A QUANTITATIVE STUDY ON EXTRACTION VERSUS DIRECT AMPLIFICATION OF TOUCH DNA SAMPLES

by

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

DNA	Deoxyribonucleic acid
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
STR	Short Tandem Repeat
Ct	Cycle Threshold
QTY	Quantity
RB	Reagent Blank
D. A	Direct Amplification

Abstract

Touch DNA, also known as Trace DNA, is an important aspect of criminal investigations, as the perpetrator is unaware of the DNA they have left behind. While there are multiple ways to extract touch DNA, it has been established that direct amplification provides exemplary results. Direct Amplification is the process of analyzing the samples by amplifying them without extraction or quantitation. However, there is no measure on how much information direct amplification provides as opposed to other widely used methods including standard extraction procedures. This study aimed to look at the differences in information obtained when processing a touch DNA sample using standard extraction procedures versus a direct amplification approach. Understanding the scope of information collected by direct amplification versus standard extraction procedures can be used to facilitate new protocols for processing touch DNA. This study focused on collecting touch DNA samples from 20 volunteers and processing the DNA using two methods. In the first half of the study, half of the samples were extracted, quantified, and amplified using the InnoXtract™, InnoQuant™ and InnoTyper™ kits, respectively, from InnoGenomics, LLC. The next part of the study included adding swabs to be directly amplified using the InnoTyper™ 21 kit. Rather than the standard extraction protocol, this method included placing the swab heads directly into the PCR tubes for amplification. Samples undergoing direct amplification are expected to show more information due to the fact that standard extraction protocols often result in loss of DNA. Data generated from capillary electrophoresis was compared by reviewing the allele peak heights. Samples exhibiting "real" and representative peaks were further reviewed to determine if the peaks were real or due to artifacts such as noise. The research findings may lead to revised protocols that can be applied to

difficult sample types, such as touch DNA, which often result in partial DNA profiles that often contain very little information.

Introduction

Biological evidence, specifically deoxyribonucleic acid (DNA), is a vital tool used in forensic biology. DNA is beneficial as it may connect the donor to a scene or weapon. Multiple types of DNA evidence are left at the scene including blood, semen, saliva. A common type of DNA left at the scene is touch DNA. Touch or Trace DNA is defined as the DNA that is left behind as a result of skin contact (1). Touch DNA is often left behind in very minute quantities, which is why this type of DNA is difficult to analyze. There are multiple factors that affect the interpretability of this DNA including the type of surface, analysis method, and potential environmental conditions that can have a larger impact on how much of the DNA is recovered.

The amount of touch DNA left behind at the scene is highly variable. It could vary based on handling time and mode of collection. Handling an item for as short as two seconds could produce a partial profile (2). Increasing the handling time to 60 seconds could result in a profile up to 98% of the time depending on the object handled (2). The amount of touch DNA recovered from a scene could vary from 0ng/ul to 160 ng/ul (3). Collection method also greatly affects the amount of DNA recovered. Using a swabbing, taping, or cutting method will significantly influence the DNA recovery. The most effective way to collect touch DNA, which was established in Templeton et al's (2013) study, is to perform a two-swab method. This is done by first swabbing with a wet swab and then a dry one. Another important factor is whether the donor is classified as "good shedder" or "bad shedder". It is thought that "good shedders" will leave behind more DNA upon contact (3). Some studies have reported that men are better shedders than women, and that children tend to leave behind more DNA than the elderly (3). There are

however, differing opinions on the reliability of shedder status. Schmidt et al. (2021) established that there is no set standard that is used when determining if an individual is a good or bad shedder. As a result, there is much variability in the classification of "good" and "bad shedders, and researchers are often hesitant to consider it as an affecting factor (4). Regardless, touch DNA remains low-template and thus is difficult to analyze and interpret.

The standard way to collect touch DNA is to swab the area of interest and perform the standard DNA profiling procedures (5). This includes extraction, quantitation, amplification, and capillary electrophoresis. During the extraction process the nucleus of the sample is broken apart to release the DNA. Quantification is meant to show how much of the DNA has been extracted from the sample. This is appropriate in typical forensic samples such as blood and semen, that have a sufficient amount of DNA. This is done to ensure that the appropriate quantity of DNA is input into the PCR, as adding too much or too little in the qPCR can negatively affect the results. However, this can be very detrimental to low template DNA samples, as this step includes removing 2ul of the sample to be placed into the qPCR (6). The 2ul of the sample consumes some of the valuable sample volume, which can further restrict results from low template samples. Following quantification, the samples are amplified and then analyzed for DNA typing. This final step results in the DNA profile of the donor, which is then used for comparison to other samples.

For touch DNA, this standard operating procedure is not favored, as the extraction step can result in the loss of DNA. It is estimated that 50 -90% of the DNA is lost during the extraction step (6). Since Touch DNA has very limiting amounts of DNA, losing up to 90% of the DNA in the sample could result in no DNA recovery. Skipping the extraction and quantitation steps and directly amplifying the samples allows for more DNA to be retained for

analysis. This generally involves adding the sample (generally a swab of the sample) directly into the PCR tube without any prior treatment. Directly amplifying the samples has proven to work with various sample types including saliva, hair, and latent prints (6).

Whether the sample was extracted or directly amplified the desired result is a DNA profile. A standard DNA profile is obtained from the analysis of short tandem repeats. Short tandem repeats are areas of repeating blocks of short sequences (7). An example of a base repeat is AAGT, this is then for example, repeated between 5 to 50 times. Studying the sizes of these blocks of repeats allows researchers to determine the identity of the donor. In order to study STRs, certain locations on the genome have been identified for analysis. Generally, STR testing kits have 21 loci markers. Alleles produced in the profile are studied to determine if the allele is a "real peak"; meaning is the peak representative of the DNA profile or is it due to stutter or background noise. After reviewing the electropherogram, if an allele peak was found to be representative of the profile, it used to determine the genotype of the donor. In low template samples such as touch DNA, it is likely that some of the profiles produced will be partial profiles. A partial profile is any DNA profile that does not produce results in all expected loci (7). Each study generates their own guidelines for determining when the profile can be classified as a full or partial profile. With low template samples, it is expected that the majority of the samples will produce partial profiles, as proven in the various studies where longer sized STR loci were harder to produce (3,7,8). Furthermore, with low template samples, the probability of drop in events, and artifacts is increased. This is due to a higher degree in stochastic variation that is present when lower amounts of DNA are amplified using a PCR (7).

Based on published studies, the Innotyper21 kit utilized in this case has been proven to be extremely sensitive. The Innotyper21 kit uses 20 bi-allelic retrotransposon markers existing

abundantly in human genome (8). The alleles have two possible states: insertion and no insertion and are termed INNULs (9). In contrast to STRs, INNULS can be detected in much shorter amplicons which makes them suitable for degraded or low template DNA samples (9). INNULs are very abundant in the human genome, and have very low mutation rates, making them more stable than STRs (9). The InnoTyper21 kit targets shorter target amplicons than those in standard STR kits at (60 – 120bp) (8). The downside of using Alu based kits, is the difference in size between the insertion and null alleles (9). The insertion allele could be 200 – 400 base pairs larger than the null allele. This makes the alleles more prone to preferential amplification and DNA degradation (9). Preferential amplification would result in one of the alleles being amplified more efficiently than the other larger allele. The size difference between the alleles could also lead to allelic drop out during PCR (9). While this might make this type of analysis not ideal for touch DNA samples, the Innotyper21 kit was manufactured and tested against degraded samples. The InnoTyper21 kit was designed to benefit from the size difference in the Alu and to detect a repeat sequence known as target site duplication (9). The TSD is a direct repeat sequence at the beginning and the end of the Alu insertion. This design greatly reduces size differences between the alleles. Employing this information, the primers in the InnoTyper21 kit were created to type highly degraded DNA samples regardless of the size of the Alu insertion (9). Kit design allowed for the insert and the null to be closely spaced (2 bp to 9 bp) for ease of capillary electrophoresis (9). The kit was also tested with degraded samples and was able to recover 95% of the profile in degraded samples, which was 1.4 times and 6.6 times higher than the MiniFiler and Identifiler Plus kits, respectively. Both the MiniFiler and Identifiler are STR based kits (9).

For this research, the allele height for both methods were planned to be compared. Ideally, the allele height for the samples will be comparable to those in the positive control. The locations of the peaks were also taken into consideration to determine if the peaks were representative or due to artifacts. This was done by using the allelic ladder peaks as a reference as to where the peaks should migrate. The positive control was used to determine if the run was successful. Finally, the negative control and reagent blanks were used to evaluate artifacts and monitor possible contamination.

The goal of this research project was to analyze samples deposited by the same donors, using two different methods. The first method was the standard method that is used in all forensic laboratories. This method included extracting, quantifying, and amplifying the samples. The second method was directly amplifying the sample. While there has been research to show the effectiveness of using direct amplification for touch DNA samples, this research aimed to show statistical differences from both methods, that were done at the same time with the same set of samples. The data was collected from 20 different volunteers for a total of 80 samples. 40 samples were manually extracted, quantified, amplified and separated using the genetic analyzer. 40 samples were directly amplified and sized. There were two main research questions in this project:

1) is there a significant difference in the amount of information generated from processing touch DNA samples using extraction versus direct amplification

2) will the swabbing time after deposition have an effect on the amount of touch DNA recovered

Finally, it is hypothesized that samples undergoing direct amplification will reveal more information in terms of the donor's genotype, as opposed to the extracted samples.

Volunteer	Extraction	Direct Amp	Sex
1	1.1,1.2	1.3,1.4	F
2	2.1,2.2	2.3,2.4	F
3	3.1,3.2	3.3,3.4	M
4	4.1,4.2	4.3,4.4	F
5	5.1,5.2	5.3,5.4	F
6	6.1,6.2	6.3,6.4	F
7	7.1,7.2	7.3,7.4	F
8	8.1,8.2	8.3,8.4	M
9	9.1,9.2	9.3,9.4	F
10	10.1,10.2	10.3,10.4	F
11	11.1,11.2	11.3,11.4	F
12	12.1,12.2	12.3,12.4	M
13	13.1,13.2	13.3,13.4	F
14	14.1,14.2	14.3,14.4	F
15	15.1,15.2	15.3,15.4	F
16	16.1,16.2	16.3,16.4	F
17	17.1,17.2	17.3,17.4	F
18	18.1,18.2	18.3,18.4	F
19	19.1,19.2	19.3,19.4	F
20	20.1,20.2	20.3,20.4	M

Table 1. Volunteers and Samples Collected

Previous Research

A study done by Templeton and Linacre (2014) demonstrated the effectiveness of using direct amplification on samples from fingerprints. Researchers carefully sterilized the area using 70% isopropanol, and 3% sodium hypochlorite. 34 Volunteers were instructed to wash their hands with warm water 15 minutes before deposition. Volunteers then deposited touch DNA samples on DNA-free plastic slides using all five digits on their dominant hand. The samples were then swabbed using nylon FLOQswab (Capan Industries) (5). The swab pre-moistened with 2 µL of 0.1% Triton X (2). A portion of the fibers from the swabs were cut using a blade. Forceps were then used to gather the small portion of the swab and apply pressure on the slides (5). The slides were swabbed 10 times horizontally and 8 times vertically. This was then repeated with a dry swab. Both portions of the swab were then placed into the same 0.2 mL PCR

tube. The swabs were then directly amplified using Profiler Plus and NGM SElect. Reference samples from buccal swabs were obtained from each volunteer. DNA profiles were generated in 71% of the samples, compared to extracted samples which did not produce any identifiable alleles (5). Profiles were deemed full when the alleles were detected above the analytical threshold (RFU) and matched the reference profile. The high-profile yield in this study is what prompted the use of the nylon flocked swabs for this research study.

Martin et al. (2018) performed a direct PCR analysis on multiple different samples including aluminum bullets, insulated wire, a circuit board, and Ziplock bags. All items were cleaned using 3% bleach before deposition. Volunteers washed their hands with warm water 15 minutes before handling the items. Volunteers were instructed to handle the items for 15seconds this process was repeated three times (10). The samples were then double swabbed, once with a 0.1% Triton™ X-100 moistened swab and once with a dry swab. Samples were then directly amplified using either the GlobalFiler®kit or the AmpFLSTR Identifiler by adding the swab heads directly into the PCR tube. Of the 48 samples that underwent extraction only 13 produced any alleles (10). Only 3 of those samples produced informative alleles. On the other hand, 77% of the 48 directly amplified samples returned a profile when analyzed using the Globafiler kit, while 100% of the samples returned a profile with the Identifiler kit (10).

It is important to note that all previous research mentioned used STR profiles as a means of testing their hypothesis while the kit that was used in this research is Alu based insertion and null (INNULS). This is important as this design can possibly prove to be more efficient in detecting low template DNA due to their shorter amplicons, which Alu based kits are better at identifying.

Materials

-InnoXtract® Hair DNA Extraction and Purification Kit (with adjusted protocol from InnoGenomics, LLC)

-InnoQuant® HY 1.2 Human and Male DNA Quantification and Degradation Assessment Kit

-InnoTyper® 21 Human DNA typing Kit

-InnoQuant Spectral Calibration Kit

-IGT ILS – 155 Size Standard

-IGT 5 Dye Matrix Standard for 3500 Series Genetic Analyzers

-I – clean nylon flocked swabs (ThomasScientific™)

-Sterile Microscope Slides

-100% ethanol

- 30% Bleach

-Scissors

- 10ul, 20ul, 200 ul, 1000 ul pipettes and pipette tips

- TE^{-4} buffer

- Hi – Di Formamide

- Plastic Cups

- Gloves

- Brown paper

- KimWipes (Kimberly-Clark Professional™ Kimtech Science™)

- Nuclease free water

- Sharpie

- Forceps

- Ruler

- Scissors

- Magnetic Stand

- Excel

- Genemapper IDX

- InnoXtract, InnoQuant, InnoTyper 21 User Guides

Methods

Sample collection:

Approval for the project was obtained through the IRB before recruitment. Volunteers were recruited through posters posted in the Forensic Science Department and through the email listserv. A total of 20 volunteers were recruited ranging between 18 – 54 years in age. The volunteer population was comprised of four males and sixteen females ranging in ethnicity. Volunteers were asked to come into the lab at a specific date and time. The lab bench was sanitized with 30% bleach and 70% ethanol. Brown paper was placed on the bench and a Kimwipe (Kimberly-Clark Professional™ Kimtech Science™) was positioned on top of the paper prior to deposition. Sterile and DNA free glass microscope slides were labeled as #.1, #.2, #.3, #.4 with the # indicating the number of volunteer. Samples ending in .1 and .2 were samples that were to be extracted and samples ending in .3 and .4 were directly amplified. The volunteers were then asked to use their index finger on their dominant hand and place it on the microscope slide. The student researcher demonstrated how to properly place the finger on the slide to ensure the most effective and consistent print. The student researcher then counted to 15 seconds before asking them to remove their finger and proceed to the next slide. A handling time of 15 seconds was chosen as both studies previously mentioned used 15 seconds. This was repeated on the 4 different slides. The KimWipe was replaced between each volunteer. All slides were left on sterilized lab benches on top of KimWipes until it was time for extraction.

Calibration:

Since the software and instruments used by InnoGenomics require different parameters the instruments in the lab were calibrated before analysis. The qPCR was calibrated using the TAMRA dye plate purchased from InnoGenomics. Furthermore, the genetic analyzer 3500 was also calibrated using the IGT 5 Dye Matrix Standard. The settings were adjusted to adhere to the protocols laid out in the InnoTyper21 protocol. A plate containing positive and negative controls was run to ensure the instrument was properly calibrated before the analysis of the samples.

Extraction:

Six hours after deposition, the samples labeled #.1 were extracted. The InnoXtract protocol was followed for all 20 samples. Samples labeled #.2 were extracted 24 hours after deposition following the same protocol. When the samples were ready for extraction, the first step was to swab the slides. The slides were swabbed using the nylon flocked swabs, which proved to be the most effective in ensuring most of the DNA was collected. In order to make sure the tips of the swabs will be fully submerged in the PCR tubes; the tips of the swabs were measured at 0.1 cm using a ruler. The swab was then bent using sterile scissors where the swab measured 0.1 cm from the tip, this is shown in figure 1. The bent tip was then moistened using nuclease free water. The swab was then applied onto the slide using heavy pressure for 30 seconds vertically and 30 seconds horizontally. The swab tips were cut and placed into 1.5 mL Eppendorf tubes for extraction. The samples were then extracted based on an adjusted InnoXtract protocol. The standard InnoXtract protocol is meant to be used with hair shaft samples. A scaled protocol was provided from InnoGenomics, LLC, however that protocol had not yet been finalized. The scaled protocol adjusted the amount of digest buffer needed for touch samples. Furthermore, the protocol required the use of a spin basket however due to limitations in resources and time, a spin basket was not used. Instead, before disposing of the substrate, forceps

were used to squeeze out all the lysates. Samples that were extracted after 6 hours were stored in 4^0C until it was time for all the samples to be quantified.

Quantification:

After all the 40 samples were extracted, the samples were quantified using the InnoQuant protocol. The standards in the InnoQuant kit were prepared in the hood based on the manufacturers protocol. The standards were set to concentrations of 25ng/ul, 2.5ng/ul, 0.3125 ng/ul, 0.0391 ng/ul, and finally 0.005 ng/ul. Standard concentrations and recommended dilution amounts are displayed in Table 2. The reaction mix was also prepared by combining the Master Mix, Primer Mix and reference dye provided in the InnoQuant kit. The appropriate volumes were calculated based on table 3. All wells including the standards, samples, and non- template controls received 18ul of the reaction mix. Per the protocol, the plate was placed into the PCR for a run of 40 cycles with the following parameters: 3 min at 95^0C then 12 seconds at 95^0 C then 30 seconds at 61^0 C. No adjustments were made to the InnoQuant protocol.

Standard	Concentration (ng/ul)	Recommended Dilution Amounts
Standard 1	20	10 µL [stock 100 ng/µL] + 40 µL Dilution Buffer A
Standard 2	2.5	10 µL [Std. 1] + 70 µL Dilution Buffer A
Standard 3	0.315	10 µL [Std. 2] + 70 µL Dilution Buffer A
Standard 4	0.0391	10 µL [Std. 3] + 70 µL Dilution Buffer A
Standard 5	0.005	10 µL [Std. 4] + 70 µL Dilution Buffer A

Table 2 Standard Concentrations

PCR Components Volume per Reaction	
InnoQuant® HY v1.3 QPCR Master Mix	10 µL
IQ HY: ROX Reference Dye (4 µM)	0.3 µL
InnoQuant® HY v1.3 Primer Mix	7.7 µL
Total Volume	**18.0 µL**

Table 3.Reaction Mix Components.

Direct Amp:

Similar to the extracted samples, the swabs were measured using the ruler and the tip of the swab was bent. The swab was then used to collect the DNA from the slides containing the sample. The samples were swabbed in an identical manner to the extracted samples (30 seconds horizontally and 30 seconds vertically). Direct amp swabs were then placed in plastic cups. Between 2 and 3 swabs were placed in each cup, ensuring that the tips did not touch as shown in figure 4. The swabs were left out to dry until all samples were ready to be amplified. This was approximately 72 hours after deposition. Once all the extracted samples were extracted and quantified, the direct amp swabs were ready to be put in the PCR tubes. The area inside the hood was sanitized. The reaction mix was prepared following the Innotyper21 protocol as displayed in table 4. The tubes were prepared with the following amounts:

Sample	Reaction Mix Volume	TE buffer volume	Test Sample Volume	Swabs (Yes or No)	Count
Direct AmpSamples (#.3 and #.4)	9 ul	16.0 µL of TE^{-4} buffer	n/a	YES	40 samples
Extracted Samples (#.1 and #.2)	9 ul	n/a	16 ul of sample from extraction	NO	40 samples
Positive Control	9ul	12 µL of TE^{-4} buffer	4.0 µL of the provided DNA Control	One control received a swab, and one control	2 samples

				was prepared with no swab	
Negative Control	9ul	16.0 µL of TE^{-4} buffer	n/a	One control received a swab, and one control was prepared with no swab	2 samples
Reagent Blanks	9ul	n/a	16 ul of sample from extraction		4 samples

Table 4. Sample Prep for Amplification

The direct amp swab tips were then placed into the PCR tubes containing the appropriate amount of reaction mix. The samples were then amplified in the HID real time PCR following the InnoTyper protocol.

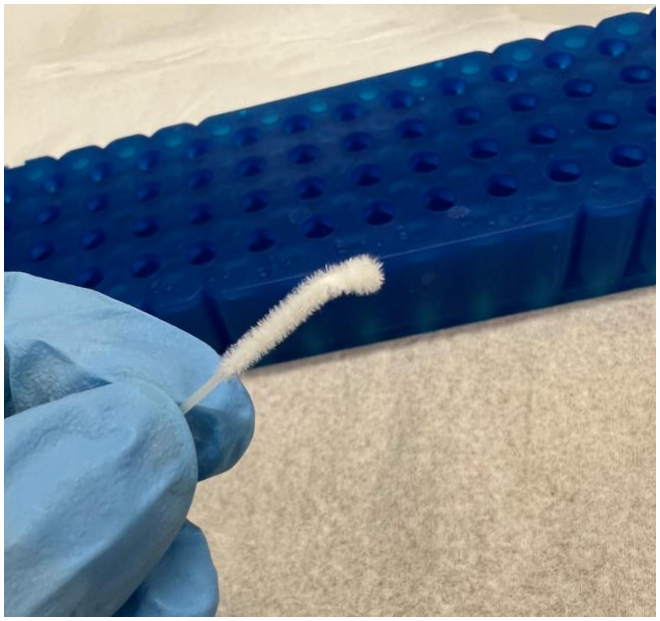

Figure 1. Bent Swab used for sample collection

Figure 2 Storage of D.A swabs until amplification

PCR Components for Multiplex	Volume per Reaction 25 µL Total Reaction Mix
InnoTyper® Primer Mix	3.5 µL
InnoTyper® Master Mix	5.0 µL
IGT DNA polymerase	0.5 µL
DNA Template/Pos. Control/TE^{-4} buffer	Up to 16.0 µL

Table 5. Components of PCR Reaction Mix

Genetic Analysis:

A 96- well plate was prepared for capillary electrophoresis. Each well received 11ul of a reaction mix composed of 10.6ul of Hi Di Formamide and 0.4ul of the ILS- 155 Internal Lane standard. Wells then received 1ul of PCR product or allelic ladder. The protocol recommended 1 allelic ladder per 23 samples. In this case, following the manufacturer's protocol, 1 allelic ladder was used for every 23 samples. The plate was heated in the thermocycler for 3 minutes at 95^0 C and then cooled on ice for another 3 minutes. The plate was then placed into the 3500 genetic analyzer. Settings were already set up based on the Innotyper21 protocol. The results were interpreted using Genemapper IDX.

Data Analysis and Interpretation

Quantitation

The results of the samples that were extracted then quantified were studied to determine how much DNA was present. The standards displayed the appropriate amount of DNA as evident in Table 1.

Standard #	Expected QTY	Resulted QTY (Row 1)	Resulted QTY (Row 2)
Standard 1	20.00	20.00	20.00

Standard 2	2.500	2.500	2.500
Standard 3	0.312	0.312	0.312
Standard 4	0.039	0.039	0.039
Standard 5	0.005	0.005	0.005

Table 6.Expected and resulted QTY results of the five standards

However, the extracted samples displayed a quantity (QTY) of 0 for all samples. This suggests that there was no DNA observed in the samples to be quantified. When comparing the threshold cycle (Ct) numbers to the standards, the mean Ct for the samples was extremely low. Threshold cycle refers to the intersection between the amplification curve and the threshold line (9). The Ct increases with decreasing amount of DNA (9). Using the standard that was set at 0.005 nanograms, as reference the mean Ct for the short target was 24.979 as displayed in Table 2. Looking at the mean Ct for all other samples, the mean is 32.595 which is much higher with no DNA detected. Furthermore, when looking at the reagent blanks, which barring any contamination, should by design have no detectable DNA present, they displayed similar mean Ct numbers to the samples that were extracted. This suggests that there was no DNA in any of the samples that were extracted.

Sample Name	Ct Mean of Short target
1.1	29.102
1.2	26.947
2.1	33.548
2.2	31.799
3.1	32.202
3.2	33.142
4.1	32.546
4.2	33.728
5.1	32.592
5.2	31.09
6.1	33.308
6.2	32.015
7.1	33.374
7.3	32.883
8.1	33.994
8.2	32.406
9.1	35.211
9.2	32.977
10.1	31.861
10.2	UND
11.1	31.96
11.2	32.915
12.1	32.31
12.2	31.788
13.1	33.635
13.2	31.778
14.1	32.92
14.2	33.219
15.1	33.396
15.2	28.216
16.1	32.496
16.3	31.207
17.1	35.49
17.2	34.439
18.1	36.21
18.2	32.454
19.1	32.612
19.2	31.241
20.1	29.843
20.2	39.525
RB 1	32.946
RB 2	31.72
RB 3	33.019
RB 4	31.542
RB Average	32.307
Average	32.625
STD 5	24.979

Table 7. Quantification Data

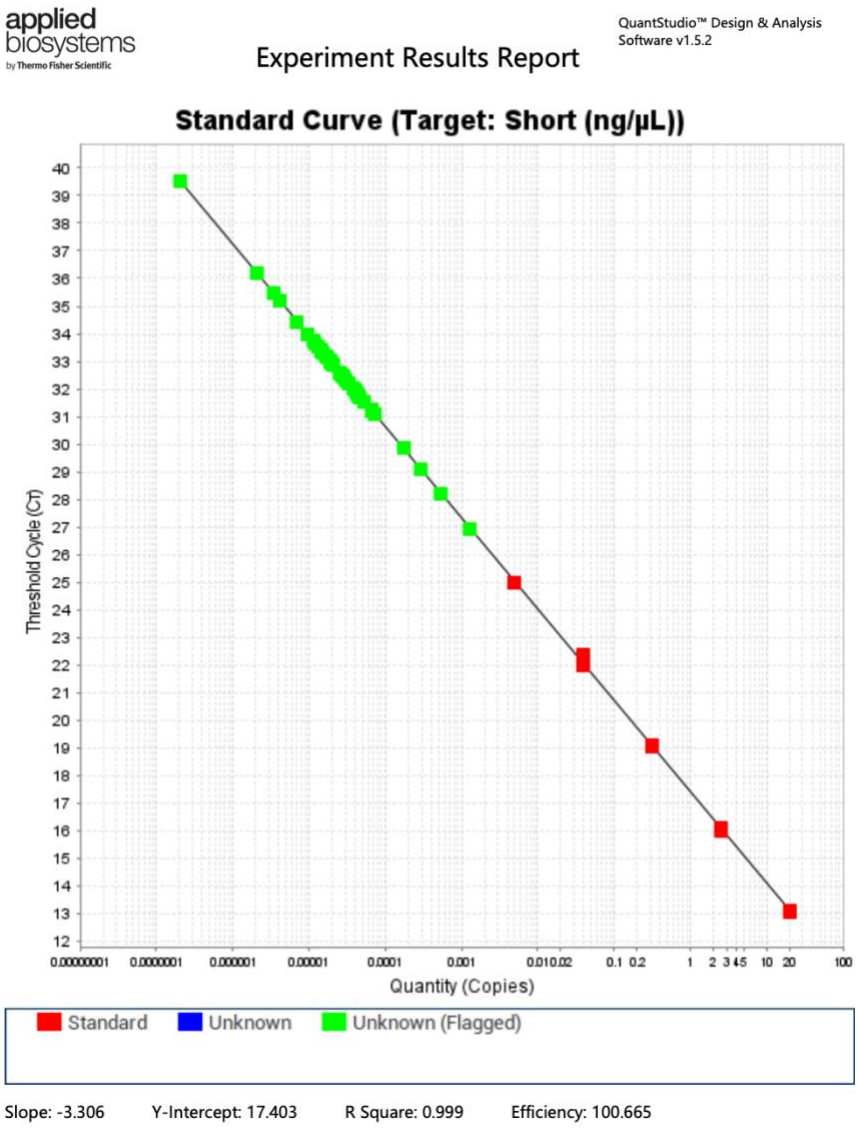

Figure 3 Standard curve of short target (ng/uL)

Figure 3 displays the short target. The red boxes are representative of the five standards. The green boxes which were 'not detected' by the qPCR are representative of the rest of the samples. The distance between the samples and the standards further shows the low levels of DNA that were present in the samples. This explains why the samples were not detected.

Furthermore, acceptable slope values for the short target based on the InnoQuant protocol are between -3.1 and -.3.6. The slope value in this case is -3.306. An acceptable R square is anything greater than 0.98 and, in this case, it was 0.999. Finally, an acceptable efficiency is anything

greater than 90% and less than 110%, in this case it was 100%. These factors further show that the run was a success in terms of the standards and the controls.

Based on the experimental design, the samples undergoing the standard procedure were also extracted at different times to see if the environmental conditions had an effect on the amount of DNA that would be present at the end. The results from the qPCR showed that the difference in extraction times had no effect on the amount of DNA present. Samples that were extracted at the six-hour mark and samples that were extracted at the twenty-four-hour mark showed similar DNA quantities after quantitation and similar results in the CE. This suggest that there was no DNA to be detected in any of the samples regardless of when they were extracted.

Genetic Analysis

The electropherograms obtained from Gene Mapper IDX were studied to determine the difference between the methods. The majority of the samples did not display any peaks in the appropriate size ranges for the alleles. However, samples that did produce peaks consistent with the size of the alleles in the allelic bins were further studied to determine if the peaks were due to artifacts and were incorrectly called. After comparing the samples from each donor, the various alleles that were called, were in fact due to noise and adventitiously fell into the allelic bins. This was determined due to the fact that the alleles being called were present in most of the samples, including the reagent blanks. If these peaks were representative of the profile, variation in their positions is expected as the samples were all collected from different volunteers. The presence of these peaks in the majority of the samples indicates that the peaks were a result of artifacts from the instrument.

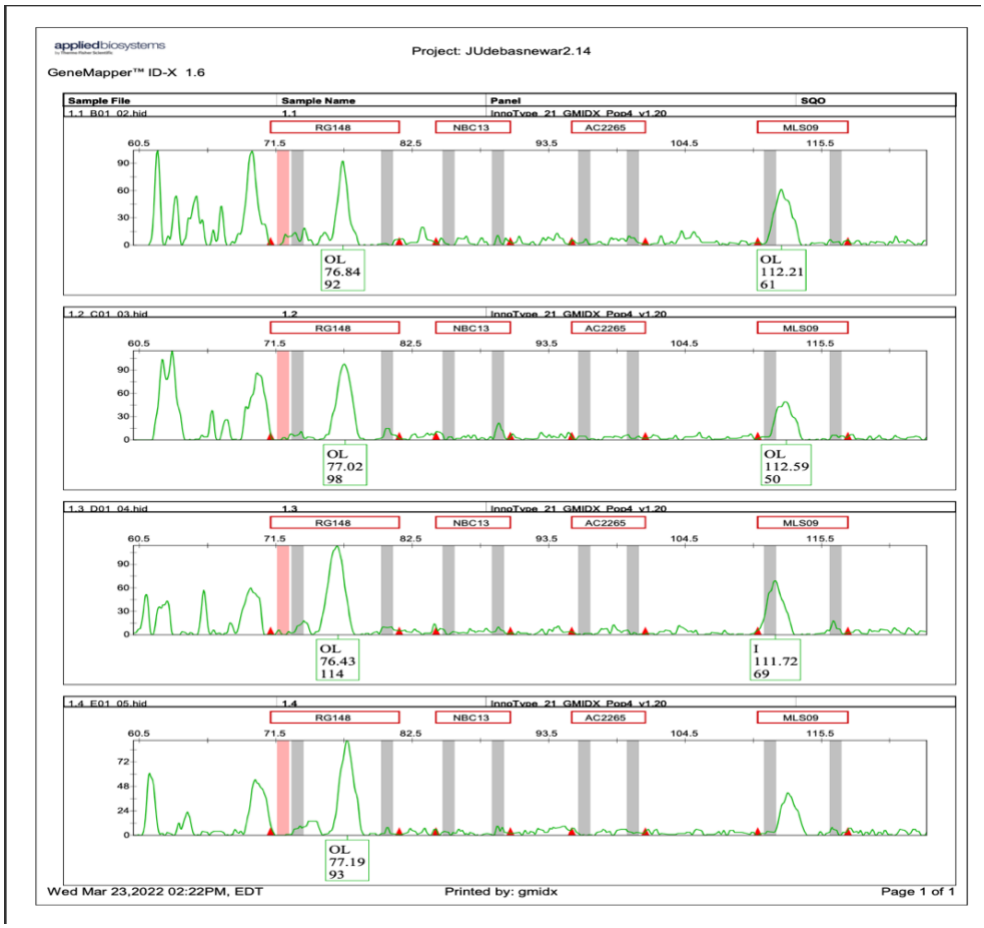

Figure 4. Electropherogram displaying all four samples from volunteer 1, separated by dye channel

As seen in figure 4 sample 1.3 displays an insertion allele at the MLS09 locus. After reviewing the other samples at the same locus, it was determined that this allele was in fact an OL peak that was incorrectly called. This was concluded as the other 3 samples displayed an OL peak at the same location. If this was a true insertion peak it would have been reflected in all the samples.

It is also important to look at the reagent blanks that were processed in parallel with the extracted and quantified samples. The purpose of the reagent blanks is to observe if there was any contamination, as by design the reagent blanks should have no DNA. In this experiment, there were four reagent blanks prepared. Figure 5 below displays the genetic profile of all four reagent blanks, that has been separated by dye channel.

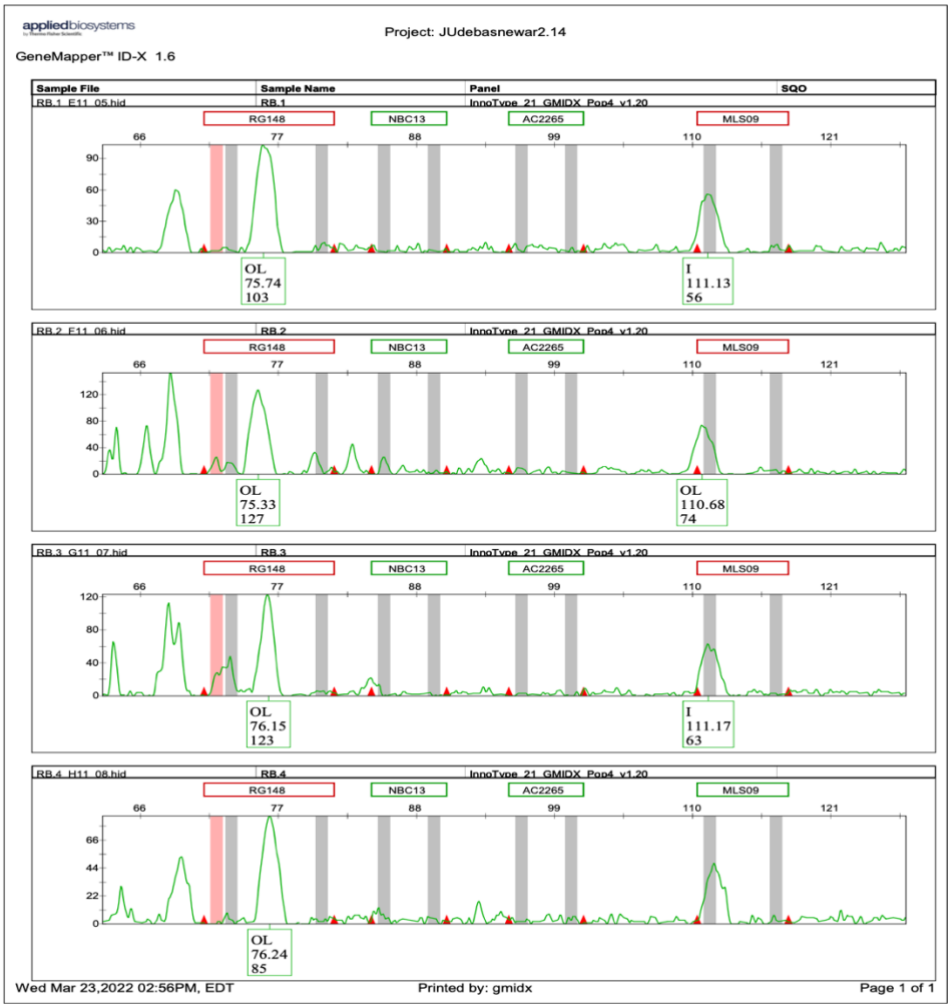

Figure 5.Electropherogram displaying all four reagent blanks, separated by dye channel

As displayed in this figure, the reagent blank did have multiple alleles. In standard cases this may indicate contamination as the reagent blank is designed to have no DNA. However, in this case it is evident that these peaks are not due to contamination. The RBs have peaks in the same

loci in all four samples. This would indicate that the peaks are due to noise or other artifacts and not due to contamination. If these peaks were due to contamination, there would be a variety in the alleles called and their location since the reagent blanks were prepared during four separate times. Having the same peaks in the same locations for all the samples is indicative that the peaks are due to noise. Furthermore, the peaks in the RBs are in the same positions as the alleles from sample 1 as displayed in Figure 4 above.

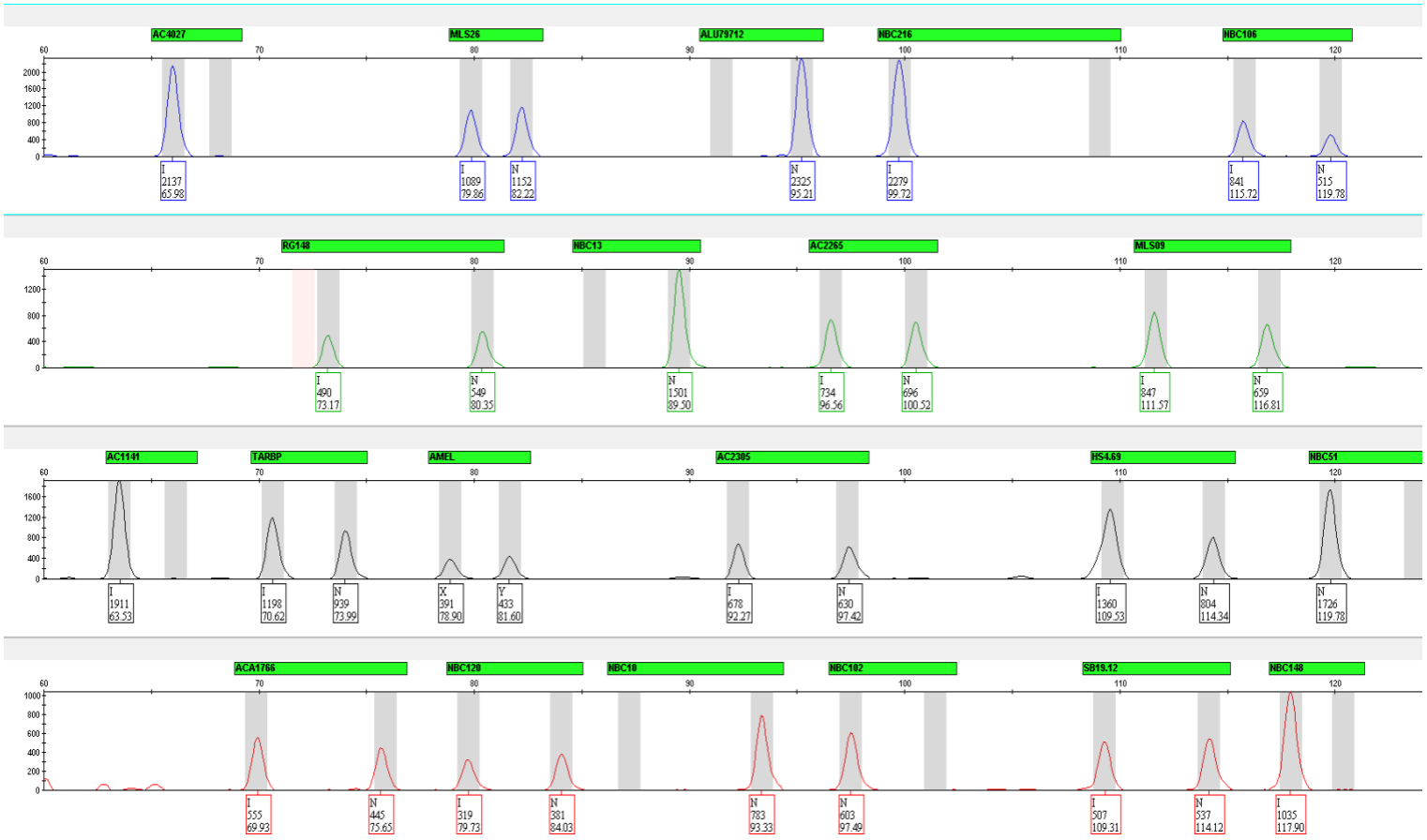

Figure 6. Positive control profile from a standard InnoTyper21 run

This figure displays a full profile of a positive control that is run on the InnoTyper kit. It is evident that the average peak heights range from 300 to 2400. Furthermore, all the peaks fall within the gray bins that are set up based on the allelic ladder.

Another factor taken into consideration was the allele height. Figure 7 below displays the positive control run in this experiment in the blue channel. It is important to examine the Y- axis scale. The peak heights for the samples are in the hundreds and thousands, similar to that displayed in the positive control obtained from a standard InnoTyper21 run (figure 6). However, when comparing the peaks from the samples, the heights are significantly lower.

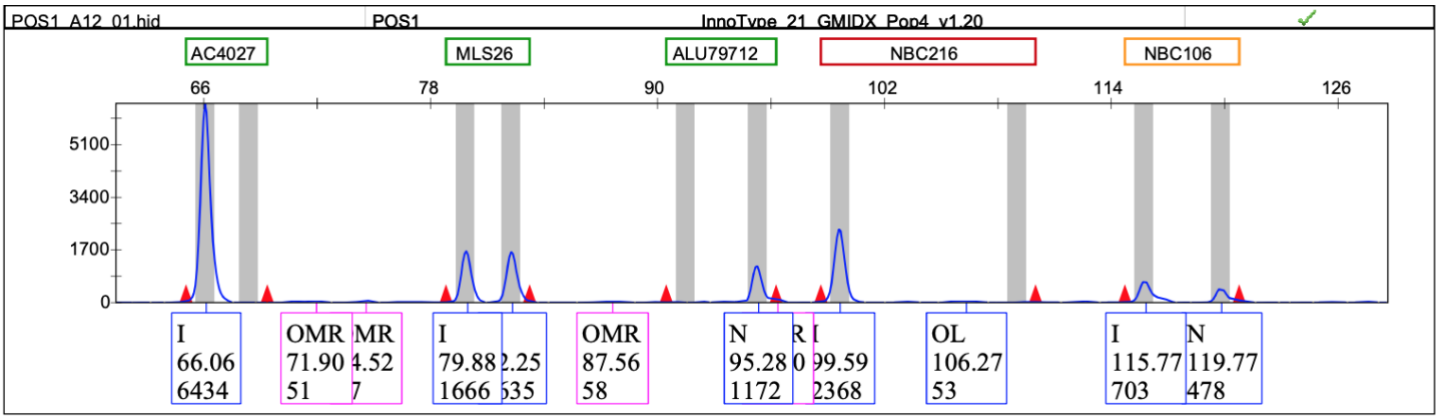

Figure 7. Electropherogram of the blue dye channel of the positive control

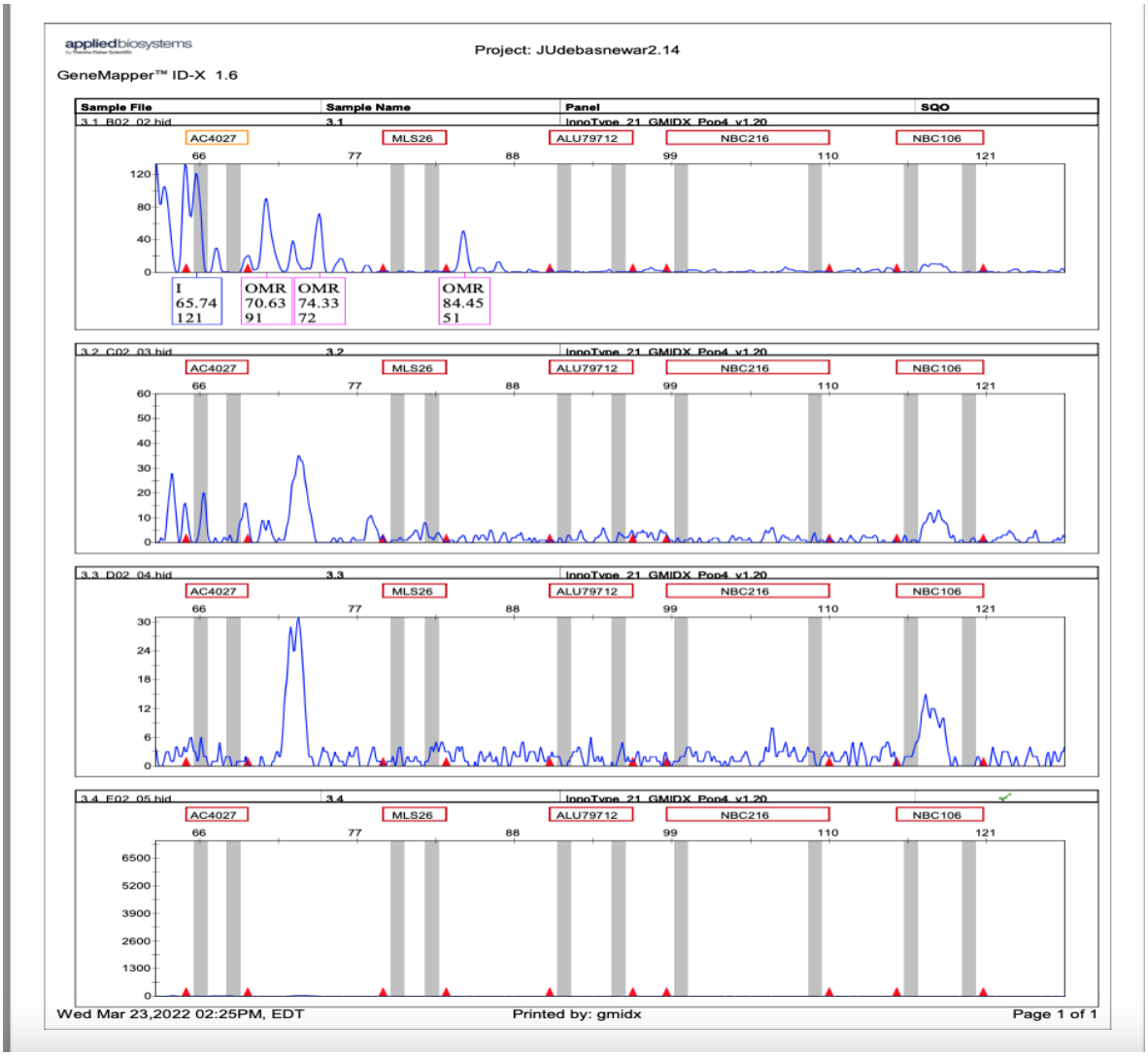

Figure 8. Blue dye channel of samples from volunteer number 3

The allele peak at the AC4027 locus is being called as an insertion allele. However, when comparing the allele height of this peak (121) to the positive control (6434), it is clear that the peak in the sample was incorrectly called due to its very low height. This peak is likely due to noise as evidenced by its low RFU.

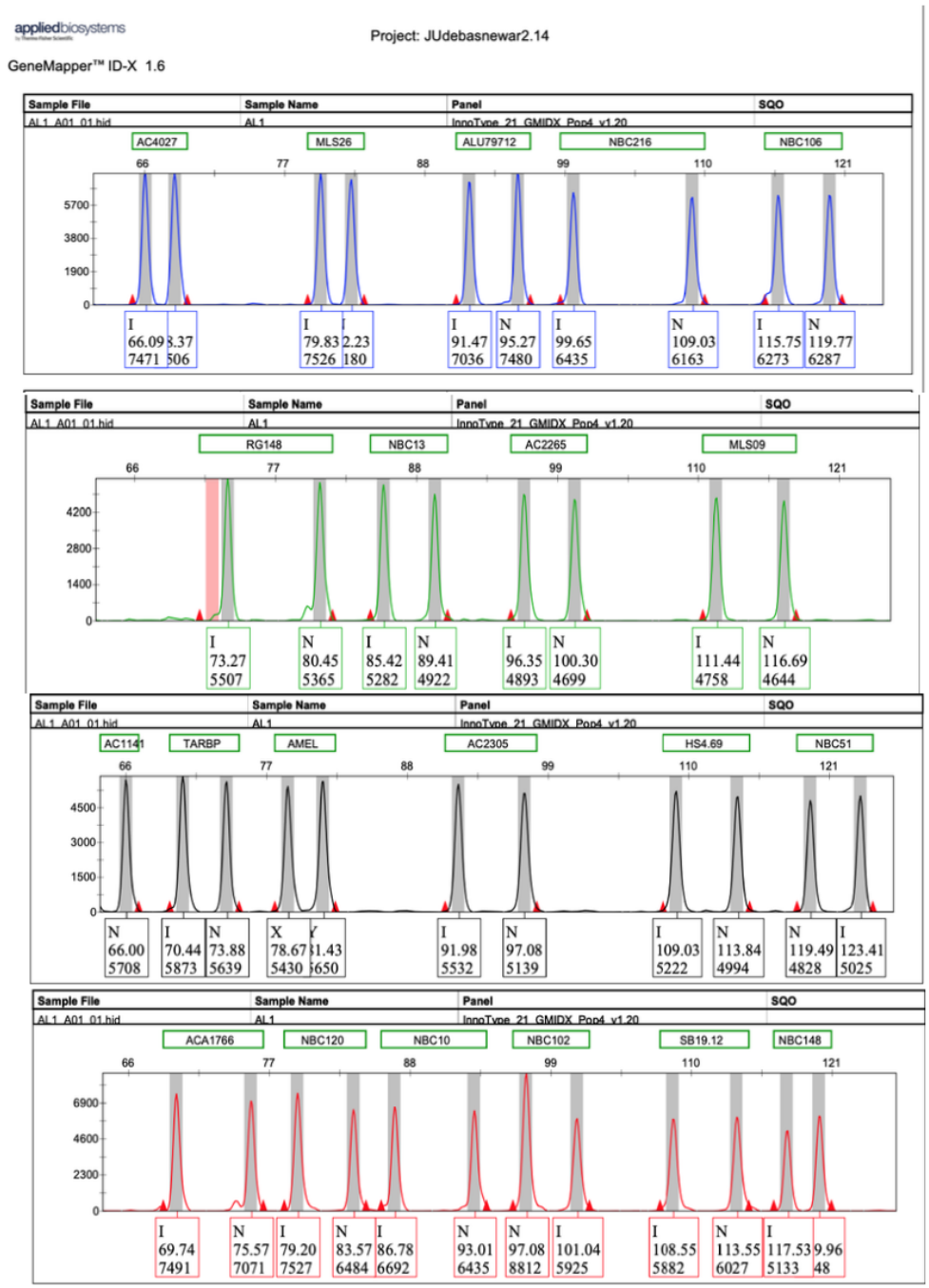

Figure 9. Allelic Ladder that was run in parallel with all samples

Figure 9 displays one of the allelic ladders. This shows that ideally all the peaks should fall within the gray bins. It is also clear that the Y – axis scale for the allelic ladder is much larger than that of the samples.

The positive control and allelic ladders produced expected results. This suggests that the run was successful. This also shows that the absence of real peaks is due to the minute amount of DNA that was present in the sample, and not due to a design error.

Research Results and Discussion

Due to the fact that the Ct means showed no significant difference between the reagent blanks and the samples, it was determined that there was no DNA in the samples to be quantified. Considering that the Ct mean for the lowest template standard was at 24, it was expected that the samples would fall around that range. Since the average for the samples was much higher, the samples cannot be considered as data, as any DNA lower than 0.005 ng is too minute.

Furthermore, the similarity in the threshold cycle mean between the extracted samples at six hours and twenty hours indicates that the difference in swabbing time did not result in DNA yields in any of the samples. The difference in swabbing time could have been studied and compared, however since there was no DNA detected a comparison was not possible. This comparison could have been important as it could indicate that no matter how early an investigator is able to swab and process the touch DNA sample, a profile will not be generated regardless.

As for the results obtained from the genetic analysis, the peak heights for all the alleles studied in the samples were too low to be considered “real” peaks. After comparing the alleles that were called to all samples donated by that volunteer, it was easy to distinguish and recognize that the peaks were due to noise. Furthermore, the disparity between the peak heights for the allelic ladder and positive control versus the samples affirmed that the sample peaks were too low to be considered as anything other than noise. The reagent blanks also corroborated that the

peaks exhibited by the samples were due to artifacts. This was evident when comparing the sample peaks and the reagent blanks at the at the same dye channel, in this case the green dye channel was used. The reagent blanks and the samples exhibited the same peaks. This was expected as with low template samples, stochastic effects and artifacts are more present. There was no pattern observed between extracted and directly amplified samples. Despite the fact that there was little information obtained from the electropherograms, neither method offered more information than the other. A possible reason for the low yield in all the samples is the short handling time. While other studies have used 15 seconds as the handling time, in this situation the same finger was used on all four slides. As a result, by the time the fourth and last sample was deposited there was less DNA on the finger, and as a result less DNA on the slide. Due to the fact that there was no DNA detected, this cannot be studied, however it is a possibility as in other studies, multiple fingers were used as opposed to just one.

Conclusion

Impact

Even though the samples were swabbed from an area where touch DNA was known to deposited, significant amount of DNA were unable to be collected. This is important as it does affirm the difficulty in processing touch DNA samples. The results from the qPCR, showed that extracted samples did not have any DNA above the lowest 0.005 ng standard before amplifying. Furthermore, the fact that samples that were swabbed as early as 6 hours after deposition did not produce any results, affirms that touch DNA is extremely low template and should be handled with care. The results showed no difference between the extracted and directly amplified samples. While, the two methods could have shown significant differences, in this case insufficient DNA was obtained to study those differences. This was validated by the fact that the

positive control showed expected results, while the sample data showed peaks demonstrative of noise.

The results of this study are helpful for future studies as they show that handling time can have a significant effect on the amount of information studied. Multiple studies have shown that touch DNA is extractable, however this study showed that in terms of glass slides, 15 seconds was not a sufficient handling time.

Limitations

Limitations in this study included not having enough resources to process reference samples, which could have been used to compare the results from the experiment. Having a reference profile for each donor, would allow the researcher to definitively determine if the alleles called were due to noise, stutter or were truly part of the donor's profile. Additionally, having the resources to repeat the procedures before amplification would have allowed the experiment to shift after noticing that no DNA was detected in the qPCR. Not having enough resources also affected the extraction process, as the spin baskets that were needed were not able to be provided. This resulted in the researcher having to use forceps to squeeze out the lysate instead of using the spin basket. Another limitation was not having the manufacturers protocol for touch samples for the InnoXtract. The protocol that was used in this experiment was an adjusted protocol from the InnoXtract protocol for extracting DNA from hair. A final limitation was the difficulty in obtaining a diverse volunteer group. The volunteers in this experiment were primarily females between the ages of 18 – 23. In order to obtain more representative results a diverse volunteer group is important. This can also be used to track any differences between sexes and/or ages.

Future Directions

Future directions of this study include repeating this experiment with STR kits to observe the difference in the effectiveness of STR versus INNUL based kits. This study can also be repeated with multiple different objects to observe if that will result in higher yields. For instance, this study could be repeated using objects that are handled more frequently in everyday lives like a cellphone or car keys as the brief amount of time the donors handled the slides could have resulted in the low yield. This study was primarily based on gathering touch DNA from latent prints; however, touch DNA can also be recovered from items of clothing. This experiment could be repeated using items of clothing to determine if the prolonged wear time would affect the amount of DNA recovered. The reagent blanks in this study helped confirm that the peaks being called were due to artifacts. This suggests that the detection limit for the samples is very low, using the results from the reagent blanks this study could be repeated with adjusted stochastic and analytical thresholds. Additionally, the use of reference samples when repeating this study with adjusted thresholds is vital, as it will allow researchers to determine if the adjusted thresholds were effective in detecting the DNA in the touch samples.

Further studies could also involve increasing the touch time of the samples. Volunteers could be asked to touch samples for a prolonged time, for example 1 minute. The samples can then be processed. Following that, the touch time could be decreased slightly, and the samples will be reprocessed. This can be used to determine at what handling time, DNA in touch samples is no longer detectable. This study can also be performed using mtDNA testing, as mtDNA is more abundant in the genome. MtDNA is also inherently more sensitive than nDNA which makes it more suitable for degraded and low template samples such as touch DNA.

Overall, this study affirmed the difficulty in processing challenging forensic samples such as touch DNA. Despite being in a controlled environment, the experiment was unable to detect

any DNA. This upholds the importance of creating updated handling and processing procedure for touch DNA samples.

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