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Analyzing the viability of direct PCR for use in conjunction with cyanoacrylate enhanced fingerprints

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Thesis submitted to the Eberly College at West Virginia University in partial fulfillment of the requirements for the degree of

Master of Science in
Forensic and Investigative Science

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Morgantown, WV
2022

Keywords: Direct PCR, Chemically enhanced fingerprints, Cyanoacrylate
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ABSTRACT

Analyzing the viability of direct PCR for use in conjunction with cyanoacrylate enhanced fingerprints

Coral M. Smith, B.S

Efficient methods for DNA analysis are desperately needed in laboratories due to the influx in DNA analysis requests. The current DNA processing methods are costly, time consuming, and involve multiple tube changes, increasing the risk of contamination. However, the use of direct PCR can simplify the DNA analysis process by eliminating the extraction, purification and quantitation steps. Presently, the FBI Quality Assurance Standard 9.4 requires all evidence samples to be quantitated. As a result, the direct PCR method of DNA processing cannot be implemented on evidence samples at this time. Direct PCR presents the opportunity to provide efficient DNA analysis, making the investigation of this process an important endeavor for the forensic community.

This study sought to evaluate the ability of direct PCR to generate DNA profiles from fingerprints that have been previously enhanced using cyanoacrylate. Traditional methods of DNA analysis used on fingerprints post chemical processing have resulted in full DNA profiles. In addition, direct PCR has been successful on a wide variety of samples containing potential inhibitors, even those visualized with various dactyloscopic powders. No studies have assessed the ability of using direct PCR to acquire DNA profiles from cyanoacrylate treated fingerprints, representing a gap in knowledge for the forensic community. An area of concern for the use of direct PCR is the loss of purification steps meant to remove PCR inhibitors, which could be problematic for analysis of chemically processed fingerprints. Therefore, the assessment of the viability of direct PCR on these types of evidence samples is of critical importance.

Participants deposited fingerprints onto glass slides that were subsequently treated with cyanoacrylate. 60 samples were processed using direct PCR and 60 samples were processed through a traditional extraction method. Results were evaluated based on percent recoverability, calculated based on the number of alleles observed over the number of alleles expected. Traditional methods were shown to have the greatest success overall with 53% of samples having at least one correct allele call, however direct PCR showed some success with only 23%. These results indicate that processing cyanoacrylate fumed fingerprint samples using direct PCR is possible and further experimentation and optimization could potentially increase these success rates.

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1.0 Introduction

Identification of people has been a goal since the time of Alphonse Bertillon and his biometric identification system, where he used anthropometry to distinguish between persons. Technology has advanced over time to techniques with incredible discriminating capabilities like fingerprinting and deoxyribonucleic acid (DNA) testing. Due to this fact, biological samples and fingerprints are some of the most common types of evidence submitted to laboratories for forensic testing. These types of samples are known for their high evidentiary value because individualizing information can potentially be obtained from them.

Fingerprints specifically can potentially provide a plethora of information by comparing the minutiae of fingerprints of a known origin to fingerprints of an unknown origin. In addition to traditional friction ridge examination, unique genetic profiles can be obtained from the DNA left behind from the owner of that fingerprint. The ability to perform multiple types of analysis on a single piece of evidence is extremely beneficial, especially when that information is independent from one another. Useful information can be extracted from the minutiae pattern on the fingerprint regardless of the DNA available and on the other hand, a genetic profile can be extrapolated from the print irrespective of its ability to be characterized.

Due to the steady increase in the backlog of DNA analysis cases, adding the responsibility of processing fingerprint swabs onto forensic laboratories could be equally as problematic as beneficial. To combat this, efficient methods for DNA analysis are desperately needed in laboratories. The current DNA processing methods are costly, time consuming, and involve multiple tube changes, increasing the risk of contamination. However, the use of direct polymerase chain reaction (direct PCR) can simplify the DNA analysis process by eliminating the extraction, purification and quantitation steps. Presently, the Federal Bureau of Investigation (FBI) Quality Assurance Standard 9.4 requires all evidence samples to be quantitated[1]. As a result, the direct PCR method of DNA analysis cannot be implemented on evidence samples at this time. This research sought to investigate the efficiency and effectiveness of the direct PCR on samples with extremely low starting quantities of DNA as well as potential PCR inhibitors.

While fingerprint evidence is extremely valuable, it is not always used to its full potential. This method was hypothesized to demonstrate the increased significance of a single fingerprint by providing the additional information of a DNA profile acquired in an efficient, effective and inexpensive manner following fingerprint analysis. Direct PCR has the potential to benefit the forensic community by serving as an efficient method of cyanoacrylate sample processing.

1.1 Goal of the study

The goal of this research was to test the viability of using the technique of direct PCR on fingerprints previously enhanced by cyanoacrylate fuming. Evaluating the ability to generate a full profile from DNA obtained via direct PCR from previously enhanced fingerprints.

2.0 Theoretical Background

2.1 DNA and Forensic Science

DNA has become an integral aspect of criminal justice and forensic science. When left by the perpetrator after a crime, it can provide unique, identifying information about them that could lead investigators in the right direction. DNA is extracted from nucleated cells and particular noncoding regions are analyzed at specific loci to develop what is called a DNA profile. DNA profiles from samples of known origins are compared to the profiles derived from samples of unknown origin for similarity. Traditionally, a body fluid sample was needed to perform this test, however with the improvement of scientific technology over the last decade has led to the improvement of the capability of forensic DNA typing to include the analysis of small amounts of DNA left by skin cells and residue.

2.1.1 Touch DNA

Touch DNA, or trace DNA, is the concept that a DNA profile can be obtained from the skin cells and residue left behind after a person comes into contact with an object. Even when there is no visible indication of body fluids, the person handling the object has left some trace behind. This trace is a complex residue that consists of nucleated cells, corneocytes and extracellular nucleic acids.[2] It also contains a combination of secretions of the sudoriferous gland, including amino acids, proteins, lipids and carbohydrates and the sebaceous glands.[3] The amount of those cells and secretions left behind by a perpetrator can vary tremendously from person to person. This variation can thus affect the ability to obtain a DNA profile because the DNA being analyzed in touch samples is originating from the nucleated cells either being sloughed off from the surface of the skin or as the fluid travels from the duct, or from cell free DNA (cfDNA) from extracellular vesicles or free floating within the fluid.[4]Some individuals can leave behind enough DNA for a full profile immediately after hand washing, deeming them a 'good shedder', while it takes others hours after handwashing to build up a concentration high enough to do that or a 'poor shedder'.[5,6] In addition to handwashing, another significant factor that effects shedder status, or the amount of DNA left behind when touching an object is hand dominance. Individuals shed more DNA from their dominant hand than their non-dominant hand[5].

At an absolute minimum, approximately seven or eight cells are needed to obtain a full DNA profile, which may be left behind after an object is touched.[7] This was proven by van Oorschot and Jones in 1996 when they successfully developed profiles from direct swabbing of hands as well as the swabbing of objects regularly handled by the user.[8] The optimum input of DNA into a PCR reaction for subsequent STR profiling however, is approximately 500 pg.[2] With each human diploid cell containing approximately 6 pg of DNA on average, approximately 80 diploid cells would be ideal.[2, 9] STR profiling from samples containing touch DNA have often shown to result in profiles of significantly lesser quality than those processed from samples with optimum DNA input.[10] This is likely due to the fact that not every contact between skin and an object leaves behind a sufficient amount of DNA. Even with the encounters that do, the DNA available is a very small quantity.[11] In a study conducted by Raymond et al, on average 1.7 ng of DNA was recovered from 252 touch DNA samples[12]. Additionally, this low success could be due

to DNA loss during extraction procedures. Optimizing these processing procedures could improve the quality of the resulting profiles.

Techniques such as developing profiles from touched objects or fingerprints can be extremely helpful in cases where very little physical evidence was recovered. It becomes essential to extract every bit of information from every piece of evidence available. This concept can be especially helpful in linking the person of interest to an item or a location. Touch samples contain miniscule amounts of DNA as compared to body fluid samples such as blood or semen, making developing full profile rather difficult. Due to this fact, optimization of a method to extract the maximum amount of DNA from touch samples is essential to the success of the process.

2.2 Current method processing DNA

Samples obtained from crime scenes often contain a lot of extraneous material that is not DNA and is not advantageous to the analysis process as a whole. To combat this issue, samples are put through a rigorous process to remove the cells from the substrate, and then isolate and purify the DNA sample to remove any potential substances that could interfere with the analysis process.

Extraction is used to isolate and purify the DNA sample in an effort to ensure the best quality profile. This is performed by eluting the DNA from the substrate in which it is attached to, lysing the cells, and then isolating the DNA. This process separates the DNA from proteins and other cellular materials. These components could potentially negatively affect the PCR process by either binding directly to the DNA or binding to the active sites of Taq polymerase, preventing its function. PCR is a sensitive process and the presence of some extraneous substances can inhibit the reaction to the point of severely reduced sensitivity or even complete amplification failure.[13] If a sample is not fully amplified, the resulting profile may contain stochastic effects such as allelic dropout or locus imbalance. Some known PCR inhibitors include but are not limited to hemoglobin, melanin, indigo dye from clothing items and phenol-chloroform.[13]

The sample is then quantitated to determine to amount of DNA present in the sample. This is an important step in the DNA analysis process for several reasons. It is a step that can assist in the troubleshooting process. If something went wrong during the extraction step, it can be detected in this quantitation step. It is a requirement set forth by the FBI's Quality Assurance Standards 9.3 that all casework samples undergo quantitation to determine the amount of human DNA present.[14] Additionally, it is an important step because most commercially available amplification kits are designed to target approximately 0.5-2.0 ng of DNA. Having more or less DNA could adversely affect DNA interpretation. For example, having too much input DNA could lead to artifacts such as split peaks, high stutter and pull up. Too little DNA input could result in no data, low level data, lack of amplification of some loci, locus imbalance, or even allelic dropout. All of these events make the profile interpretation challenging and should be avoided as much as possible.

Based on the results from quantitation, the sample may need to be diluted or concentrated going into the amplification stage. PCR amplification is the process of replicating a specific region of DNA to make many copies. At each cycle, the amount of DNA strands doubles, creating an exponential increase in PCR product. Approximately a billion copes of a specific sequence are required for the sample to proceed to capillary electrophoresis. [13] This process is

depicted below in Figure 3 taken from the Fundamentals of Forensic DNA Typing by John M. Butler.[13]

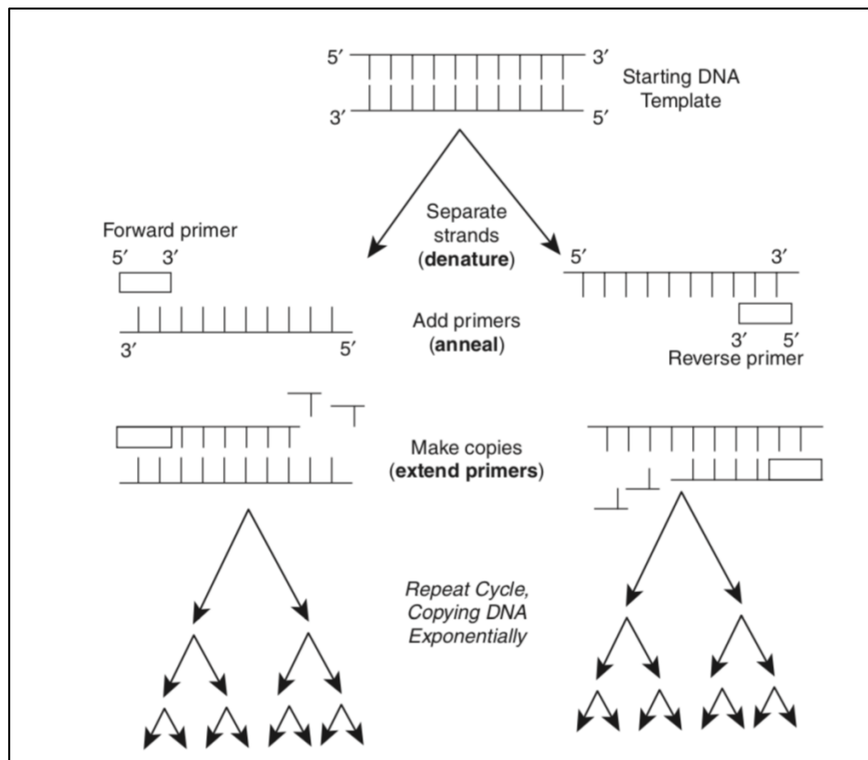

Figure 3. This diagram depicts the PCR process. The double stranded template strand is denatured and separated into two separate strands. Primers then anneal to the end of the region to be amplified. DNA polymerase the makes copies of the targeted sequence by extending the primers[13]

Overall, the sample processing method currently implemented on forensic casework samples is a time consuming and costly process. Even though it is an effective technique, it has the potential to introduce contamination due to the multiple tube changes involved. This method can also be detrimental to samples that potentially contain low amounts of DNA because of the frequent discarding of the supernatant. This supernatant could contain trace amounts of DNA that would be useful in a sample with a small amount of DNA, such as touch DNA.

2.2.1 Combined DNA Indexing System

The Combined DNA Indexing System, abbreviated CODIS, is the federal database software of the NDIS or National DNA Indexing System. It contains over 6.5 million STR profiles from all 50 United States. This database allows a questioned profile to be searched against DNA profiles recovered from crime scenes, previous offenders and other suspects.[13,15] The use of this system has proven to be an invaluable tool for agencies at the local, state and federal level.[16] The Federal Bureau of Investigation, FBI regulates the samples that are able to be uploaded into this database. In order to be considered eligible, a profile must meet certain

requirements explained in the NDIS Operational Procedures Manual. Some of those requirements include having been processed in accordance with the FBI Quality Assurance Standards. All of the 20 CODIS Core Loci must have been attempted, with a minimum of eight of the thirteen original CODIS Core having allele calls. In addition to that, the allele calls at those eight loci need to have a match rarity of at least one in ten million.[17]

2.2.2 Direct PCR

Direct PCR is an area of DNA analysis that is becoming increasingly popular in the research community because of the potential benefits it offers. Essentially, this technique eliminates the extraction, purification and quantitation steps which could potentially be detrimental in cases with low starting amounts of DNA. Instead, the sample is taken directly into the amplification phase of the process.

The traditional extraction step involves the use of several reagents and multiple tube changes. In addition to opening the sample up to contamination, the steps involved in extraction can contribute to a significant loss in DNA.[18,2] This is because of several tube changes and wash steps that discard the supernatant, which could contain genetic material. Direct PCR allows the full quantity of DNA present in the original sample to be amplified. This method has been proven to yield significantly higher amount of DNA going into capillary electrophoresis. All cellular material captured originally is carried into the amplification process, ensuring that low level samples have the best chance of leading to a full profile. In fact, direct PCR has shown high success rates with samples containing extremely low starting quantities of DNA.[19] A success rate of 100% full STR profiles was obtained from direct PCR processing of a single hair in the anagen growth phase, demonstrating the success and applicability of this technique on samples that would have otherwise not been tested.[19]

In a study conducted by Ottens et al in 2013, researchers found an average loss of DNA of over 83% when extracting with the Promega IQ kit and a loss of over 71% of starting DNA when extracted using the QIAGEN Micro kit.[18] When some evidence items have starting quantities with extreme low amounts, this conclusion is concerning. They concluded that only 17 cells were required to obtain full profiles when using direct PCR as compared to 250 starting cells needed to obtain a full profile using the aforementioned DNA extraction kits.[18]

In another study completed by Templeton et al in 2015, control DNA was deposited onto various surfaces and collected. Samples that underwent direct PCR consistently resulted in higher average relative fluorescent units (RFUs) than their extracted counterparts. [11] Overall, the study demonstrated that direct PCR increased the yield of PCR products, therefore increasing the success of the profile as a whole.[11] Additionally, a study was conducted by Thankiatkrai et al where DNA was recovered from spent shell casings. Utilizing the direct PCR method led to improved STR profiles as compared to extracted samples.[20]

Financially, implementing direct PCR would save laboratories money in multiple ways. This method significantly reduces the amount of time it takes from sample evaluation to visualization of the profile. A profile can be analyzed within a matter of hours compared to the 24-72 hours traditional processing takes, increasing the turnaround time for a case. This method would also reduce the amount of resources required to process each sample. Traditional DNA processing utilizes several different reagents in the extraction and quantitation steps. Since those steps are circumvented in direct PCR, the laboratory would save money on those consumables.

One of the concerns about this method is that potential contaminants and inhibitors to the PCR reaction are never removed because the sample does not go through a purification process during extraction. Crime scene samples are most likely going to contain impurities from not only the area which they were taken from, but even the substrate they are stained on. Therefore, it is thought that these samples will experience problems during amplification because substances such as indigo dye or other inhibitors will interfere with the reaction and the targeted DNA will not amplify properly. In a study completed by Forensic Science Service Tasmania in Australia, blood was collected from various items known to contain inhibitors and processed via direct PCR. [21] Complete profiles were obtained at an 80% success rate with samples processed at a reduced volume [21].Another study tested the viability of using direct PCR on samples that underwent presumptive testing. The blood samples were treated with luminol, acid phosphatase test was used on semen and the Phedebas test was used on saliva samples. [22] 74 out of the 90 samples tested (82%) with this method yielded full profiles of high quality, further substantiating the notion that direct PCR is a more robust method than originally thought .[22]

Direct PCR has already been implemented into some laboratories for use on reference samples, however it cannot currently be used on evidence samples due to several concerns. The Quality Assurance Standards set forth by the Federal Bureau of Investigation specifically state in Standard 9.4 that all forensic samples must be quantitated before nuclear DNA amplification.[14] Quantitation is an important step in the process for several reasons. It is a step that can assist in the troubleshooting process. If an issue occurred during the extraction step, such as a low level DNA or contamination, it may be able to be detected in this step. Additionally, it is an important step because most commercially available amplification kits are designed to target approximately 0.5-2.0 ng of DNA. If more or less DNA is present in the sample, it could adversely affect DNA interpretation. Excessive input DNA could lead to artifacts such as split peaks, high stutter and pull up. Too little DNA input could result in no data, low level data, lack of amplification of some loci, locus imbalance, or even allelic dropout. All of these events make the profile interpretation challenging and should be avoided as much as possible. Although there are risks to utilizing the direct PCR procedure, when it comes to touch DNA samples the potential benefits out way the possible negative consequences.

2.3 Optimization of sample collection

Since the samples that would benefit the most from being processed via direct PCR are low-yield, touch DNA evidence, it is important to optimize the collection procedure in an effort to maximize the quantity of DNA collected from the source. Copan Industries has developed a new swab type designed to maximize efficiency in collection of samples. Contrary to a traditional swab, FLOQSwabs®, pictured in *Figure 4* from COPAN USA's product website*,* do not have an internal core to trap the specimen. Instead, nylon fibers are arranged in a perpendicular fashion to keep the sample near the swab head.[23] This design has been suggested to promote the effective release of sample from the fibers, therefore increasing DNA recovery.

Figure 4. 4N6 FLOQSwabs® are designed with a nylon flocked head to improve DNA recovery from various surfaces[23]

In a study conducted by The George Washington University, the ability of 4N6FLOQSwabs® were compared to traditional cotton swabs by depositing DNA onto various surfaces and collecting samples with each swab type.[24] A 980% increase in the recovery of DNA was seen by the samples collected using the 4N6FLOQ swabs when extracted using the PrepFiler® kit, and a 50% increase when extracted with the DNA IQ kit.[24] An additional study compared the performance of four different swabs in a controlled environment. [25] A reference swab was compared to the COPAN 4N6FLOQSwabs®, the Purtitian FAB-MINI-AP, and the Sarstedt Forensic Swab for the collection of touch DNA from various surfaces. It was found that the FLOQ® swabs recovered the highest amount of DNA from the various materials as compared to the other swabs and were the most convenient to use.[25]

The microFLOQ® direct swabs, pictured in *Figure 5* taken from COPAN USA's product website, are designed in the same fashion as the 4N6FLOQ®, except for a lysis buffer embedded into the tip for use with direct PCR.[26] The tip of the swab is designed to be the ideal size to collect the amount of DNA needed. The tip is scored and will break off directly into the standard 96 well plate used in PCR, streamlining the entire process.[26]

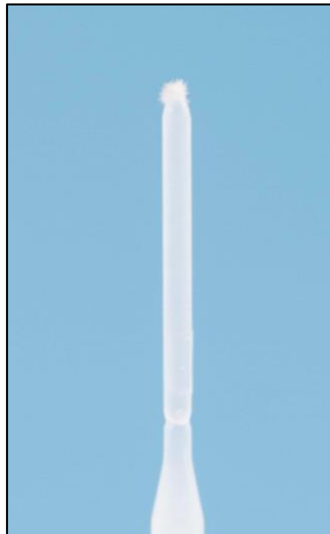

Figure 5. The microFLOQ® direct swab is designed to maximize the efficiency of sample processing. The nylon flocked swab head is scored to break off into a standard 96 well plate used for PCR[26]

2.4 Fingerprint Enhancement Techniques

2.4.1 Dusting

A common technique for visualizing latent, or unseen, fingerprints is dusting. This process involves using a soft filament or glass fiber brush to lightly apply a thin layer of powder which physically adheres to the moisture from the oily and aqueous components of the fingerprint residue.[3] This powder can contain a wide range of components based on the specific manufacturer, but always contains the essential components of a pigment and a binder.[3] Pigments such as carbon black, talc, kaolin and aluminum are used to provide the color contrast against the background surface. Binders such as iron powder, lycopodium and gum Arabic are used to promote adhesion to the residue of the fingerprint. This technique enhances the appearance of the fingerprint, allowing it to be visualized and examined.[3]

Fingerprints enhanced using the dusting technique have successfully been processed for DNA using traditional methods.[27] [28] [29] Direct PCR has also been used to recover DNA from fingerprints previously enhanced with dactyloscopic powders. Fingerprints were dusted with either white hadonite, silver aluminum, HiFi Volcano silk black or black magnetic fingerprint power and subsequently processed using a FLOQswab® and direct PCR. Full profiles were able to be developed from prints dusted with each different powder, further solidifying that the sample does not need to be free of impurities to successfully yield a profile using direct PCR. [30]

2.4.2 Chemical Developers

2.4.2.1 Cyanoacrylate Fuming

Cyanoacrylate fuming is a technique that was discovered in the 1950s by scientists who were trying to develop a new polymer to be utilized in the aircraft industry. Today, it is commonly performed on latent fingerprints found on non-porous surfaces like metal, plastic glass, and even coated papers.[3] When processing fingerprints, cyanoacrylate is heated up until it vaporizes in an enclosed chamber like the one pictured in *Figure 6* taken from the Air Science product website.[31] These vapors are cyanoacrylate monomers that interact with the initiators withing the complex fingerprint residue such as water-soluble amines and carboxylic groups. A second vaporized monomer attaches to the monomer now attached to the residue forming a dimer. This reaction progresses, adding more monomers on, forming a polymer. The process continues until cyanoacrylate has run out or the reaction is physically terminated by stopping the vaporization of the glue. This polymerization process forms a hard, white substance on the friction ridge marks. The fingerprint can be visualized as is, with various lighting techniques, or further enhanced using fingerprint powders or dye stain like Rhodamine 6G and basic yellow 40.[32]

DNA profiles have been successfully recovered from samples that have been treated using this chemical and extracted and processed traditionally.[33] Additionally, treating touch samples with cyanoacrylate has shown to actually increase the quantity of DNA recovered from the object.[34] [35] Full profiles were obtained from metal cables handled by the donor and then subsequently treated with cyanoacrylate.[34]

Figure 6. The Safefume 360 Automated Cyanoacrylate Fuming Chamber used to treat latent fingerprints on non-porous surfaces[31]

3.0 Methods:

3.1 Experiment Overview:

After West Virginia University Internal Review Board (IRB) approval and informed consent, five participants deposited their fingerprints from their pointer middle and ring finger of their right hand onto a DNA free glass slide. This occurred three times per round for a total of four rounds. 15 samples per group per round were processed for a total of 60 direct PCR samples and 60 traditionally extracted samples.

3.2 Validation Study

A study was conducted to determine a validated analytical threshold for West Virginia University's Applied Biosystems ®3500 Genetic Analyzer. To calculate the appropriate analytical threshold, 36 no-template controls (NTC) were amplified with GlobalFiler™ PCR Amplification Kit utilizing half reactions. Samples were run on the Applied Biosystems ®3500 Genetic Analyzer utilizing a 30kV and 100 second injection time. Resulting electropherograms were analyzed using GeneMapper™ IDX version 1.4.

3.3 Reference Sample Collection

Each participant was asked to submit a buccal swab to be processed as a reference to obtain their DNA profile. Participants fasted for thirty minutes prior to collection. A sterile cotton swab was rubbed and rotated on the inside of the participants cheek for 30 seconds. The swab was then placed inside a sterile collection tube until processing. A reference DNA profile was obtained from each sample utilizing the West Virginia University Organic Extraction Protocol, eluted at 100 µL. Quantitation was performed utilizing the Quantifiler™ Trio kit in conjunction with the Applied Biosystems ®7500 Real Time PCR instrument. Amplification was completed using the GlobalFiler™ PCR Amplification kit utilizing the Applied Biosystems GeneAmp ® PCR system 9700. Capillary electrophoresis was performed on the Applied Biosystems ®3500 Genetic Analyzer. Analysis was completed following the respective manufacturer's guidelines as well as West Virginia University's DNA Laboratory Protocols.

3.4 Experimental outline

3.4.1 Fingerprint deposition procedure

Five participants deposited their fingerprints based on the procedure outlined below. Each participant deposited fingerprints from their pointer, middle and ring fingers of their right hand onto the DNA-free glass slide with dimensions of two inches by three inches. They did this by pressing their fingers onto the slide at the same time using medium pressure. The surface was deemed DNA free after irradiating it under UV light for thirty minutes. After deposition, the samples were stored in DNA-free petri dishes until treatment.

3.4.2 Cyanoacrylate treatment

Sample slides were placed into binder clips that had been irradiated under UV light and hung in the Safefume 360 Automated Cyanoacrylate Fuming Chamber (pictured in *Figure 6*) for fifteen minutes at 22°C and 80% humidity using Microburst Cyanoacrylate Glue from Evident. At the conclusion of the cycle, samples were placed back into their respective petri dishes for storage until sampling.

3.4.3 Sampling

Traditional extraction samples were swabbed using COPAN 4N6FLOQSwab® moistened with 40µL of acetone, adding additional acetone as necessary when the swab became dry and was no longer picking up the sample. Slides were swabbed until the fingerprint was no longer visible. Direct PCR samples were swabbed using COPAN microFLOQ® direct swabs moistened with 2µL of acetone, adding additional acetone as necessary when the swab became dry and was no longer picking up the sample. Slides were swabbed until the swab was saturated with sample and could not pick up any more.

3.4.4 Traditionally Extraction samples

3.4.4.1 Extraction

Traditionally extracted samples were eluted to a volume of 40µL using West Virginia University's Organic Extraction Protocol which can be found in *Appendix A*. Samples were frozen at -20 C temperature until further analysis.

3.4.4.2 Quantitation

Samples were quantitated using Quantifiler™ Trio kit, full reactions on the Applied Biosystems ®7500 Real Time PCR instrument.

3.4.4.3 Concentration

Any samples with a concentration of less than 0.067ng/µL of DNA were concentrated using Microcon® Centrifugal Filter and TE buffer following West Virginia University's Concentration Protocol which can be seen in *Appendix B*. The concentration procedure was performed until the sample volume was approximately 15µL for all samples regardless of whether or not the quantity was between the concentrations of 0.001ng/µL to 0.067ng/µL in order to target 1ng of DNA for amplification.

3.4.4.4 Amplification

All traditionally extracted samples were amplified using the GlobalFiler™ PCR Amplification kit following manufacturers guidelines at 29 cycles. Rounds 1 and 2 were amplified on the Bio-Rad T100 Thermocycler. Rounds 4 and 5 were amplified on the Applied Biosystems VeritiPro™ Thermocycler. All samples were stored at -10 C until further analysis.

3.4.5 Direct Amplification Samples

3.4.5.1 Incubation

COPAN microFLOQ® direct swabs were incubated in 150µL of Prep N Go Buffer™. The published protocol suggests 400µL however due to the specialized swabs, using 200µL of buffer fully covered the swab and created a less dilute sample which is more conducive to low template DNA.

3.4.5.2 Amplification

Samples were amplified using the GlobalFiler Express™ kit, following the manufacturer's protocol at 28 cycles. Rounds 1 and 2 were amplified on the on the Bio-Rad T100 Thermocycler. Due to instrument issues, Rounds 4 and 5 were amplified on the Applied Biosystems VeritiPro™ Thermocycler.

3.4.6 Separation

Both traditionally extracted and direct amplification samples were separated on the Applied Biosystems ®3500 Genetic Analyzer with an 8 capillary, 36 cm array. LIZ 600 size standard and POP 7 polymer were used. A 30kV and 10 second injection time was used.

3.4.7 Interpretation

Resulting electropherograms were analyzed using GeneMapper™ IDX Version 1.4 at the West Virginia University's DNA Laboratory's validated analytical threshold of 100 RFU.

4.0 Results

4.1 Analytical Threshold Determination

Upon analysis of the resulting 36 NTC electropherograms, four samples were seen to have allele calls with heights above 158 RFU (Relative Fluorescent Units) and were therefore eliminated from the data set. The remaining thirty-two samples were analyzed at one RFU, the sizing tables were downloaded. The height of the data points (in RFU) in the range of 72 base pairs to 400 base pairs were considered in the calculations for the blue, green, yellow and purple dye channels. The height of the data points (in RFU) in the range of 72 base pairs to 460 base pairs were considered in the calculations for the red dye channel. Mean, standard deviation, mean plus three times the standard deviation and mean plus ten times the standard deviation was calculated for each dye channel. Based on this analysis, the decision was made to use the most conservative option of mean plus ten times the standard deviation. The nosiest dye channel was determined to be yellow with mean plus ten times the standard deviation determined to be 100 RFU. The analytical threshold value was set to 100 RFU across all dye channels. Results are displayed in *Table 1.*

Table 1. The peak heights (in RFU) of the data points of 36 no template controls (NTC) were analyzed from 72-400 base pairs. Mean plus three times the standard deviation and mean plus ten times the standard deviations were calculated.

Dye Channel	**Mean +3*SD**	**Mean +10*SD**
Blue	16	39
Green	27	64
Yellow	34	100
Red	21	48
Purple	29	70

4.2 Profile Interpretation

Since the allele calls of the fingerprint depositor was known for each sample, profiles were analyzed and interpreted to determine drop out and drop-in. Drop-in was defined by an allele above the analytical threshold that did not belong to the fingerprint depositor and was not deemed to be contamination. Dropout was defined by a locus missing either one or both of the alleles of the fingerprint depositor. When calculating allelic dropout, only samples containing at least one true allele call were included. Alleles deemed to be contamination due to presence in the extraction blanks or substrate controls were excluded from both drop in and drop out calculations unless they belonged to the fingerprint depositor. The results are displayed in *Table 2*.

Table 2. Allelic drop in and dropout across individual loci in samples processed through traditional extraction using the GlobalFiler™ PCR Amplification Kit and direct PCR using GlobalFiler™ Express PCRAmplification Kit. Amelogenin, Y indel and DYS391 were excluded from these calculations. Averages were rounded to the nearest whole number.

		Traditional*			**Direct†**		
Locus	**Base pair size**	**% of samples with drop-in**	**% alleles dropped out**	**% of samples with dropout**	**% of samples with drop-in**	**% alleles dropped out**	**% of samples with dropout**
D2S441	75.0-113.5	23	11	13	22	75	100
D10S1248	80.0-132.0	3	63	67	4	78	81
D22S1045	83.5-126.5	6	41	47	4	78	88
D3S1358	90.5-146.5	6	44	50	0	88	100
D8S1179	108.5-176.5	13	15	20	0	81	88
D19S433	115.5-173.5	19	22	20	0	88	88
D5S818	133.5-189.5	3	56	60	0	73	69
vWA	151.0-215.0	6	51	53	0	88	88
D1S1656	154.0-209.5	6	65	77	4	93	100
TH01	174.0-219.5	6	9	10	0	55	56
D21S11	179.5-246.5	10	4	43	17	86	88
D13S317	197.0-249.0	6	92	97	0	81	88
D12S391	211.0-270.5	3	60	70	0	80	94
D16S539	221.5-273.5	3	64	70	0	91	100
FGA	221.0-380.0	13	33	47	0	84	94
D18S51	255.5-347.5	0	88	93	0	75	94
D7S820	265.5-304.5	3	86	97	0	84	94
D2S1338	275.5-355.5	3	44	47	0	83	94
CSF1PO	277.0-325.0	0	87	90	0	88	81
SE33	306.0-444.0	3	97	97	0	78	94
TPOX	332.5-384.5	3	61	63	0	91	88
Overall Average		7	54	59	2	82	89

** Traditional samples included in drop-in calculation: N=31, traditional samples included in dropout calculation: N=30.*

†Direct samples included in drop in calculation: N=23, direct samples included in dropout calculation: N=16.

The results discussed below are approximate values, as the averages presented above in Table 2 are rounded to the nearest whole number. Overall, the sample set processed using direct PCR displayed the highest amount of allelic dropout with 89% of samples having at least one allele dropout. Four loci (D2S441, D3S1358, D1S1656, D16S539) had dropout of at least one allele in all samples processed through direct PCR. Dropout seems to be equally distributed regardless of loci size in direct PCR. Samples processed traditionally however had a dropout rate 30% lower than that seen in direct PCR at 59%. Percent of dropout was seen at a higher rate at larger loci. For example, the locus with the largest possible base pair, SE33, saw a dropout rate of 97% of samples with 97% of alleles dropped out, where the locus with the smallest possible base pair size only saw 13% of samples with dropout and 11 % of alleles dropping out. In terms of drop-in, traditionally extracted samples saw the most instances of alleles being called that did not belonging to the depositor (7%) and compared to the drop-in rate of direct PCR (3%). Generally, the smaller loci saw the most instances of allelic drop-in. For example, the locus with the smallest possible allele, D2S441, saw the highest rate of drop-in in both the traditionally extracted samples (23%) and the direct PCR samples (22%). That locus accounts for approximately a quarter of the alleles that dropped in across all samples. In contrast, the locus with the largest possible allele, TPOX, saw the lowest instances of drop-in with regard to traditionally extracted samples (3%) and direct PCR samples (0%). These conclusions were made based on the patterns and trends seen in these samples, however the sample size is small and amplification rate was low in most direct PCR samples overall.

Two of the profiles obtained were determined to be mixed profiles from two contributors; one being the depositor and one being an unknown source This was determined based on the presence of more than two alleles at multiple loci.

Overall, out of the 60 samples processed traditionally, only 32 samples had at least one correct allele call and only 14 of those had success rates above 50%. Traditionally processed samples had an overall success rate of 53%. Direct PCR on the other hand, had success rates much lower at a total overall success rate of 23% and only 2 profiles with success rates above 50%. These results are depicted in *Table 3.* Alleles were present in several of the substrate controls, indicating that further optimization of the process of treating samples with cyanoacrylate before DNA sampling is necessary.

According to Tennessee Bureau of Investigation Forensics Services Division: Forensic Biology STR Typing Manual, random match probabilities are calculated for profiles that have at least one allele called at a minimum of seven loci[36]. Assuming these guidelines, a total of 27 profiles would qualify; 6 from direct PCR and 21 from traditionally processed samples. These results are depicted in *Table 3.*

Table 3. Percentage of profile recovered for each method calculated by dividing the number of alleles called that belonged to the fingerprint depositor divided by the expected alleles for that depositor (N=60 for each treatment group)

Percent recovery (%)	**Traditional**	**Direct**
1-20	9	9
21-40	6	2

41-60	6	2
61-80	6	1
81-100	5 [2 full profiles]	-
Total profiles*	32 [53%]	14 [23%]
Overall reportability†	21/60 [35%]	6/60 [10%]

** 'Total profiles' is equal to the number of profiles with at least one correct allele call. Amelogenin, Y indel and DYS391 were excluded from these calculations.*
†Reportability determined based on a minimum of seven out of the twenty loci having at least one allele called.

As mentioned in section *2.3.1 Combined DNA Indexing System*, samples must meet requirement to be eligible for entry into the database. The profiles processed via direct PCR do not follow the FBI Quality Assurance Standards, specifically QAS 9.4 because these samples were not quantitated. Excluding that factor, out of the 120 total samples processed across both groups (traditionally extracted and direct PCR) only seven profiles are eligible to be 'uploaded' into CODIS in terms of meeting loci and match rarity requirements. Only one of those profiles was processed via direct PCR, resulting in an approximate success rate of 2% in terms of uploadability. Six profiles from the traditional category were 'upoadable', resulting in a 10% success rate. All of the profiles would need to be searched under the Moderate Stringency category due to instances of allelic dropout.[37] This means that a locus containing a 10 allele would match a profile containing a 10 or a 10, 11, or a 10, 11, 12.[37] The random match probabilities of those aforementioned samples are listed in *Table 4.* Probabilities were calculated using the 2017 NIST 1036 Revised U.S Population Dataset of allele frequencies, using population substructure correction applied to homozygous genotypes as recommended in NRC II 4.1.

Table 4: Profiles deemed 'uploadable' to CODIS software and the associated match statistic using all called alleles and using the alleles called at a minimum of 8 of the original 13 CODIS Core loci. Allele frequencies taken from the 2017 NIST 1036 Revised U.S Population Dataset of allele frequencies using population substructure correction applied to homozygous genotypes as recommended in NRC II 4.1.

Participant Sample†	**Population**	**Match Statistic**	**Match Statistic (For CORE Loci)***
1T	*Caucasian*	3.84×10^{20}	9.28×10^{10}
	Af. Am	6.50×10^{22}	4.95×10^{12}
	Asian	1.06×10^{24}	1.05×10^{13}
	Hispanic	9.22×10^{21}	1.02×10^{12}
3T	*Caucasian*	1.79×10^{22}	5.85×10^{11}
	Af. Am	7.46×10^{16}	2.36×10^{9}
	Asian	1.83×10^{21}	1.26×10^{13}
	Hispanic	3.67×10^{19}	2.38×10^{10}
3T	*Caucasian*	2.78×10^{33}	3.02×10^{16}
	Af. Am	1.46×10^{26}	4.76×10^{13}
	Asian	4.95×10^{34}	1.45×10^{18}

	Hispanic	6.05 x 10^{30}	1.60 x 10^{15}
3T	*Caucasian*	4.46 x 10^{34}	4.84 x 10^{17}
	Af. Am	2.17 x 10^{27}	7.07 x 10^{14}
	Asian	4.95 x 10^{35}	1.46 x 10^{19}
	Hispanic	1.52 x 10^{32}	4.00 x 10^{16}
4T	*Caucasian*	1.15 x 10^{32}	4.47 x 10^{17}
	Af. Am	5.85 x 10^{34}	1.67 x 10^{19}
	Asian	9.88 x 10^{29}	5.12 x 10^{16}
	Hispanic	4.76 x 10^{32}	5.76 x 10^{17}
4D	*Caucasian*	2.55 x 10^{15}	2.3 x 10^{09}
	Af. Am	1.58 x 10^{16}	1.4 x 10^{10}
	Asian	1.42 x 10^{12}	1.88 x 10^{10}
	Hispanic	4.60 x 10^{15}	2.44 x 10^{09}
5T	*Caucasian*	1.93 x 10^{18}	2.85 x 10^{9}
	Af. Am	7.01 x 10^{21}	3.22 x 10^{11}
	Asian	1.81 x 10^{20}	6.64 x 10^{09}
	Hispanic	2.31 x 10^{19}	6.62 x 10^{09}

†T designated samples processed through traditional extraction; D designates samples processed with direct PCR.
**Match Statistic (For CORE loci) is the random match probability calculated from the loci included in the 13 original CODIS core loci.*

5.0 Discussion and Conclusions:

This preliminary study was conducted to evaluate the inhibitory effects of cyanoacrylate on the process of direct PCR. The ability to analyze the individualizing information from fingerprints and then be able to sample that same fingerprint for DNA evidence is immeasurable. The ability to cut the time to process these samples significantly could potentially revolutionize the workflow for DNA laboratories.

The traditionally extracted and processed fingerprints had much better success than those processed using direct PCR. Traditional samples had an overall success rate of 53% as depicted in *Table 3*. Over half of the profiles had at least one correct allele call. Although traditionally process samples saw higher success rates, there were several direct samples with success rates greater than 40% showing that while PCR may be inhibited, the mild inhibition is not detrimental to the ability to gain an informative DNA profile. Processing samples in this fashion is possible and further experimentation and optimization could potentially increase these success rates. Experimentation with a larger sample pool should be conducted.

One issue encountered throughout the project was in the sampling stage. Direct PCR samples were collected using microFLOQ® direct swabs as pictured in *Figure 5.* By design, these swabs have an extremely small sampling head. Due to the reduced surface area, the swab became saturated with cyanoacrylate and sample quickly. Visually, some fingerprint residue was left behind and thus some DNA could have potentially been left behind. Future studies could potentially further optimize the sampling procedure. One way to do this could be to use the double swab method with the microFLOQ® direct swabs. This would entail moistening a swab with acetone and then sampling the fingerprint, then following that with a dry swab. Both swabs would be processed together. Another potential option would be using the COPAN 4N6FLOQSwabs® pictured in *Figure 4* for the direct PCR method instead of the microFLOQ®

direct swabs. This would allow for a greater surface area to capture more of the sample and therefore the maximum amount of DNA.

Using cyanoacrylate treatment as a method for the enhancement of latent fingerprints is often followed by a dye stain to further improve the visualization of the pattern and minutiae detail, as discussed in Section *2.5.2.1 Cyanoacrylate Fuming*. There is the potential that these chemicals may potentially inhibit PCR as well as serve as a potential source of contamination. A study similar to this one should be conducted on cyanoacrylate and subsequently dye-stained fingerprints.

In some profiles from both the traditionally processed and direct PCR processed prints, there were alleles present that did not belong to the contributor. This was expected as samples used in this study were designed to mimic that of samples that could be found at a crime scene, while maintaining some control. In other words, the surface where the fingerprints were deposited was clean, however participants were not instructed or regulated on pre-deposition activities. Additionally, time after handwashing, showering or objects touched was not noted or regulated. Therefore, it is possible that participants had just washed their hands, washing away most of the DNA that had built up. It is also possible that the individual just touched a commonly touched surface containing the skin cells and DNA from past users of the object, leading to secondary or tertiary transfer of DNA to the glass slide before treatment, leading to drop-in alleles. Additionally, even though samples were irradiated under UV light and deemed DNA-free, the surfaces were not verified to be such. It is possible that there was remaining DNA before fingerprint deposition that was captured during sampling. This is possible for fingerprint samples as well as the substrate control. It is also possible that drop-in alleles were acquired post deposition. Fingerprints were deposited under a fume hood, with samples from each contributor in the same air space during collection time for that round. It is possible that the drop-in seen in the substrate control or drop-in seen in some samples originated in this fashion and not from secondary of tertiary transfer. Moreover, the process of cyanoacrylate fuming itself is subject to contributing to contamination. The cyanoacrylate fuming chamber used (pictured in *Figure 7*), is an open enclosure with all samples exposed to the same circulating air. It is possible that some drop-in alleles originated from this step and that should be taken into account when evaluating the applicability of this method in conjunction with DNA testing.

Overall, the results of this study align with the findings of similar studies. Using cyanoacrylate as a means of enhancing a latent fingerprint is not detrimental to the recovery of informative DNA profiles. A study by Khuu et al tested the efficiency of recovering DNA from fingerprints treated with three different methods; PolyCyano UV, Lumicyano and cyanoacrylate. Four participants deposited five sets of fingerprints onto glass fingerprint slides. Two fingerprints from each set were spiked with washed saliva cells from a separate donor. All were treated with their respective cyanoacrylate treatments. Low concentrations of DNA were recovered after extraction with the QIAamp Mini Kit, and partial profiles were observed in only two samples, with the rest of the samples showing no informative information. Profiles were not able to be developed from fingerprint donors in spiked samples, however 100% of the alleles of the saliva cells in the spiked sample were called. Although this study did not see high success rates, with purely touch DNA samples, they were able to recover some alleles from spiked samples post cyanoacrylate treatment[35]. However, in concordance with the results of this study, degradation was seen in a higher rate the larger the loci size. Another study by Tempelton et al., tested the inhibitory effects of dactyloscopic powders on direct PCR. They concluded that the powders tested did not inhibit PCR and 61% of resulting profiles we considered ‘uploadable’ to

the Australian Database, NCIDD. Overall, direct PCR has proved to be a more robust method than originally thought.

Fingerprints are a commonly encountered evidence type in criminal cases and individualizing information can be gained from the analysis of minutiae points within the friction ridge pattern. Historically, there is a difficult choice to be made as to whether or not to enhance the fingerprint for analysis, or swab the area for potential touch DNA. This choice can be particularly difficult, especially when there is limited evidence found or available. However, it has been shown in numerous studies that DNA can successfully be recovered from fingerprints enhanced by various methods when processed through traditional extraction workflows. In these traditional methods, contaminants, extraneous substances, and potential PCR inhibitors are filtered out, leaving the DNA sample purified. These steps are not a part of the direct PCR process. Instead, the sample is briefly incubated and sent directly into the amplification cycle. The sample is not purified and everything, including potential PCR inhibitors are included in the reaction. This method has proven beneficial for reference samples and research in this area is flourishing on the use of this method on various sample types. One of the most enticing benefits to this method is the drastically reduced sample processing time. This could be a means to reduce the exponentially increasing backlog in DNA labs across the country. Additionally, due to the elimination of multiple steps, the need for several costly consumables and reagents is eliminated, saving laboratories money in the long run. Overall, the need for research into direct PCR is clear. If this method can be established as reliable and robust for a vast range of sample types, it can potentially be incorporated into the regular workflow of crime labs across the country. Although this was a proof-of-concept study, promising results were seen. Traditional extraction methods were shown to be the most successful, but success was seen in samples processed via direct PCR, meaning the potential for increasing that success rate exists. Further optimization and experimentation is needed to validate these observations.

6. 0 Appendices

Appendix A: Organic Extraction Protocol

1. Place the sample cutting or swab in a sterile 1.5 mL **microcentrifuge tube**.
2. Add 500µL of **stain extraction buffer** and 15 µL of **Proteinase K**. Pulse vortex for 10 seconds and then briefly centrifuge (8,000 rpm for 1 minute).

   NOTE: Add enough stain extraction buffer to cover the entire cutting or swab. This may be more than 500µL
3. Incubate sample at 56C for 2-24 hours.
4. Spin in centrifuge for 8,000 rpm for 30 seconds to force condensation into the bottom of the tube.

5. Remove substrate and transfer to a spin basket and fresh 1.5mL **microcentrifuge tube**. Spin in microcentrifuge at 14,000 rpm for 3 minutes. Retain substrate until completion of all typing. Transfer stain extract from swab into the original incubation tube with the rest of the extract.
6. In a fume hood, add 500µL of **Phenol Chloroform: Isoamyl Alcohol** to the stain extract. Pulse vortex to attain milky emulsion.
7. Spin the tube in a microcentrifuge at 14,000 rpm for 3 minutes. There should be two clearly separated layers.
8. Remove the bottom organic layer and the interface from the tube and discard into a dedicated waste container.
9. Repeat steps 6-8 until middle layer is clear (at least 2 times for buccal swabs).
10. Add 100µL of **TE buffer** to a **Microcon micro-concentrator unit**. Transfer the aqueous phase (top layer) from the tube in step 9 to the concentrator unit. Avoid pipetting any of the organic phase (colored yellow) or interface from the tube into the concentrator unit. Discard tube containing organic phase (bottom layer) and interface into a dedicated waste container.
11. Cap the micro-concentrator unit and spin in a microcentrifuge at 500 rcf for DNA Fast Flow or 14000 rcf for 30K for at least 10 minutes (additional spin time may be required).
12. Carefully remove the concentrator unit from the Microcon centrifuge and discard the fluid from the microcentrifuge tube. Return the concentrator to the top of the microcentrifuge tube (Only remove the micro-concentrator if there is too much fluid in the tube).
13. Add 200µL of **TE buffer** to the concentrator unit. Cap and spin the micro-concentrator unit in a microcentrifuge at 500 rcf for DNA Fast Flow or 14000 rcf for 30Kfor at least 10 minutes (additional spin time may be required).
14. Add **TE buffer** to the micro-concentrator unit to bring the total volume between 20-200µL. Remove the concentrator from the microcentrifuge tube and carefully invert the concentrator onto a fresh, sterile, labeled **microcentrifuge tube**.
15. Spin the micro-concentrator unit in a microcentrifuge at 1000 rcf for 3minutes.
16. Store samples at 5C or frozen. When ready to use, vortex and spin for 5 seconds.

Appendix B: West Virginia University Concentration Protocol

1. Add 5µL of nuclease free water or TE buffer to the membrane of the filter to trap the DNA. If the filter is dry, it won't work properly.
2. Add all eluted DNA to the membrane in the filter.
3. Centrifuge at 2000rpm for 6 minutes (or 3000rpm for 3 minutes if filtering takes too long).
4. Carefully flip the filter into a clean tube (so that the membrane is facing into the tube; filter sits inverted in the new tube) and throw away the original tube with the original tube with the flow through. The new tube is the DNA reception tube.

    a. Steps 2-5 tend to need to be repeated to attain the target volume if the target volume is more than a few µL. If this is the case, keep the same flowthrough tube and DNA reception tube & label them for their respective samples to avoid confusion with having a new set of tubes each round of steps. This flowthrough liquid, flowthrough tube, and filter will ultimately be discarded at the end of the concentration process.
5. Centrifuge with the cap open (it should not be able to shut if the membrane is inverted), at 3000 rpm for 3 minutes (or 3500 rpm for 2.5 minutes if filtering takes too long).
    a. At the beginning of this step, the membrane in the filter contains the DNA. At the end of this step, the liquid in the DNA reception tube contains the DNA. Don't accidentally throw either away.
6. If DNA extract volume is more than the target volume, repeat steps 2-5 until the target volume or less is acquired.
    a. If there is less than the target volume, add nuclease free water or TE buffer to a separate clean tube and compare this tube and the DNA extract to approximate the volume.
    b. Alternatively, use a pipette appropriate for the target volume and measure the sample present in the DNA reception tube to determine the current volume.
7. Once the desired volume has been attained, the filter and flowthrough tube(s) can be discarded, while the labeled DNA reception tube with the final DNA concentrate can be saved and either stored or utilized for amplification.

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