Optimization of a DNA Extraction Protocol for the Beckman Coulter Biomek® NX^{P} Laboratory Automation Workstation

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Abstract

DNA analysis plays an integral role in forensic science investigations. This results in a high volume of samples requiring DNA testing. Due to the growing need for backlog reduction, these additional samples can inundate an already lengthy process (1). The DNA workflow consists of several steps that may be automated to minimize hands-on time for analysts and increase sample throughput in the lab (2). Extraction can be one of the most time-consuming steps in this process. Extraction consists of removing cellsby agitating them from the substrate and lysing the cell membranes to release the DNA into solution. The DNA must then be purified. Several automated platforms have been developed or adapted to address this. One such platform is the Beckman Coulter Biomek® NX^{P} Automation Workstation, a liquid handling system for which Promega® Corporation has developed a DNA extraction protocol using their DNA IQ™ system (3). This method enables the processing of 96 samples per run and requires very little hands-on time from analysts. Solubilization, however, must be performed on samples prior to robotic handling.

Several experiments were conducted to develop a protocol that will maximize DNA yield and minimize sample contamination. During the pilot study, we were able to determine the optimal incubation temperatures and lengths of sample incubation periods. Several comparison studies between Promega® DNA IQ™ spin baskets and the Qiagen® Investigator® Lyse&Spin baskets were also performed to determine the best spin basket for use in the protocol. In addition, optimization studies were performed on the Biomek® NX^{P} protocol. This included a tip comparison study for reliable transferring of large volumes and determining the best elution volume to account for sample loss due to evaporation and transfer. During the course of this process, troubleshooting of the Biomek® NX^{P} was performed to minimize the potential for

contamination and address instrument errors that resulted in a forced abortion of the extraction. Tip touch and aspiration steps not in the original protocol were incorporated and adjustments to the instrument itself were made to prevent tubing interference and account for expansion of deep well plates when heated.

Based on the experimental results, it was determined that a digestion using the high yield buffer with an incubation temperature of 56°C for one hour in high yield buffer produces comparable amounts of DNA to the original protocol which required a more involved process with 56°C for 30 minutes, 99°C for 10 minutes, and 4°C for 10 minutes. Furthermore, using a Qiagen® Investigator® Lyse&Spin basket yields comparable DNA to a Promega® DNA IQ™ spin basket and eliminates the transfer step. It was also found that the use of p1000 tips produce comparable quantities of DNA to p200 tips when transferring large volumes of sample. Finally, an elution volume of 37 µL was chosen for a target final volume of 27-28 µL.

Therefore, the protocol was modified to incorporate these components and applied to further studies on the Biomek® NX^P. Further studies may be performed to determine the best buffer volume in this study and the role it will play in potential contamination during purification on the instrument.

Introduction

The role that DNA analysis plays in forensic science has been growing since its introduction to the field with Alec Jeffrey's "DNA fingerprinting" in the late 20^{th} century. However, this technique required large quantities of sample and produced results with limited powers of discrimination. Advancements in technology have led to increasing capabilities, resulting in a higher demand for testing of DNA evidence (2). Current testing can obtain results

from items people have simply touched while often still producing useful, informative profiles (4). Furthermore, legislation in different states has led to the collection and testing of DNA samples from arrestees in addition to convicted offenders. Due to this testing combined with other factors within the criminal justice system, the volume of DNA samples processed by laboratories has risen to the extent that a backlog of thousands of cases has built up across the country (1). The need to reduce this backlog while continuing to meet current casework demands has resulted in laboratories turning to automation to decrease the amount of hands-on time analysts must spend on samples.

There are several steps in the DNA workflow that can be automated. This workflow typically consists of extraction, quantitation, normalization, amplification, capillary electrophoresis, and analysis. Various labs have automated extraction, quantitation and amplification set-up, and normalization. Some have moved towards an almost fully automated process, even using expert systems to analyze data (2). One of the steps where automation can save the most time is extraction. This step tends to involve repeated washes, lengthy incubation periods, and extensive pipetting. When extraction is automated, analysts can prepare samples for extraction, set up the instrument, and leave samples to extract while performing other duties. Beyond reducing the hands-on time required by analysts, this should also reduce human error inherent in a manually performed procedure.

It must be noted that automation is not well-suited to all extraction methodologies. Those that require more involved steps or centrifugation are not easily performed by liquid handling systems. Though some instruments may have this capability, there has been an increasing trend in the use of paramagnetic, DNA-binding bead based extraction methods to make automation more easily achieved. These methods utilize beads with DNA-binding surfaces, often silica,

which may be drawn toward a magnet to separate them from the lysate (5). This allows for simple washes without centrifugation to isolate the DNA. Furthermore, the nature of the isolation permits a more thorough removal of impurities than many older methods. One of these is the DNA IQ™ system from Promega® which uses a paramagnetic resin to bind and purify DNA. DNA IQ™ has been shown to remove PCR inhibitors that could affect downstream processes as well as or better than other extraction kits (6).

Several platforms have been developed for automation of this system, many of which have been adopted by forensic labs across the country. These include the Tecan Freedom EVO®, Biomek® 2000, Biomek® 3000, and Biomek® NX^{P} (3,7-9). Promega® has worked with these companies that developed these automated workstations to create protocols that yield comparable amounts of DNA to similar methods. Prior to use of the new method in the laboratory, it must be optimized to produce the maximum possible DNA yield and validated to verify this yield is comparable to or better than the methods previously employed by the laboratory (10). Laboratories must then further consider the cost of the technology, the time it will take for extraction, and the ease with which it can be used in comparison to others.

The New York City Office of Chief Medical Examiner (NYC OCME) recently evaluated the DNA IQ™ system on the Biomek® NX^{P}. The project began in late 2014, however, the instrument initially had many issues that caused concern for its use in casework. This included liquid leaking from the pipette tips during transfer steps and liquid collecting on the pipette tip filters due to leaking within the liquid displacement system. Modifications of steps to the protocol such as pauses and additional aspirations, as well as the use of smaller tubing in the instrument, reduced these problems so the validation could proceed. Once the instrumentation

was functional, a digestion protocol was developed to prepare samples prior to extraction on the Biomek® NX^{P}.

In order to optimize this protocol, several studies were conducted varying individual parameters. The NYC OCME developed a protocol using a digestion buffer, consisting of 0.05% SDS and proteinase K, in place of the casework buffer produced by Promega® that yielded comparable results and therefore was used in further studies. In addition, a comparison of the DNA IQ™ spin basket to the Qiagen® Lyse&Spin basket was conducted to potentially eliminate the need for a time consuming substrate transfer step. Incubation times and temperatures were varied based on alternate protocols to determine which produced the most DNA and were most compatible with the rest of the method.

In order to standardize DNA analysis across the country, the Scientific Working Group on DNA Analysis Methods (SWGDAM) was formed. It is comprised of scientists representing forensic labs at each level of government, all of whom approve SWGDAM publications prior to their release. This group develops guidelines for all appropriate aspects of DNA analysis to provide a means of improving quality assurance and minimizing any compromising of evidence. According to SWGDAM guidelines, validation of an extraction method should include at least a contamination assessment, sensitivity study, repeatability study, and reproducibility study on known/non-probative samples (10). Therefore, a plan for the validation of the DNA IQ™ system on the Biomek® NX^{P} was developed in accordance with these guidelines.

Materials and Methods

DNA Workflow

Extraction

Digestion of samples was performed according to a protocol adapted in each experiment listed below. The subsequent extraction was performed with Promega® DNA IQ™ on the Beckman Coulter Biomek® NX^P Laboratory Automation Workstation (Beckman Coulter, Fullerton, CA).

Quantification

Quantification was performed with Quantifiler® Trio according to the NYC OCME Standard Operating Procedures for STR Analysis (11). Two sets of standards and one no template control (NTC) were added for each run. Plates were run on the Applied Biosystems® 7500 Real-Time PCR System (Life Technologies™, Foster City, CA).

Amplification

Amplification was performed using the Promega® Powerplex® Fusion system at half reactions. This uses half the amount of master mix recommended in the Powerplex® Fusion protocol (12). The amplification was carried out on the Applied Biosystems® GeneAmp® PCR System 9700 thermalcycler (Life Technologies™, Foster City, CA).

Capillary Electrophoresis

The samples amplified with Powerplex® Fusion were evaluated with capillary electrophoresis on the Applied Biosystems® 3130*xl* Genetic Analyzer (Life Technologies™, Foster City, CA).

Analysis

Analyses of capillary electrophoresis data were performed using GeneMarker® HID software v2.7.1 (SoftGenetics®, State College,PA).

Instrument settings

Elution volume study

Samples in casework often have to be microconned following extraction for purification, concentration, or both. The target elution volume for microcons at the NYC OCME is approximately 25 µL. When eluting on the Biomek® NX^P, the measured elution volumes are consistently lower than the setting in the program. To account for dead space between the tip and tube as well as evaporation due to the open plate in the final incubation step, the program was set to elute 37 µL. A plate with eight wells filled with 200 µL 0.05% SDS was run with DNA IQ™ on the Biomek® NX^P and the eluate was measured with a pipette.

p200 v p1000 tips

A master mix of high yield digestion buffer composed of 16 µL proteinase K and 384 µL 0.05% SDS per reaction with sufficient excess to account for loss due to evaporation and transfer was prepared. The master mix was then added to eight Eppendorf® 1.5 mL microcentrifuge tubes, 400 µL per tube. Ten microliters of a 1:10 dilution of HemaCare® BioResearch whole blood was then added to seven of these tubes, leaving one blank to act as an extraction negative. These samples were incubated in a Thermomixer® at 56°C for 30 minutes with shaking at 1400 rpm, 99°C for 10 minutes with no shaking, and 4°C for 10 minutes with no shaking. They were then manually transferred to a 2.2 mL square deep well plate for extraction on the Biomek®

NX^P. For each sample, 190 µL was run using p200 tips in the volume reduction step of the DNA IQ™ protocol, and 190 µL was run using p1000 tips in this step. The extracts were then quantified with Quantifiler® Trio and compared.

High Yield Digestion Optimization

Incubation time and temperature studies

The original digestion protocol developed for the lysis of samples prior to extraction with DNA IQ™ on the Biomek® NX^P included an incubation of 56°C 30 minutes, 99°C 10 minutes, and 4°C 10 minutes. The purpose of the last two steps is to inactivate proteinase K in the digestion buffer. This required a screw down rack to ensure tubes remained closed at 99°C, and included inconvenient transfers of tubes to and from this rack. Given that DNA IQ™ should purify out proteinase K, it was theorized that these steps were unnecessary. Therefore, a comparison of the original protocol to a protocol without the 99°C and 4°C incubation steps was performed.

Twelve samples of whole blood were digested with each protocol, 6 with a 1:5 dilution of blood and 6 with a 1:100 dilution of blood. Ten microliters of the dilution was added to a 1/3 swab cutting and digested in 200 µL digestion buffer (8 µL proteinase K and 192 µL 0.05% SDS). An extraction negative was run with each digestion protocol. Following the incubation, the swabs were transferred to Promega® DNA IQ™ spin baskets which were placed back in the original microcentrifuge tube. They were then centrifuged at 16,100 x rcf for 2 minutes to removed liquid from the substrate. All samples were extracted together with DNA IQ™ on the Biomek® NX^P and quantified with Quantifiler® Trio.

In addition, this same experiment was run to compare a digestion protocol with incubations of 56°C 30 minutes, 99°C 10 minutes, and 4°C 10 minutes with a protocol with an incubation of 56°C 1 hour.

Spin Basket Comparison Study

The original high yield digestion protocol developed uses the Promega® DNA IQ™ spin baskets to remove excess liquid from substrates. This requires a transfer of the substrate that exposes it to a greater potential for contamination and requires a large amount of the analyst's time. This is particularly due to the need to clean the forceps used in transfer with bleach, water, and ethanol, between samples. Other companies have developed spin baskets in which the digestion may be performed in the spin basket itself. Then, following the incubation steps, centrifugation of the tube and basket ensemble opens the basket allowing the lysate to pass through while the substrate remains, eliminating the need for a transfer step.

One such basket is the Qiagen® Lyse&Spin basket (13). To compare the DNA yield obtained from use of these spin baskets to the DNA IQ™ spin baskets, an experiment was performed. Twelve samples were prepared for digestion in each spin basket type as previously discussed in the incubation comparison protocol. The recovery tube used for all spin baskets remained the Promega® ClickFit 1.5 mL tube, as this was the size compatible with the available heating platforms.

An additional modification was made to the protocol testing the Qiagen® Lyse&Spin basket. In order to account for the temperature difference caused by the increased distance from the heat source, the samples were incubated at 65°C instead of 56°C. The samples were then extracted with DNA IQ™ Biomek® NX^{P} and quantified with Quantifiler® Trio for analysis.

Comparison of Original Protocol and Modified Protocol with Altered Spin Basket/Incubation

In order to ensure the modified protocol yielded comparable quantities of DNA to the original protocol, a comparison was performed. Samples were prepared as in the spin basket comparison studies. The original protocol was run for one set and a modified protocol for the other. The original protocol used Promega® DNA IQ™ spin baskets and 30 minute 56°C, 10 minute 99°C, and 10 minute 4°C incubations. The modified protocol used Qiagen® Lyse&Spin baskets in Promega® ClickFit tubes and a 65°C incubation for one hour. The samples were then extracted with DNA IQ™ on the Biomek® NX^P and quantified with Quantifiler® Trio for analysis.

Validation

Cross-Contamination Study

To begin the validation, 96 samples were prepared with the modified protocol. Forty-eight of these samples were negatives with no swab or sample. The other 48 were made by pipetting 10 µL of whole blood onto 1/3 swab cuttings. A buffer volume of 400 µL was used for the digestion, as this is the maximum permitted by the protocol and has the most potential for cross contamination in a deep well plate. The final centrifuge step was performed at 16,100 x rcf for five minutes. Following the centrifugation, the caps with spin baskets attached were cut from the bottom of the tube and discarded. The lysates were transferred to the Biomek® NX^P using a transfer protocol and were set up in a checkerboard pattern (Figure 1).

The samples were then extracted with DNA IQ™ Biomek® NX^P and quantified with Quantifiler® Trio for analysis.

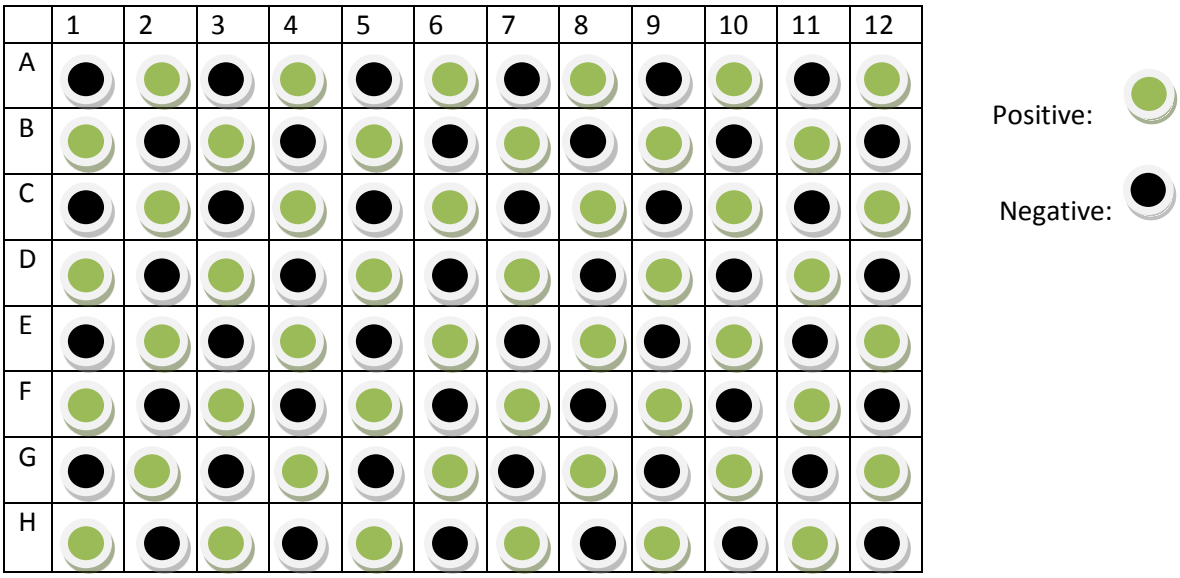

Figure 1. Checkerboard set-up for first cross contamination study.

Second Cross-Contamination Study

In order to determine if cross-contamination was occurring while samples were extracted in the Biomek, a study was performed with control DNA added directly to a deep well plate. Control DNA from Promega®, male and female, was diluted to approximately 50 ng/µL and 2 µL were added to a deep well plate in a checkerboard pattern, alternating columns of male and female (Figure 2). Each well also contained 400 µL of digestion buffer pipetted directly into the plate.

The samples were then extracted with DNA IQ™ Biomek® NX^{P} and quantified with Quantifiler® Trio for analysis.

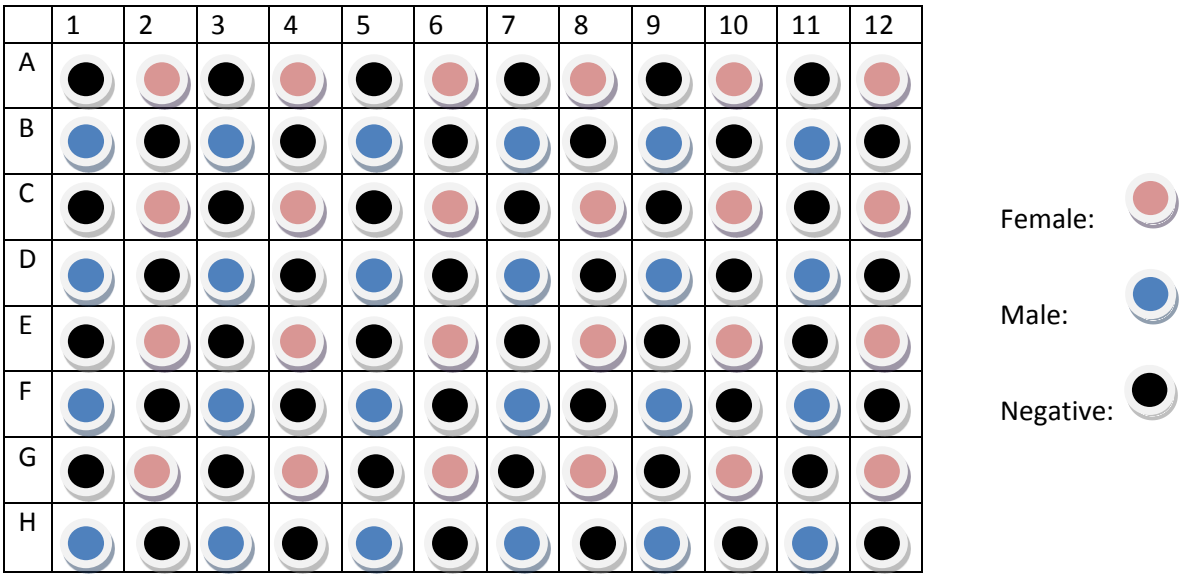

Figure 2. Checkerboard set-up for second cross contamination study.

Results

Elution Volume Study

The average elution volume when the Biomek® NX^{P} software was set to elute at 37 µL was 28.5 µL with a standard deviation of 1.30 µL.

p200 vs p1000 tips

The values from this study as determined by Quantifiler® Trio and the elution volume were averaged and compared for the 1:10 whole blood dilution (Figure 3). The average DNA yield for the protocol using the p200 tips for transfer in the volume reduction step of the extraction was 649 pg with a standard deviation of 170 pg. The average DNA yield for the protocol using the p1000 tips was 1,060 pg with a standard deviation of 175 pg. A paired t-test was used to compare the results and produced a p value of 0.0017.

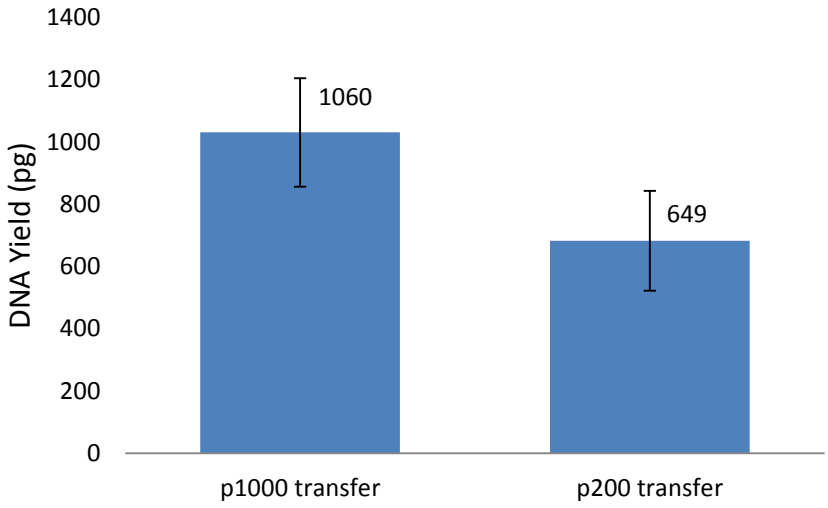

Figure 3. Comparison of DNA yields from extractions using p200 or p1000 tips in the volume reduction step of the protocol for DNA IQ™ on the Biomek® NX^{P}.

Incubation Time and Temperature Studies

The first incubation time and temperature study compared using the original incubation period, a 30 minute 56°C incubation, a 10 minute 99°C incubation, and a 10 minute 4°C incubation, with only using a 30 minute 56°C incubation period. The values from this study as determined by Quantifiler® Trio and the elution volume were averaged and compared for both the 1:5 and 1:100 dilutions of whole blood (Figures 4-5). The average DNA yield using the original incubation on a 1:5 dilution of whole blood was 23,085 pg with a standard deviation of 8986 pg. The average DNA yield using only a 30 minute 56°C incubation on a 1:5 dilution of whole blood was 19732 pg with a standard deviation of 9123 pg (Figure 4). The average DNA yield using the original incubation on a 1:100 dilution of whole blood was 134 pg with a standard deviation of 39 pg. The average DNA yield using only a 30 minute 56°C incubation on a 1:100 dilution of whole blood was 259 pg with a standard deviation of 84 pg (Figure 5).

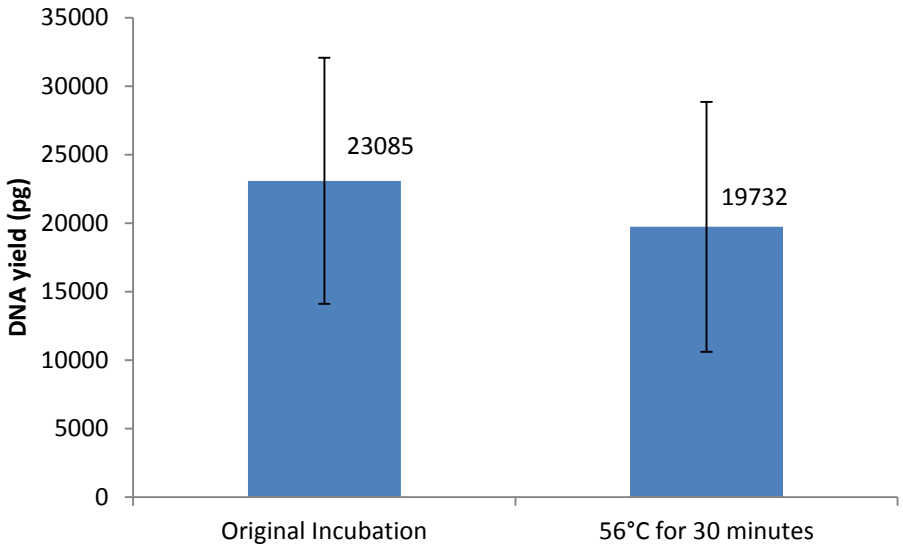

Figure 4. Comparison of DNA yield from a 1:5 dilution of whole blood using previous incubation protocol and a modified incubation protocol.

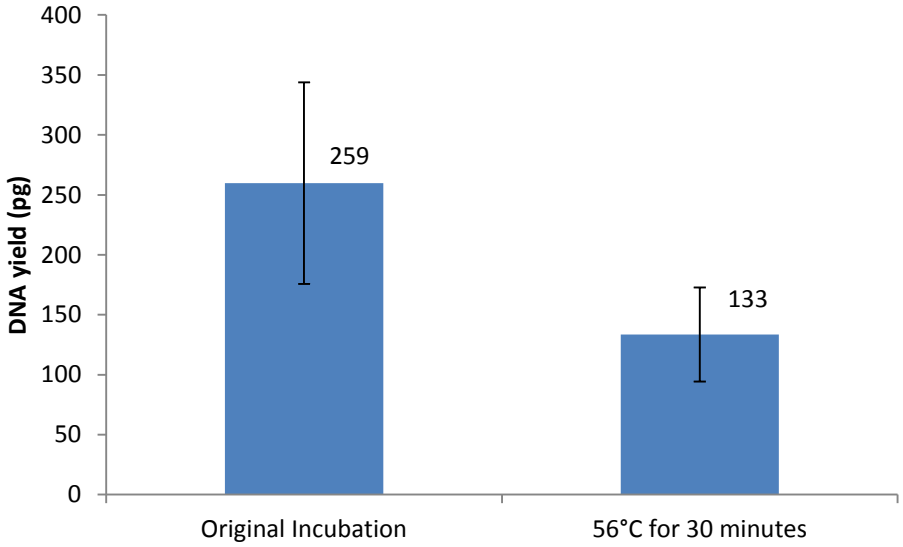

Figure 5. Comparison of DNA yield from a 1:100 dilution of whole blood using previous incubation protocol and a modified incubation protocol.

This study was repeated comparing the original incubation period to a 56°C incubation for 1 hour. The values from this study as determined by Quantifiler® Trio and the elution volume were averaged and compared for both the 1:5 and 1:100 dilutions of whole blood (Figures 6-7). The average DNA yield using the original incubation on a 1:5 dilution of whole

blood was 23325 pg with a standard deviation of 29381 pg. The average DNA yield using a 1 hour 56°C incubation on a 1:5 dilution of whole blood was 1848 pg with a standard deviation of 1009 pg (Figure 6). The average DNA yield using the original incubation on a 1:100 dilution of whole blood was 38.1 pg with a standard deviation of 14.5 pg. The average DNA yield using a 1 hour 56°C incubation on a 1:100 dilution of whole blood was 68.8 pg with a standard deviation of 24.8 pg (Figure 7).

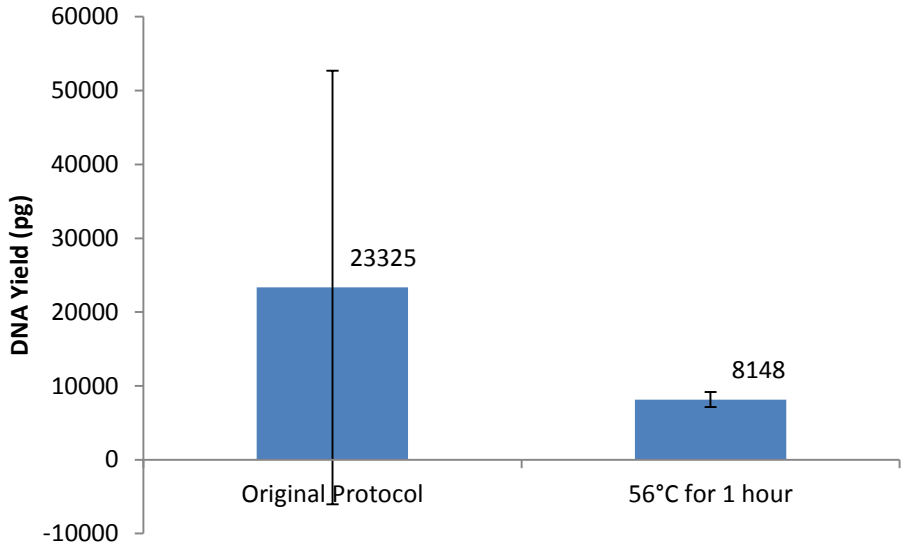

Figure 6. Comparison of DNA yield from a 1:5 dilution of whole blood using previous incubation protocol and a modified incubation protocol.

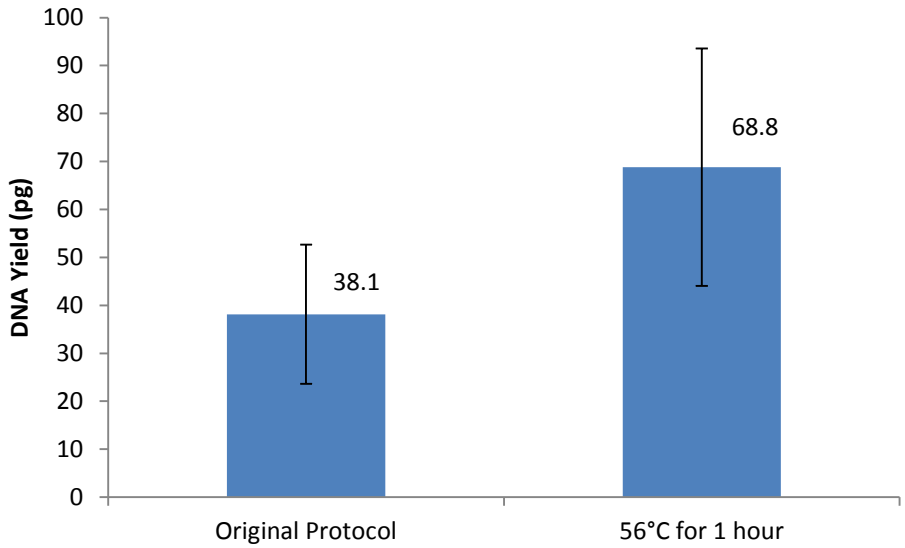

Figure 7. Comparison of DNA yield from a 1:100 dilution of whole blood using previous incubation protocol and a modified incubation protocol.

Spin Basket Comparison

A comparison of the spin baskets was conducted using the original incubation protocol of 56°C for 30 minutes, 99°C for 10 minutes, and 4°C for ten minutes. Samples were digested either in the Qiagen® Lyse&Spin baskets or the Promega® DNA IQ™ spin baskets with 1:5 or 1:100 dilutions of whole blood. The DNA yield was determined based on elution volume and analysis results from Quantifiler® Trio. For the 1:100 dilution, the average DNA yield from the Qiagen® Lyse&Spin basket digestion was 6,367 pg with a standard deviation of 930 pg. For the Promega® DNA IQ™ spin baskets, the average DNA yield was 17,482 pg with a standard deviation of 6802 pg. For the 1:5 dilution the Lyse&Spin baskets produced an average DNA yield of 358 pg with a standard deviation of 61.0 pg, and the DNA IQ™ yielded an average of 337 pg with a standard deviation of 26.9 pg. It should be noted that in this study, there was some incomplete flow through lysate in the Qiagen® Lyse&Spin baskets that could affect yield.

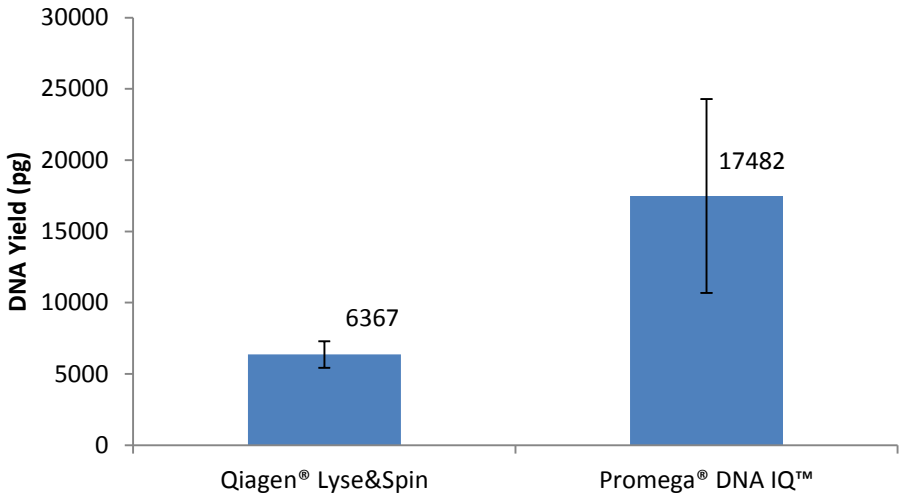

Figure 8. Comparison of DNA yield from a 1:5 dilution of whole blood using Qiagen® Lyse&Spin Baskets and Promega® DNA IQ™.

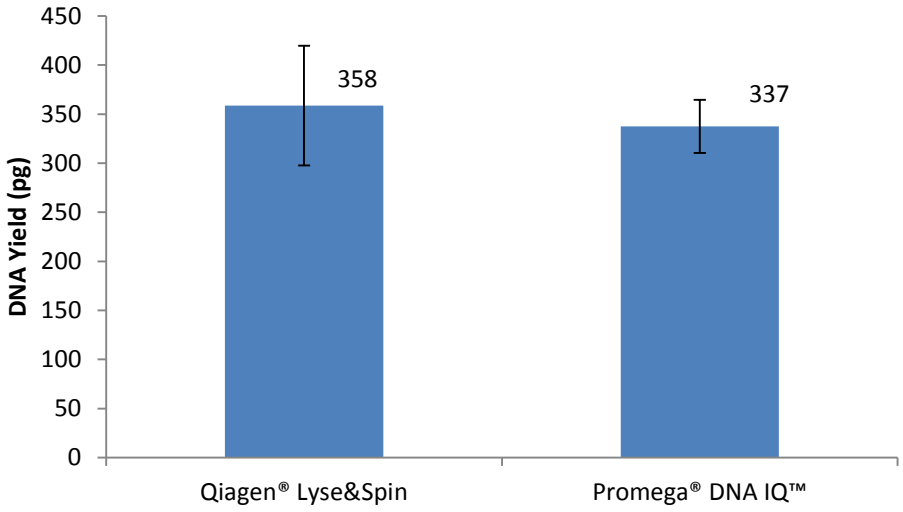

Figure 9. Comparison of DNA yield from a 1:100 dilution of whole blood using Qiagen® Lyse&Spin Baskets and Promega® DNA IQ™.

Comparison of Original Protocol and Modified Protocol

This study compared the original protocol and a modified protocol using a 56°C incubation for 1 hour and Qiagen® Lyse&Spin baskets. The values from this study as determined by Quantifiler® Trio and the elution volume were averaged and compared for both the 1:5 and 1:100 dilutions of whole blood (Figures 10-11).The average DNA yield for the 1:5 dilution with the original protocol was 11,004 pg with a standard deviation of 4950 pg. The

average DNA yield for the 1:5 dilution with the modified protocol was 21,813 pg with a standard deviation of 11,256 pg. The average DNA yield for the 1:100 dilution with the original protocol was 156 pg with a standard deviation of 134 pg. The average DNA yield for the 1:100 dilution with the modified protocol was 362 pg with a standard deviation of 223 pg.

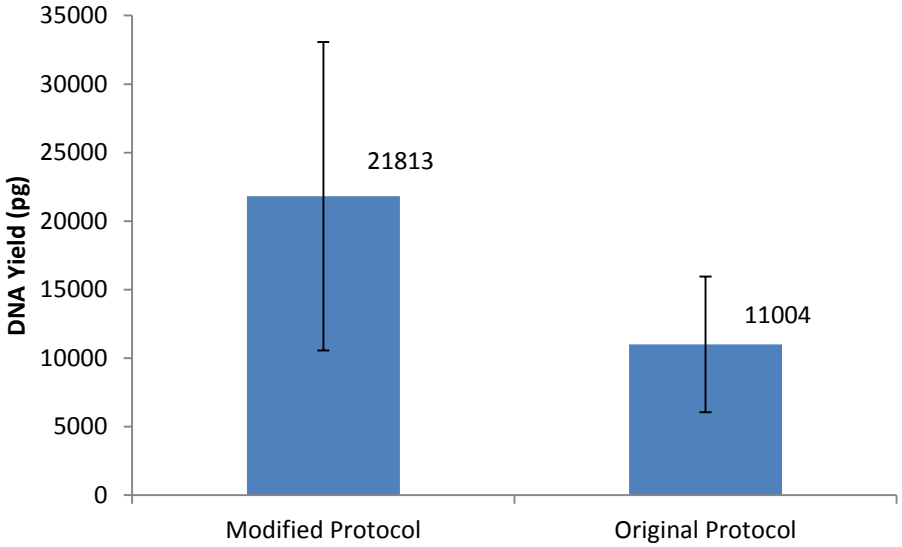

Figure 10. Comparison of DNA yield from a 1:5 dilution of whole blood using the modified and original digestion protocols.

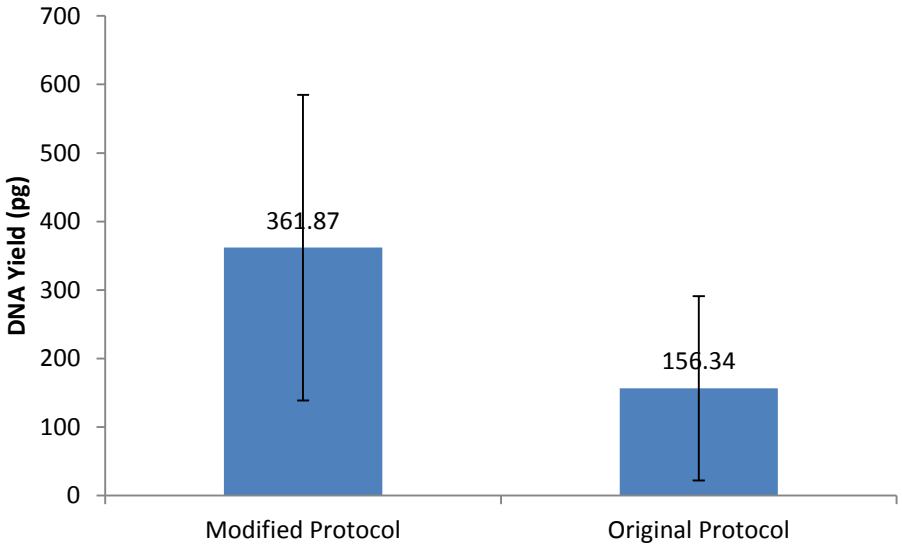

Figure 11. Comparison of DNA yield from a 1:100 dilution of whole blood using the modified and original digestion protocols.

Cross Contamination Study 1

For a negative control to be considered clean, as per OCME SOPs, it must have a concentration of less than 0.2 pg/μL measured in the quantification step. Analysis with Quantifiler® Trio identified 3 wells that should be negative with DNA in excess of this limit. These samples were amplified with Powerplex® Fusion for identification. Two wells contained alleles that could have come from the positive control. A third well contained an unknown profile not consistent with the positive control or lab personnel.

Cross Contamination Study 2

Analysis with Quantifiler® Trio identified 1 well that should be negative with DNA in excess of the 0.2 pg/μL limit. This well was positive for male DNA. At this point in the research, the project was discontinued.

Discussion and Conclusions

Instrument Settings

The first goals of the project consisted of optimizing the instrument settings for DNA extraction on the Biomek® NX^P. The study conducted to determine the best setting for the elution volume used a setting of 37 μL for the elution volume and yielded results within an acceptable range of 27-28 μL which was the target volume. Given the variability of results, it was determined it should not be lowered much further. The second study compared the use of the p200 or p1000 tips in transferring large volumes of sample in the volume reduction step of the DNA IQ™ protocol. There was some concern the wider bore of the p1000 tip would make it more likely to remove DNA-containing resin when discarding waste. However, significantly

higher DNA yields were obtained when using the p1000 tips for this step than when using the p200 tips. This may be due to a more complete transfer of resin to the processing plate.

Based on the results of these studies, both the 37 µL elution volume setting and p1000 tips in the volume reduction step were added to the protocol for DNA IQ™ on the Biomek® NX^P.

High Yield Digestion Protocol

Several studies were conducted to optimize the high yield digestion protocol to maximize ease of use and minimize the possibility of contamination. These included several comparison studies. For these studies, the whole blood used lacked an anti-coagulant. To make samples as homogeneous as possible, blood was first transferred away from large clots and vortexed prior to addition to dilutions. These dilutions were then vortexed before addition to each sample. However, large standard deviations suggest some smaller clots remained.

One focus of the comparison studies was to determine the best incubation times and temperatures for the digestion. The original protocol included a 30 minute 56°C, 10 minute 99°C, and 10 minute 4°C incubation. This was not only long, but required a significant amount of hands-on time from analysts. In addition to transferring samples, they had to be placed in a screw-down rack to ensure lids remained closed at high temperatures between the 56°C and 99°C incubations. Furthermore, these racks were not compatible with other spin baskets being considered for the study. The purpose of the 99°C and 4°C incubations is to inactivate the enzyme proteinase K, so it cannot affect results later on. As DNA IQ™ is both an extraction and

purification method, it should remove proteinase K, making its inactivation no longer a concern (6).

Therefore, a study was conducted to determine the effect of removing these additional incubation steps. The results of this study suggested comparable DNA yields at higher concentrations of DNA, but showed a significant difference at low concentrations. A potential explanation of this is that there was insufficient proteinase K activity to fully denature nucleases harmful to DNA. A longer 56°C incubation of 1 hour was then tested. This yielded comparable amounts of DNA at lower concentrations, but highly variable quantities at higher concentrations. The high standard deviation observed here may have been due to clots added to some of the samples resulting in higher than expected quantities of DNA. This could account for the extreme variability, particularly as the initial testing of 56°C for 30 minutes yielded comparable amounts of DNA in the 1:5 dilution study to the old protocol.

In addition to incubation period testing, two spin baskets were compared, the Promega® DNA IQ™ spin basket and the Qiagen® Lyse&Spin basket. The results of this study showed comparable DNA yields for the 1:100 dilution samples, but the DNA IQ™ spin baskets showed significantly more DNA yield for the 1:5 dilution samples. It was observed during the course of this study that the lysate was not flowing through the Lyse&Spin baskets in its entirety for several samples. This would make a larger difference in more concentrated samples where more volume lost equates with larger quantities of DNA lost. Alterations to the centrifugation step were made to try to account for this, increasing the length and speed to improve flow-through.

Finally, both the 56°C 1 hour incubation and Qiagen® Lyse&Spin baskets were incorporated into a modified protocol for comparison to the original protocol. This study

showed comparable DNA yields between the two protocols for both concentrations tested and was adopted prior to beginning the cross-contamination study.

In the cross-contamination study, 48 positive and 48 negative samples were extracted in a checkerboard pattern. Quantification showed three of the negative samples contained DNA. Amplification and analysis showed the positive control as the possible source for two of the samples, though no definitive conclusions could be made as few alleles were identified. The third well contained several alleles not consistent with the positive control or any profiles on record in the lab. This suggests possible contamination of a consumable, or some other source.

To identify if contamination were occurring in the digestion or the extraction on the Biomek® NX^P, pre-extracted DNA was added directly to a deep well plate for purification on the instrument. If contamination occurred at this step, it should still occur in this experiment. One well showed contamination. As the Biomek® NX^P has been in the process of optimization and validation for several years, it was determined by management at the NYC OCME that this combined with various software and hardware errors was sufficient cause to discontinue the project.

It was deemed possible that shaking of the deep well plate transferred small droplets from one well to another. It is also possible some small dripping occurred of droplets clinging to the sides of pipette tips. For more information, a study could be conducted with 48 different positive controls to make identification of the cross-contamination source simpler. To identify any contamination of consumables, the digestion would have to be performed with the replacement of individual consumables until the unknown profile no longer occurred.

Other future studies include the review and testing of alternate automation platforms. There are currently several in use in various forensic labs across the country that may be considered for the NYC OCME.

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References

1. Nelson, M, Chase, R, DePalma, L, National Institute of Justice, US Department of Justice, Office of Justice Programs, & United States of America. (2013). *Making Sense of DNA Backlogs, 2012—Myths vs. Reality*.
2. Roewer, L. DNA fingerprinting in forensics: past, present, future. Investigative Genetics 2013: 4(1): 22.
3. Greenspoon SA, Ackroyd-Isales A, Thomas JT, Jenkins B. Optimization and Validation of the Biomek® NX for the Automation of Forensic Sample Processing. Proceedings of 21st International Symposium on Human Identification; 2010, Madison, WI.
4. Van Oorschot, RA, Ballantyne, KN, Mitchell, RJ. Forensic trace DNA: a review. Investigative Genetics 2010: 1(1):14
5. Nagy, M, Otremba, P, Krüger, C, Bergner-Greiner, S, Anders, P, Henske, B, Roewer, L. Optimization and validation of a fully automated silica-coated magnetic beads purification technology in forensics. Forensic science international 2005:152(1):13-22.
6. Hu, Q, Liu, Y, Yi, S, & Huang, D. A comparison of four methods for PCR inhibitor removal. Forensic Science International: Genetics 2015:16:94-97.
7. Frégeau, C J, Lett, CM, & Fourney, RM. Validation of a DNA IQ™-based extraction method for TECAN robotic liquid handling workstations for processing casework. Forensic Science International: Genetics 2010: 4(5): 292-304.
8. Greenspoon, SA, Ban, JD, Sykes, K, Ballard, EJ, Edler, SS, Baisden, M, Covington, BL. Application of the BioMekR 2000 laboratory automation workstation and the DNA IQ™ system to the extraction of forensic casework samples. Journal of Forensic Science 2004: 49(1): 29-39.

9. Stangegaard, M, Meijer, PJ, Børsting, C, Hansen, AJ, Morling, N. Biomek® 3000 The Workhorse in an Automated Accredited Forensic Genetic Laboratory. Journal of laboratory automation 2012: 17(5): 378-386.
10. Scientific Working Group on DNA Analysis Methods. Validation Guidelines for DNA Analysis Methods. December 2012. Available: http://media.wix.com/ugd/4344b0_cbc27d16dcb64fd88cb36ab2a2a25e4c.pdf
11. New York City Office of Chief Medical Examiner. Quantifiler® Trio DNA Quantification Kit. Approved May 2015. Forensic Biology Protocols for Forensic STR Analysis. New York, NY, www.nyc.gov/ocme.
12. Promega®. Powerplex® Fusion System Technical Manual. Revised March 2015.
13. Qiagen®. Investigator® Lyse&Spin Basket Kit Handbook. August 2013.