15	0.77

	1500 cells (Slide)	1500 cells (Swab)	1500 cells (Direct)
1	4.50	3.23	7.48
2	4.40	3.91	6.75
3	4.33	3.08	4.60
4	4.78	4.14	3.64
5	4.33	5.13	4.57
6	4.18	3.44	7.10
7	4.76	4.81	3.13
8	5.10	6.08	7.25
9	7.60	6.40	6.61
10	4.82	4.85	4.61
11	3.75	8.32	6.78
12	4.74	7.29	4.90
13	6.20	4.87	7.25
14	5.80	8.58	6.61
15	3.50	5.67	6.00
16	6.15	4.36	4.95
17	6.20	4.39	4.85
18	5.80	4.86	5.60
19	3.50	5.67	7.50
20	3.56	4.36	4.35
Mean	4.90	5.17	5.73
SD	1.09	1.54	1.37

FS-3 mock fingerprints dried on slide as white residue:

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