FY 2009 Forensic DNA Unit Efficiency Improvement

"A More Efficient Means to Collect & Process Reference DNA Samples"

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For an electronic copy of this report that includes hyperlinks to additional research data (data summary tables, individual .pdf files of electropherograms for each sample, etc.) please send a written request to:

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Abstract

Federal funding made available by the National Institute of Justice (NIJ) through the DNA Initiative helped states and local governments significantly increase the capacity of their DNA laboratories between 2005 and 2008 [2]. At the same time, the demand for DNA testing continues to rise, thus outweighing the capacity of some crime laboratories to process the increased number of samples being received. The demand is coming from two primary sources: (1) the increased amount of DNA evidence that is collected in criminal cases and (2) the expanded effort to collect DNA samples from convicted felons and arrested persons [2].

All states and the federal government have laws that require collecting DNA from convicted offenders [2]. The federal government also requires collecting DNA from arrestees, a trend rapidly growing in many states. With nearly every state pursuing legislative expansion of their Combined DNA Index System (CODIS) databases, the need to implement the most efficient DNA testing methods possible is paramount. In addition, with the current economic situation affecting most every state, it is equally imperative to develop a cost effective means of performing DNA analysis on these samples. The Oklahoma State Bureau of Investigation (OSBI) has proposed a research study that will provide a solution to both of these needs.

The objective of this research project was 1) to implement a new DNA buccal collection kit that is universal in use, provides a higher success rate on the first analysis attempt, all at a significant reduction in cost, and 2) develop a technique to process buccal swabs using the Identifiler® Direct amplification kit. This kit eliminates the need for the extraction step in the DNA analysis process, but is specifically designed for use on FTA® cards.

For objective 1, the OSBI researched the advantages and disadvantages of several types of DNA collection kits. From this, a new buccal collection kit was developed that combines the best qualities of several different collection kits into one. This “All-In-One™” DNA collection kit is simple to use, provides reliable results, all at an affordable cost. The simplicity of the kit design allows for any law enforcement agency to properly collect known reference DNA

samples without any transfer steps or drying steps required. This direct DNA collection method helps to ensure that sufficient DNA is present, allowing the laboratory to obtain a full DNA profile on the first analysis attempt, thus reducing the time and cost of unnecessary re-testing. In addition, the cost of the buccal collection kit is a fraction of the cost of most kits currently used by CODIS laboratories. OSBI is currently seeking opportunities to make this new buccal collection kit available to CODIS, forensic, and paternity testing laboratories.

For objective 2, the OSBI tested many different techniques that would allow for the direct amplification of buccal swabs. These techniques involved taking an entire swab head, lysing in various reagents, and using an aliquot of the liquid lysate for amplification. Some techniques tested worked well, others did not. However, it was demonstrated that direct amplification of buccal swabs is possible, thus eliminating the time and cost associated with the extraction step of the DNA analysis process.

By implementing these improvements, any forensic laboratory, regardless of size, can increase their current efficiency so they are better prepared to handle an increase in sample submissions without creating a backlog. This will ensure the CODIS database is utilized to its full potential, helping to solve past crimes as well as preventing future crimes to the highest extent possible.

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Executive Summary

OSBI Laboratory

Established in 1925, the Oklahoma State Bureau of Investigation (OSBI) is the general investigative agency of the State of Oklahoma. Serving a population in excess of 3.6 million citizens, the OSBI provides service and support of law enforcement throughout the state. The OSBI is composed of five separate divisions, including Investigative Services, Criminalistics Services, Informational Services, Administrative Services, and Information Technology Services.

The Criminalistics Service Division offers services in forensic biology (serology and DNA), trace evidence, marijuana/drug identification, toxicology, firearms/toolmarks, latent prints, shoe and tire impressions, and technical crime scene processing. The Division also maintains the Combined DNA Index System (CODIS), an automated firearms identification system (IBIS), an automated fingerprint identification system (AFIS), and an automated paint identification database called Paint Data Query (PDQ).

OSBI maintains one full service forensic laboratory, and four regional forensic laboratories that provide specific services. Each of these five facilities is accredited under the ASCLD/LAB Program, following the requirements of the FBI's Quality Assurance Standards for both forensic and database testing laboratories.

OSBI's CODIS Unit

The OSBI's Combined DNA Index System (CODIS) Unit has witnessed firsthand what a powerful tool the CODIS database is. Although small compared to some states, the Oklahoma CODIS database contains ~105,000 DNA profiles, producing over 940 hits in its 15 year history. These investigative leads have helped solve homicides, rapes, and other violent and non-violent crimes.

The initial Oklahoma database law only allowed DNA samples to be collected from persons convicted of certain violent felony crimes. Subsequent years brought legislative expansion of the database, eventually leading to DNA samples being collected for all felony convictions, certain misdemeanor convictions, and upon arrest for illegal immigrants. Each legislative expansion brought additional DNA samples to the laboratory for analysis, but typically, no additional funding was provided for the increased amount of DNA testing. This has caused the OSBI to rely heavily on NIJ and other federal grants to provide the means to collect and process these offender samples.

While it has proven extremely difficult to obtain state funding for CODIS, there can be no argument about the success obtained with each expansion of the database. After burglary was added as a qualifying offense, the number of hits doubled. After the expansion to all felony convictions, the number of hits obtained doubled again. In addition, the OSBI has seen firsthand that many of the "less violent" offenders often hit to unsolved violent crimes. Roughly a third of all hits obtained from an offender collected following a burglary conviction have solved more violent offenses such as homicide, rape, and robbery. With this information at hand, the OSBI does not want the cost of DNA testing to become a reason for legislators to stop pursuing their database expansion efforts.

In order to meet the mission of the OSBI, the goal of the CODIS Unit is to ensure that offender DNA profiles are entered into the database within 30 days of receiving the sample. However, based on past experience, the CODIS Unit realizes that to *consistently* meet this goal, the Unit must be prepared to respond to increases in sample submission with little or no increase in instrumentation, staff, and funding. In order to accomplish this, the CODIS Unit has re-evaluated nearly every step in the analysis process searching for the most cost efficient methods that can be implemented. This evaluation has led to many bottlenecks being identified, many of which have already been addressed, including 1) implementing a web based LIMS system that allows law enforcement agencies to log offender information into the LIMS

system once the samples are received at the laboratory, 2) validating the use of a reduced volume of the amplification reagent, thus allowing two times as many samples to be analyzed per amplification kit, 3) validating more robust equipment designed for higher through put, and 4) purchasing expert system software that will be used to analyze data from offender database samples, thus reducing the amount of time it takes for technical review.

Other State CODIS Laboratories

In 2008, OSBI conducted a poll of all 50 State CODIS Laboratories inquiring about the type of procedures they had in place to process offender samples, if their laboratory had a backlog, and if they anticipated future expansion in their database laws. Nearly half of the states responded which provided the necessary insight into other state facilities. Nearly half of the responding states indicated they had a backlog (ranging anywhere from ~1,000 to as many as ~30,000 samples). For some facilities, this led to turn-around times as high as 1 year from the time the offender sample was collected until the DNA profile was entered into the CODIS database. When asked why, many states indicated it was partially because of inefficiencies in their current collection and analysis procedures. Nearly half of these state facilities also indicated that an expansion in their state database law would cause a backlog of samples to occur if they were not provided the necessary funding and additional personnel. Since expanding database laws is a growing trend across the forensic community, laboratories should not solely look at purchasing more equipment and hiring more analysts, but rather, they should re-evaluate their current procedures being used in an effort to eliminate inefficiencies. With decreases in economic funding, and an increase in sample submissions, a cheaper means of providing high quality DNA results is critical.

Research Proposal

The OSBI has identified two key opportunities for improvement: 1) implementing a new DNA collection kit that is universal in use, provides a higher success rate on the first analysis

attempt, all at a significant reduction in cost, and 2) eliminating the need for the extraction phase of the DNA analysis process, thus greatly increasing throughput and significantly reducing the cost of analysis. By implementing these combined improvements, laboratories will be prepared to handle an increased number of samples with the passing of database expansion laws. In addition, this will help ensure the CODIS database is utilized to its full potential and that future crimes are prevented to the highest extent possible.

Improvement 1: Implement a New DNA Collection Kit

Most states rely on law enforcement officials at correctional facilities, sheriff's offices, and/or municipal police agencies to collect DNA samples. Whether being used as a known reference sample in a criminal case or for entry into the CODIS DNA database, proper collection of these samples is imperative in order to provide a good DNA profile. DNA collection kits come in all shapes and sizes, each with their own advantages and disadvantages. Among the most popular kits used by state laboratories include blood vials, Whatman® FTA® cards, and the BODE™ Buccal DNA Collector™.

Blood Samples

Blood provides an excellent source of DNA, but requires a specialized medical staff to collect the samples. This is not always feasible or even possible for all collection facilities. In addition, unless the blood sample is spotted onto another material (such as cotton cloth) and allowed to air dry, the blood vials must be refrigerated, requiring a specialized storage location until the sample is tested. With more states expanding their laws to include collection of DNA from arrested individuals, all law enforcement agencies need to have the ability to collect a DNA sample, making blood samples a highly impractical means of collection. However, the cost advantages of a blood collection kit far outweigh some of the other DNA collection kits.

Buccal Samples

For most forensic and paternity testing laboratories, buccal samples are rapidly becoming the specimen of choice when collecting known reference DNA samples. Buccal samples are collected by swabbing the inside of the person's mouth, thus removing epithelial cells from the inside of the cheek. These cells provide an excellent source of DNA, and the collection method does not require a specialized medical staff. This non-evasive procedure uses no needles, contains little biohazard waste, and permits sample retention at room temperature for many years.

Whatman® FTA® cards

One of more popular buccal collection methods is the FTA® card by Whatman®. A sterile foam swab is rubbed along the inside of the individual's mouth for ~30 seconds, then blotted onto the FTA® card [3]. The DNA cells are transferred from the swab to the FTA® card, and the swab is thrown away. Once dry, FTA® samples can be safely stored at room temperature for several years without sample degradation [3]. Whatman® FTA® cards are chemically treated with proprietary reagents that lyse cells upon contact, causing the release of the DNA. During DNA analysis, the laboratory takes a small punch from the FTA® card and begins the extraction process. Whatman® FTA® cards are compatible with any DNA extraction protocol used in forensic laboratories [3].

While buccal on FTA® provide the ability for all agencies to collect a sample, often times they do not produce a full DNA profile on the first analysis attempt, requiring the laboratory to re-analyze the samples using a modified analysis technique. The main reason these samples fail during DNA analysis is because the collection technique requires transferring DNA from a foam swab to the FTA® paper. If this transfer step is done incorrectly, the DNA remains on the foam swab (which is thrown away) and not on the FTA® paper (which is analyzed by the laboratory).

Another disadvantage of the FTA® cards is the cost. Whatman® offers a variety of buccal collectors, all at a significantly higher cost than a blood sample. However, FTA® buccal cards are among the most popular collection kits utilized by state CODIS laboratories. When taking into account the increased cost of the FTA® cards and the potential for sample failure, the FTA® buccal card is a highly inefficient means of sample collection.

Bode™ Buccal DNA Collector™

Another popular buccal collection method is the Bode™ Buccal DNA Collector™. This collector is an easy to use, non-evasive device that collects cheek cell samples in one simple step [4]. The advantage of this system is the sample is collected directly on to the cotton paper without a transfer step. Once collected, the sample is placed into a transport pouch that contains desiccant which allows for short term storage of the sample. An archival tray is available for long term storage of the Buccal DNA Collectors. During DNA analysis, the laboratory takes a small punch from the cotton paper and begins the extraction process. The Bode™ Buccal DNA Collector™ is compatible with most DNA extraction protocols used in forensic laboratories.

The main disadvantage of the Bode™ Buccal DNA Collector is the extremely high cost. However, many state CODIS laboratories use these kits to collect offender database samples. As with the FTA® cards, the cost of the Bode™ Buccal DNA Collector makes it a highly inefficient means of sample collection.

Swabs

Swabs are becoming a more popular buccal collection method among forensic and paternity testing laboratories. This non-evasive collection technique uses a cotton or polypropylene swab similar to ordinary Q-tips to collect the epithelial (cheek) cells. The three main benefits to swabs are 1) most all forensic and paternity testing laboratories already have protocols in place to process DNA from swabs, thus requiring no new validations or protocols, and 2) swabs are significantly cheaper than other DNA collection devices, making them an

attractive alternative for any DNA testing laboratory, and 3) the sample is collected directly onto the swab without the transfer step, thus producing a high level of success on the first analysis attempt. One State CODIS Laboratory reported "they get a full DNA profile on the first try, every time, no exceptions". While this may not be the case for all laboratories, these types of results are what every lab strive to achieve. Successful analysis begins with successful collection of the DNA sample.

The disadvantage of swabs is they must be allowed to air dry before being placed into an evidence envelope. If not, the wet swab will leak through the outer package, potentially contaminating other DNA samples. Most DNA collection facilities will sample multiple individuals at a time, and line up the wet swabs in drying racks before packaging. This too is another potential source of sample to sample contamination or even sample switches. The wet swabs can be placed directly into cardboard swab boxes, but these packages are bulky and require more long term storage space.

"All-in-One™" DNA Collection Kit

Prior to this grant award, the OSBI researched the advantages and disadvantages of several types of DNA collection kits. From this, a new buccal collection kit has been developed that combines the best qualities of several different collection kits into one. This "All-In-One™" DNA collection kit is simple to use, provides reliable results, all at an affordable cost. The simplicity of the kit design allows for any law enforcement agency to properly collect known reference DNA samples without any transfer steps or drying steps required. This direct DNA collection method helps to ensure that sufficient DNA is present, allowing the laboratory to obtain a full DNA profile on the first analysis attempt, thus reducing the time and cost of unnecessary re-testing. In addition, the cost of the buccal collection kit is a fraction of the cost of most kits currently used by CODIS laboratories. OSBI is currently seeking opportunities to make this new buccal collection kit available to CODIS, forensic, and paternity testing laboratories.

Improvement 2: Eliminate the Extraction Step of the DNA Analysis Process

There are many different extraction techniques that can be utilized to process DNA samples. The type of extraction technique used varies from state to state, with each technique having its own advantages and disadvantages. Regardless of which technique is being utilized, the reagents used can be very expensive, and it can take several hours to complete the extraction process. Until recently, this extraction step was necessary to prepare the samples for amplification.

DNA Extraction

Like a large number of CODIS laboratories, the OSBI currently uses Promega's DNA IQ® Kit for extraction and purification of offender DNA database samples. Punches from FTA® cards (buccal) are placed into a 96-well reaction plate (Promega's SlickPrep plate), lysed in a buffer solution at ~95°C for ~1 hour, and extracted using a magnetic resin. Using a robotics workstation to perform the extraction, the entire process takes ~2.5 hours per plate of samples (88 samples total) at the cost of ~$2.50 per sample.

Previous to the 2009 DNA Efficiency Improvement Award, the OSBI conducted research using the same DNA IQ® extraction technique on buccal swabs instead of FTA® cards. Following the same technique described above, the buccal swabs were extracted by placing the entire swab head into a 96-well reaction plate (Promega's SlickPrep plate), lysed in a buffer solution at ~95°C for ~1 hour, and extracted using a magnetic resin. Using a robotics workstation to perform the extraction, the entire process takes ~2.5 hours per plate of samples (88 samples total) at the cost of ~$2.50 per sample.

Utilizing the DNA IQ® extraction technique, both FTA® cards and buccal swabs yielded good DNA results on the first analysis attempt. In a side-by-side study of the two sample types, the buccal swab samples produced a full DNA profile (on the first analysis attempt) on 100% of the samples analyzed, compared to 89% for the FTA® samples. In addition, the buccal swab

samples showed fewer artifacts (minus A, pull-up, elevated stutter) than the FTA® samples, providing cleaner DNA profiles and easier data analysis. When combined with the significant cost savings of the collection kit, switching from FTA® cards to buccal swabs is something that should be considered by any laboratory.

Identifiler®Direct PCR Amplification Kit

Applied Biosystems™ developed the AmpFℓSTR® Identifiler® Direct PCR Amplification Kit specifically to address the DNA extraction and purification bottleneck that exists in the processing of single-source samples, including criminal DNA database samples, paternity samples, and casework reference samples.[1] The Identifiler® Direct kit amplifies the 16 loci included in the Identifiler® kit from single source samples spotted on FTA® cards, without the need to perform any DNA extraction or purification. Blood or buccal samples on FTA® cards can be punched into PCR plates or tubes, and taken directly to PCR amplification, without any loss in resulting data quality.[1] By eliminating the tedious steps involved in DNA extraction and purification, automation of the process becomes much easier, and requires a less sophisticated and less expensive robot (or simply done without robotics). While the time to result is greatly reduced, so is the potential for sample contamination or other procedural errors that can occur during the process.

Research Conducted

While the Identifiler® Direct kit offers a tremendous advantage to laboratories using FTA® cards, it is specifically designed for laboratories that utilize FTA cards, thus eliminating a large portion of laboratories in the world. With this in mind, the OSBI proposed to validate Identifiler® Direct for use on buccal swabs. Since we have already discussed some of the advantages of buccal swabs over FTA® cards, this proposed technique would provide the most cost effective means of processing known reference samples.

42 different lysing methods were attempted in this research project. Each method tested the effects of varying the lysing reagent used, lysing temperature, lysing time, lysing reagent volume, and the actual size of the swab head used to collect the DNA sample. The research eliminated several different techniques and narrowed the focus to one specific method. Samples from this method were used in a side-by-side comparison to FTA® samples. The results from this research demonstrated that, although the buccal samples showed good results from direct amplification, the FTA® showed better results. Due to the limitations of the research study, several additional buccal swab methods have been suggested for future research studies, something we highly encourage pursuit of. The FTA® samples worked very well with the Identifiler® Direct kit, with 100% of the samples tested yielding a full DNA full profile (CODIS uploadable) on the first analysis attempt.

In a recent DNA conference it was announced that Applied Biosystem's has been conducting research on a technique that would allow the Identifiler® Direct kit to be used on buccal swabs. According to the information presented at this conference, a technique has been successfully validated and is due to be released to the forensic community in the very near future.

Conclusion

Any forensic laboratory, regardless of size, can increase their efficiency and prepare to handle an increased number of sample submissions without creating a backlog. In doing so, laboratories should not solely look at purchasing more equipment and hiring more analysts, but rather re-evaluate their current procedures being used in an effort to eliminate inefficiencies.

Two key weaknesses in the current DNA analysis methods have been identified. If improved, the cost of analysis per sample and the time it takes to process these samples with drastically decrease. Whether a CODIS database sample or a known sample in a criminal case, these new methodologies provide a more efficient and cost effective means of performing DNA analysis on known reference samples. This will have a significant impact on the overall backlog of criminal cases.

Main Body

Introduction

There are many different extraction techniques that can be utilized to process DNA samples. The type of extraction technique used varies from state to state, with each technique having its own advantages and disadvantages. Regardless of which technique is being utilized, the reagents used can be very expensive, and it can take several hours to complete the extraction process. Until recently, this extraction step was necessary to prepare the samples for amplification.

Applied Biosystems™ developed the AmpFℓSTR® Identifiler® Direct PCR Amplification Kit specifically to address the DNA extraction and purification bottleneck that exists in the processing of single-source samples, including criminal DNA database samples, paternity samples, and casework reference samples.[1] The Identifiler® Direct kit amplifies the 16 loci included in the Identifiler® kit from single source samples spotted on FTA® cards, without the need to perform any DNA extraction or purification. Blood or buccal samples on FTA® cards can be punched into PCR plates or tubes, and taken directly to PCR amplification, without any loss in resulting data quality.[1] By eliminating the tedious steps involved in DNA extraction and purification, automation of the process becomes much easier, and requires a less sophisticated and less expensive robot (or simply done without robotics). While the time to result is greatly reduced, so is the potential for sample contamination or other procedural errors that can occur during the process.

While the Identifiler® Direct kit offers a tremendous advantage to laboratories using FTA® cards, it is specifically designed for laboratories that utilize FTA cards, thus eliminating a large portion of laboratories in the world. With this in mind, the OSBI proposed to validate Identifiler® Direct for use on buccal swabs. Since we have already discussed some of the advantages of buccal swabs over FTA® cards, this proposed technique would provide the most cost effective means of processing known reference samples.

Methods

FTA® cards contain chemicals that lyse cells when they come into contact with the paper, thus making amplification directly from the sample possible. Since a buccal swab does not contain these types of chemicals, an initial lysis step was performed. Once completed, the liquid lysate containing the DNA can be transferred to PCR plates or tubes, combined with the Identifiler® Direct reagents, and taken directly to PCR amplification. Although the lysing step is still involved, the extraction and purification steps are removed, thus providing a significant cost savings and greatly reducing analysis time.

When analyzing FTA® cards, the user takes a small punch of the sample and places it directly into an amplification tube or a 96-well amplification plate. The Identifiler® Direct reagents are then added and the samples are amplified on a thermalcycler. One of the problems encountered in this technique is that when the samples are placed into the tube or well, static buildup in the plastic tube/plate causes the FTA® punch to "jump around". This is especially problematic when using an open amplification plate, as samples from one well can "jump" into another well, which causes sample-to-sample contamination. To remedy this problem, the Identifiler® Direct amplification reagents can be added to the tube/plate first, followed by the addition of the FTA® punches. However, even if static is not a problem, it is very easy to inadvertently place a sample punch into an adjacent well. This technique opens the opportunity for unnecessary laboratory sample errors. The punch used must also be cleaned between samples to avoid any DNA carryover from the previous sample.

For the buccal swabs, it was decided that instead of taking a punch of the buccal swab, the entire swab head would be used. This not only prevents the static build-up problem but also allows for a more efficient means of setting up the samples. One entire 96-well plate can be setup in ~15 minutes using a whole buccal swab, as compared to ~45 minutes using FTA® punches. The entire swab head was placed into a 96-well plate (Promega's SlickPrep plate) and the swab stick was broken off at the swab end (snapped off). The preferred lysing solution is then added to each well, and the samples are incubated in a water bath at various

temperatures for varying amounts of time. One of the benefits of using an entire swab head is the small amount of time it takes to setup an entire plate. In addition, the problem of inadvertently placing the sample into the wrong well is averted, because it is impossible to fit two swab heads into the same well. Finally, the entire swab head is being used instead of a cutting or punch, the sample punch does not need to be cleaned between samples, saving time and averting potential sample-to-sample contamination.

For studies 1 through 5, each method analyzed four buccal and one reagent blank. The buccal swabs were collected from volunteers whose DNA profile was already known. Following each specific lysing method, the samples were quantitated using the Applied Biosystems™ Quantifiler Human DNA Quantitation Kit and a 7500 Real Time PCR instrument to determine the overall yield. Although direct amplification eliminates the quantitation step of the DNA analysis process, this information was used to help determine which lysing methods provided the best DNA yield, and to identify any potential inhibitors that may be present. Varying amounts of lysate (ranging from 1.0µl to 5.0µl) were transferred to a 1.5ml amplification tube, followed by the addition of 25µl of the Identifiler® Direct amplification reagents. PCR amplification was performed on a 9700 thermalcycler at 28 cycles, following the same thermalcycler conditions as outlined in the cycle number study (see Table 1). Following amplification, the samples were prepared for genetic analysis by combining 1µl of amplified DNA template to 9µl of a formamide/size standard mixture, and injected at varying times (either 5 or 10 seconds) on a 3130 Genetic Analyzer (4 capillary).

After each study was conducted, the data from each method tested was calculated and compared. If any method did not produce the desired results, modifications to any of the varying parameters listed above were made, and additional studies were preformed. Once (and if) a desired method was obtained, a side-by-side comparison would be made between the buccal swab method and the FTA card method. The following is a summary of the results obtained in this research study.

Results

Cycle Number Study

As recommended by Applied Biosystems™, the first step was to conduct a brief sensitivity study to determine the optimal PCR cycle number for use with the Identifiler® Direct kit. 26 previously profiled offender database samples (buccal on FTA) were used for this study. Following the Identifiler® Direct kit procedures, a single 1.2mm punch was taken from each FTA® sample and placed into a 96-well PCR plate. This process was repeated for a total of 3 plates, one for each amplification cycle to be tested. 25µl of the Identifiler® Direct Reaction Mix were added to the sample wells, and each of the three plates were amplified using a different cycle number (26, 27, and 28 cycles) to determine the optimum for use. PCR amplification was performed on a dual well 9700 thermalcycler following the recommended PCR cycling conditions as outlined in Table 1. The samples were prepared for genetic analysis by combining 1µl of amplified DNA template to 9µl of a formamide/size standard mixture. All three plates were injected for 5 seconds on 3730 DNA Analyzer (48 capillary).

Table 1: PCR Cycling Conditions

Initial Incubation Step	**Cycle (26, 27, or 28 cycles)**			**Final Extension**	**Final Hold**
	Denature	**Anneal**	**Extend**		
Hold	Cycle			Hold	Hold
95°C	94°C	59°C	72°C	60°C	4°C
11 minutes	20 seconds	2 minutes	1 minute	25 minutes	∞

The optimal PCR cycle number should generate profiles with heterozygous peak heights of ~1,000 to 3000 rfu, with minimal occurrences of artifacts (minus A, excessive stutter, etc.) or allelic drop-out events. Applied Biosystems™ notes that since unpurified samples are being amplified, a greater variation in peak height from sample to sample is expected (as compared to purified samples). The results from all three plates were calculated and compared. No artifacts were observed on any sample, and full profiles were obtained at 26, 27, and 28 cycles. The average heterozygous peak height ratios were 2012 rfu at 26 cycles, 2329 rfu at 27 cycles, and 4120 rfu at 28 cycles.

The results of the cycle number study demonstrated that the Identifiler® Direct amplification kit works as expected on FTA® samples. Although the average heterozygous rfu value was higher than desired at 28 cycles, this was the optimal PCR cycle number selected for the remainder of the research study. This would ensure the maximum amount of DNA data would be obtained from the swab samples. Now that the optimal PCR cycle number had been determined, the next step was to identify the optimal lysing method that would allow for direct amplification of swabs.

Study 1

With little to no research found that discusses using the Identifiler® Direct amplification kit on cotton swabs, multiple variables and analysis techniques needed to be explored. In study 1 we evaluated the effects of using different lysing reagents, different volumes for the lysing reagents, different lysing temperatures, and different lysing times. Although many different variables are being tested at one time, the intent behind this initial study was to identify a trend in the data to help narrow the focus of the research for future studies conducted. Ten different lysing methods were evaluated in study 1 (see Table 2). All samples were amplified using 2.5µl of sample lysate, and were injected on the 3130 Genetic analyzer once at 5 seconds and once at 10 seconds. No method tested produced the desired results; however, the study eliminated several different techniques and narrowed the scope of the research for future studies conducted.

Table 2: Lysing methods used for Study 1

Method	Swab Head Size	Lysing Solution	Lysis Solution Volume (µl)	Lysing Temp. (°C)	Lysing Time (minutes)
1	Large	DNA IQ® Lysis Buffer	400	70	15
2	Large	Stain Extraction Buffer	400	70	15
3	Large	Stain Extraction Buffer + ProK	400 + 10	70	15
4	Large	$T.E.^{-4}$	400	70	15
5	Large	$T.E.^{-4}$	400	70	30
6	Large	$T.E.^{-4}$	400	90	15
7	Large	H_2O	400	70	30
8	Large	H_2O	400	90	15
9	Large	$T.E.^{-4}$	300	70	15
10	Large	$T.E.^{-4}$	200	70	15

Method 1

The samples lysed with the DNA IQ® Lysis Buffer showed no results. One of the major ingredients in the DNA IQ® Lysis Buffer is a chemical called guanidine. Guanidine has been found to be a major source of inhibition in upstream PCR amplification. Due to this, the DNA IQ® protocol includes at least three mandatory wash steps to rid the extract of all traces of guanidine. After discussions with the Promega Corporation, they confirmed that they would fully expect complete inhibition to occur with even just 1µl of the lysis buffer added to the PCR reaction, resulting in no amplification. No additional studies were conducted using DNA IQ® Lysis Buffer.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 1	1	Inhibited	-	No data	-	-	-
				No data	-	-	-
	2	Inhibited	-	Bad injection	-	-	-
				No data	-	-	-
	3	Inhibited	-	No data	-	-	-
				No data	-	-	-
	4	Inhibited	-	No data	-	-	-
				No data	-	-	-
Average:		-	-		-	-	-

Method 2

The samples lysed with the Stain Extraction Buffer (SEB) showed no results. The Dithiotreitol (DTT) in the SEB is suspected to be inhibiting the amplification. The SEB concentration used is 2% which should be tolerable according to Applied Biosystems™. Additional testing with SEB was conducted in study 2.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 2	1	Inhibited	-	No data	-	-	-
				No data	-	-	-
	2	Inhibited	-	No data	-	-	-
				No data	-	-	-
	3	Inhibited	-	No data	-	-	-
				No data	-	-	-
	4	Inhibited	-	No data	-	-	-
				No data	-	-	-
Average:		-	-		-	-	-

Method 3

The samples lysed with SEB/ProK showed no results. The DTT and/or the ProK are suspected to be inhibiting the amplification. The SEB concentration used is 2% which should be tolerable according to Applied Biosystems™. Additional testing with SEB was conducted in study 2.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 3	1	Inhibited	-	No data	-	-	-
				No data	-	-	-
	2	Inhibited	-	No data	-	-	-
				No data	-	-	-
	3	Inhibited	-	No data	-	-	-
				No data	-	-	-
	4	Inhibited	-	No data	-	-	-
				No data	-	-	-
Average:		-	-			-	-

Method 4

The samples lysed with T.E.$^{-4}$ showed some potential. Although all samples contained dropout, there were good allele peaks present both above and below the 100 rfu threshold. Since this method showed some potential, it was explored further in later studies. However, based on the low quantitation results it was apparent that additional modifications needed to be made to the lysis solution volume, lysing temperature, and/or the lysing time.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 4	1	0.134	0.335	Dropout x12 loci	-	174	-
				Dropout x8 loci	-	205	78.70%
	2	0.065	0.162	Dropout x12 loci	-	116	-
				Dropout x8 loci	-	155	85.31%
	3	0.278	0.070	No data (peaks below threshold)	-	-	-
				Dropout x14 loci	-	121	-
	4	0.088	0.219	No data (peaks below threshold)	-	-	-
				Dropout x15 loci	-	101	-
Average:		**0.079**	**0.196**		**-**	**145**	**82.01%**

Method 5

Although the samples in method 5 were lysed 15 minutes longer than in study 5 (at the same temperature), the quantitation yield was half as much. This can be attributed to either pipetting errors during quantitation, or varying amounts of DNA present on the swabs used in each study. Although some DNA peaks were observed, it was apparent that additional modifications needed to be made to the lysis solution volume, lysing temperature, and/or the lysing time.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 5	1	0.006	0.016	No data	-	-	-
				No data	-	-	-
	2	0.052	0.131	Dropout x12 loci	-	142	-
				Dropout x10 loci	-	166	99.04%
	3	0.063	0.157	Dropout x14 loci	-	129	84.68%
				Dropout x13 loci	-	155	84.03%
	4	0.004	0.010	No data	-	-	-
				No data	-	-	-
Average:		**0.031**	**0.078**		**-**	**148**	**89.25%**

Method 6

Method 6 again used T.E.$^{-4}$ as the lysing reagent, but the lysing temperature was increased to 90°C. None of the samples showed peaks above the detection threshold. This study proved that increasing the lysing temperature alone was not sufficient.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 6	1	0.004	0.010	No data	-	-	-
				No data	-	-	-
	2	0.003	0.009	No data (peaks below threshold)	-	-	-
				No data (peaks below threshold)	-	-	-
	3	0.017	0.042	No data (peaks below threshold)	-	-	-
				Dropout x15 loci	-	114	-
	4	0.001	0.004	No data	-	-	-
				No data	-	-	-
Average:		**0.006**	**0.016**		**-**	**114**	**-**

Method 7

The samples lysed with ultrapure de-ionized water showed no results. It does not appear this is a good lysis reagent under the conditions used.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 7	1	undetected	-	No data	-	-	-
				No data	-	-	-
	2	0.001	0.002	No data	-	-	-
				No data	-	-	-
	3	0.010	0.025	No data	-	-	-
				No data	-	-	-
	4	0.002	0.004	No data	-	-	-
				No data	-	-	-
Average:		**0.004**	**0.010**		**-**	**-**	**-**

Method 8

The samples lysed with ultrapure de-ionized water showed no results. It does not appear this is a good lysis reagent under the conditions used.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 8	1	0.002	0.005	No data	-	-	-
				No data	-	-	-
	2	0.003	0.007	No data (peaks below threshold)	-	-	-
				No data (peaks below threshold)	-	-	-
	3	0.009	0.022	No data (peaks below threshold)	-	-	-
				No data (peaks below threshold)	-	-	-
	4	0.003	0.008	No data	-	-	-
				No data	-	-	-
Average:		**0.004**	**0.010**		**-**	**-**	**-**

Method 9

There was a wide range in the quantitation results of the four samples, which ultimately had a significant impact in the overall DNA profile obtained. Only two of the four samples showed peaks above the peak detection threshold, which indicated the method still needed to be modified.

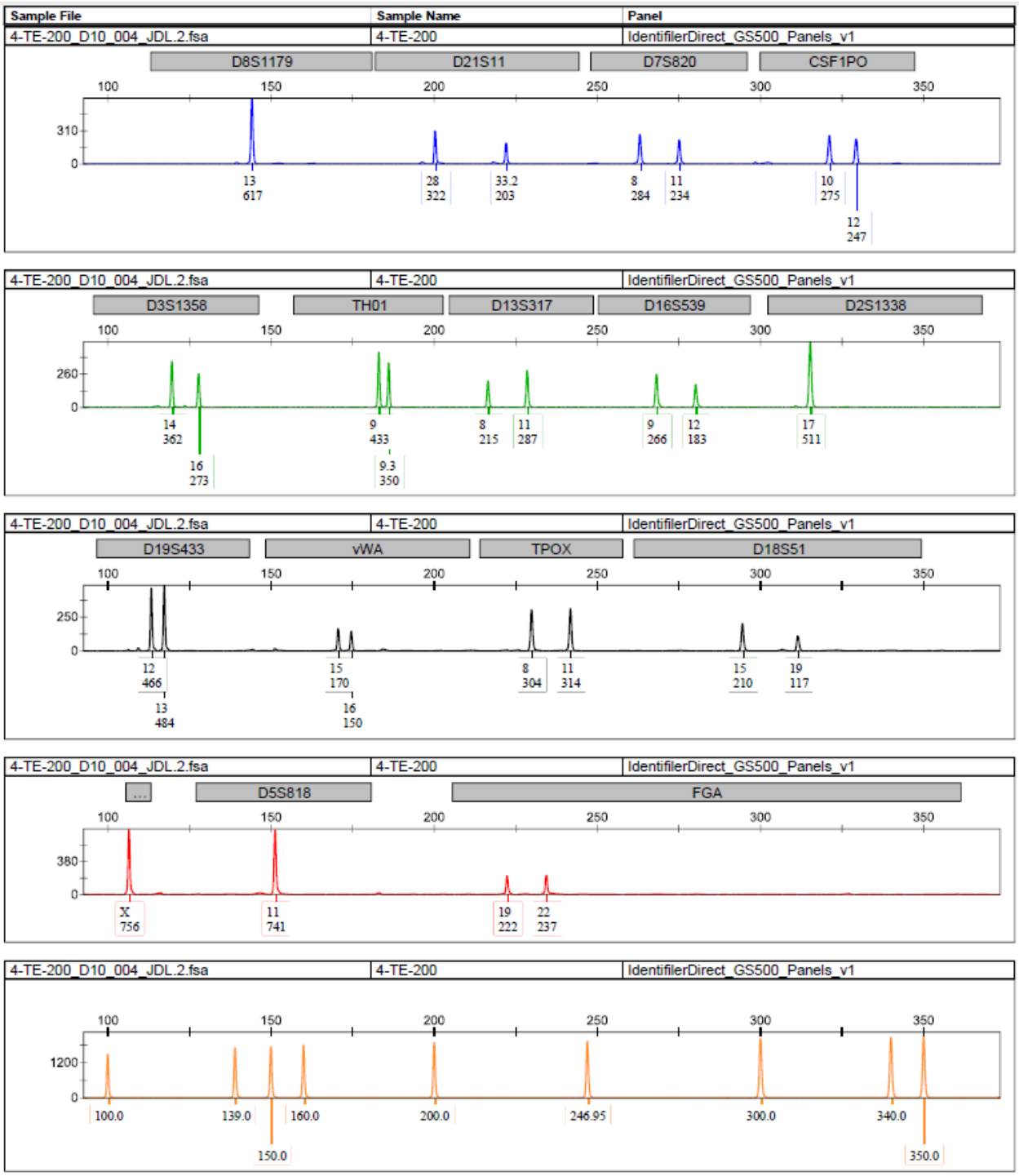
	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 9	1	0.090	0.224	Dropout x8 loci	-	174	88.48%
				Dropout x3 loci	-	254	87.34%
	2	0.025	0.061	No data	-	-	-
				No data (peaks below threshold)	-	-	-
	3	0.045	0.112	Bad Injection	-	-	-
				Bad Injection	-	-	-
	4	0.052	0.131	Dropout x11 loci	-	136	92.29%
				Dropout x7 loci	-	179	87.50%
Average:		**0.053**	**0.132**			**186**	**88.90%**

Method 10

Once again, the wide range in the quantitation results of the four samples ultimately had a significant impact in the overall DNA profiles obtained. Three of the four samples showed peaks above the peak detection threshold, with one sample providing a full DNA profile. This proves that with the right amount of DNA template, the direct amplification from buccal swabs is possible. However, it is apparent that a lysing method needs to be developed that produces a more consistent yield of DNA from sample to sample. This will be explored in future studies.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 10	1	0.085	0.213	Dropout x10 loci	-	172	84.70%
				Dropout x5 loci	-	217	84.29%
	2	0.012	0.029	No data	-	-	-
				No data (peaks below threshold)	-	-	-
	3	0.032	0.081	Dropout x15 loci	-	121	-
				Dropout x15 loci	-	117	-
	4	0.126	0.315	Dropout x1 locus	-	226	82.82%
				OK	-	330	80.49%
Average:		**0.064**	**0.160**			**197**	**83.08%**

Method 10, Sample #4, Injection 2 of 2 (10 seconds)



Study 2

In study 1 we demonstrated that direct amplification from a swab was possible, but the optimal lysing technique had not been obtained. In study 2 we continued to explore different lysing techniques by modifying the lysing reagents, lysing reagent volumes, lysing temperatures, and lysing times. Eleven different lysing methods were tested for study 2 (see Table 3). All samples were amplified using 2.5µl of sample lysate, and were injected on the 3130 Genetic analyzer twice at 10 seconds.

Table 3: Lysing methods used for Study 2

Method	Swab Head Size	Lysing Solution	Lysis Solution Volume (µl)	Lysing Temp. (°C)	Lysing Time (minutes)
11	Large	T.E.$^{-4}$	200	70	60
12	Large	T.E.$^{-4}$	200	95	15
13	Large	T.E.$^{-4}$	200	95	60
14	Large	T.E.$^{-4}$	300	70	60
15	Large	T.E.$^{-4}$	300	95	15
16	Large	T.E.$^{-4}$	300	95	60
17	Large	T.E.$^{-4}$	400	70	60
18	Large	T.E.$^{-4}$	400	95	60
19	Large	Stain Extraction Buffer (no DTT)	400	95	60
20	Large	Stain Extraction Buffer (no DTT)	300	95	60
21	Large	Stain Extraction Buffer (no DTT)	200	95	60

Results obtained from study 2 eliminated several more techniques, and narrowed the scope of the research even further. Data showed that, for maximum yield, 95°C appears to be the optimal lysing temperature and 60 minutes appears to be the optimum lysing time. This lysing combination was used for the remainder of the research. However, the preferred lysing reagent volume is still unclear. Methods 13, 16, and 18 produced full profiles but had varying ranges in peak heights, peak height ratios, artifacts, and amount of preferential amplification. As discussed in Study 1, the wide range in the quantitation results observed between samples ultimately had a significant impact in the overall DNA profiles obtained. A method still needs to be developed that produces a more consistent yield of DNA from sample to sample. This will be explored further in study 3.

Method 11

The wide range in the quantitation results of the four samples ultimately had a significant impact in the overall DNA profiles obtained. Three of the four samples showed peaks above the peak detection threshold, with one sample providing a full DNA profile (along with a significant amount of pull-up and split-peaks). Additional methods still need to be explored.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 11	1	0.228	0.570	Bad peak morphology	Yes	7031	95.15%
				Bad peak morphology	Yes	6786	95.81%
	2	0.024	0.061	No data (peaks below threshold)	-	-	-
				No data (peaks below threshold)	-	-	-
	3	0.107	0.268	Dropout x1 locus	-	944	85.23%
				Dropout x1 locus	-	811	84.17%
	4	0.096	0.241	Dropout x15 loci	-	160	-
				Dropout x15 loci	-	150	-
Average:		**0.114**	**0.285**			**2647**	**90.09%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 12

The wide range in the quantitation results of the four samples ultimately had a significant impact in the overall DNA profiles obtained. Three of the four samples showed peaks above the peak detection threshold, but no sample provided a full DNA profile. Additional methods still need to be explored.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 12	1	0.012	0.029	Dropout x14 loci	-	130	72.25%
				Dropout x15 loci	-	135	78.62%
	2	0.006	0.016	Dropout x14 loci	-	154	68.59%
				Dropout x15 loci	-	160	-
	3	0.020	0.051	Dropout x11 loci	-	456	85.74%
				Dropout x11 loci	-	419	87.67%
	4	0.005	0.012	No data	-	-	-
				No data	-	-	-
Average:		**0.011**	**0.027**			**242**	**78.57%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 13

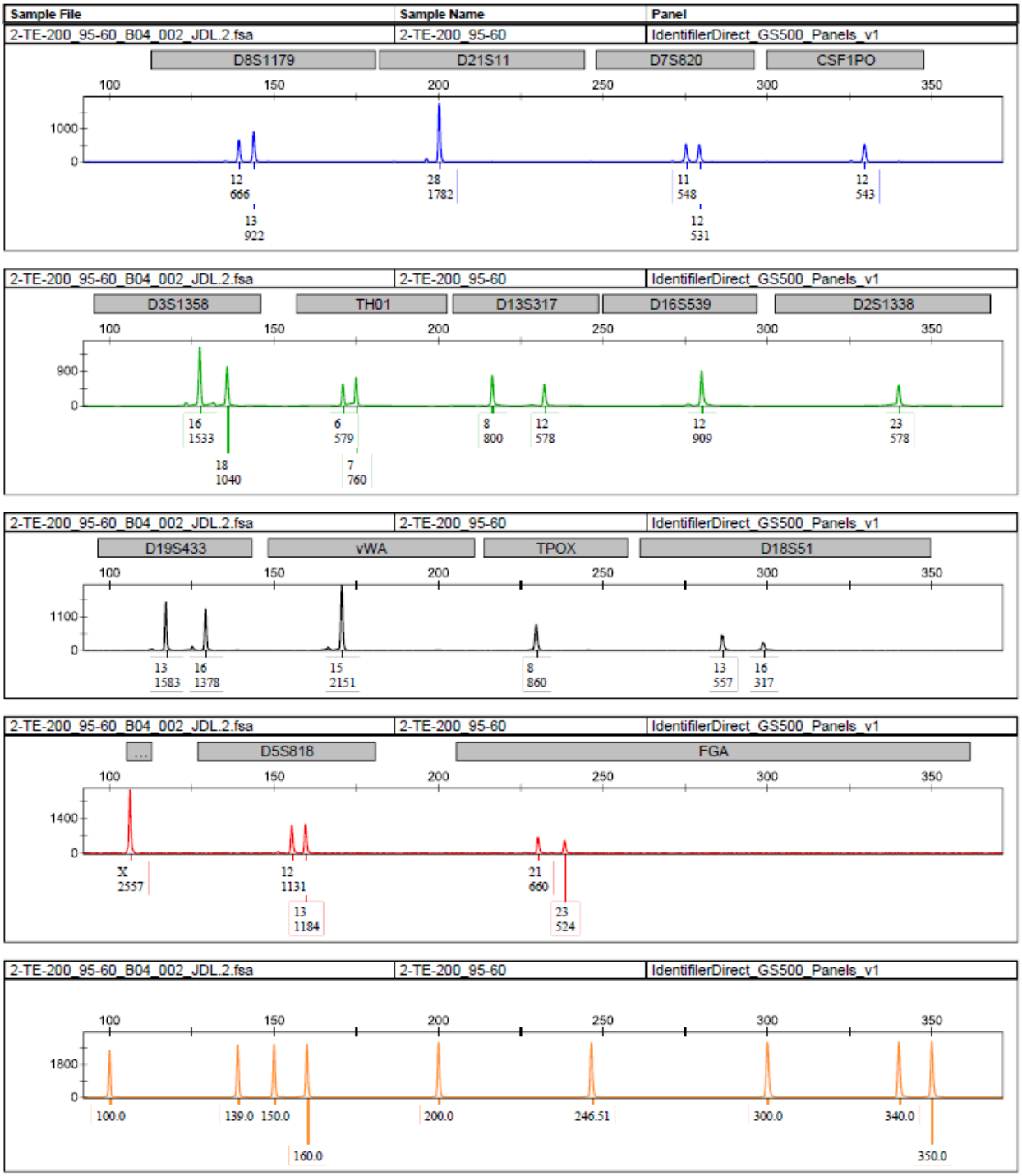
All four samples produced a full DNA profile, the best results obtained thus far. One of the samples had peak heights above the detection limit of the 3130, which caused artifacts to be observed. However, this same sample injected on a 3730 DNA analyzer would more than likely produce a clean DNA profile. This method will be explored further in future studies.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 13	1	0.318	0.795	OK	-	2634	84.17%
				OK	-	2104	83.50%
	2	0.039	0.099	OK	-	1068	78.27%
				OK	-	987	78.25%
	3	0.415	1.038	OK	Yes	3927	86.17%
				OK	Yes	3785	86.47%
	4	0.168	0.420	Unexplained peak	Yes	6340	89.37%
				Unexplained peak	Yes	5925	91.11%
Average:		**0.235**	**0.588**		**-**	**3346**	**84.66%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 13, Sample #2, Injection 2 of 2 (10 seconds)



Method 14

Only one sample produced a full DNA profile, but the peaks were so far off scale that many artifacts were also present. The remaining three samples had some peaks present as well, but did not produce a full DNA profile.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 14	1	0.058	0.145	Dropout x1 locus	-	1373	83.55%
				Dropout x1 locus	-	1293	84.49%
	2	0.032	0.080	Dropout x 12 loci	-	146	94.77%
				Dropout x12 loci	-	133	89.77%
	3	0.153	0.383	Dropout x11 loci	-	238	73.62%
				Dropout x11 loci	-	217	73.62%
	4	0.130	0.325	Unexplained peak	Yes	6193	87.39%
				Unexplained peak	Yes	6010	88.77%
Average:		**0.093**	**0.233**			**1950**	**84.50%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 15

Some samples had peaks above the peak detection threshold, but no sample produced a full DNA profile. When looking at the low quantitation values for these 4 samples, these results are expected.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 15	1	0.027	0.068	Dropout x13 loci	-	225	75.26%
				Dropout x13 loci	-	228	78.07%
	2	0.002	0.004	Dropout x15 loci	-	124	-
				Dropout x15 loci	-	110	-
	3	0.038	0.096	Dropout x14 loci	-	126	88.98%
				Dropout x14 loci	-	119	97.22%
	4	0.007	0.018	No data	-	-	-
				No data	-	-	-
Average:		**0.019**	**0.046**			**155**	**84.88%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 16

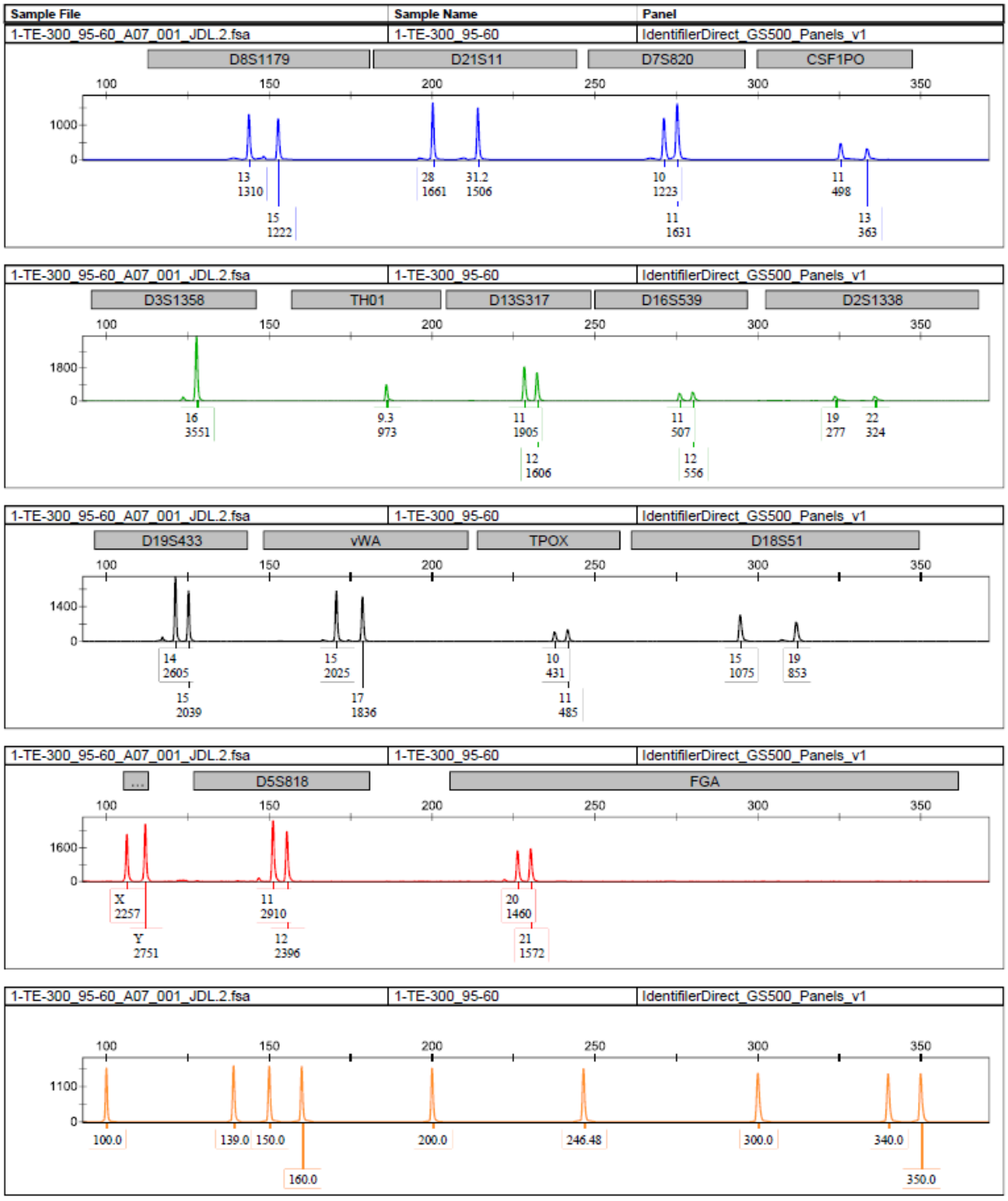
Three of the four samples produced a full DNA profile, and the one remaining sample had dropout at only one locus. The peak heights were the best overall observed thus far, keeping the samples free from artifacts. This method will be explored further in future studies.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 16	1	0.119	0.298	OK	-	1697	85.29%
				OK	-	1460	84.80%
	2	0.160	0.400	OK	-	1547	89.30%
				OK	-	1556	89.43%
	3	0.184	0.460	Dropout x1 locus	-	1064	88.16%
				Dropout x1 locus	-	974	87.32%
	4	0.051	0.127	OK	-	1233	83.71%
				Dropout x2 loci	-	1077	85.42%
Average:		**0.128**	**0.321**			**1326**	**86.68%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 16, Sample #1, Injection 2 of 2 (10 seconds)



Method 17

Only one sample produced a full DNA profile, but due to the data being off scale, artifacts (pull-up) were also present. Quantitation results for the remaining three samples were very low, which explains why they did not provide a full DNA profile.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 17	1	0.120	0.300	OK	Yes	4510	90.07%
				OK	Yes	5164	91.62%
	2	0.018	0.045	No data	-	-	-
				No data	-	-	-
	3	0.137	0.343	Dropout x13 loci	-	187	92.46%
				Dropout x13 loci	-	190	89.25%
	4	0.074	0.186	No data	-	-	-
				No data	-	-	-
Average:		**0.087**	**0.218**			**2513**	**90.85%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 18

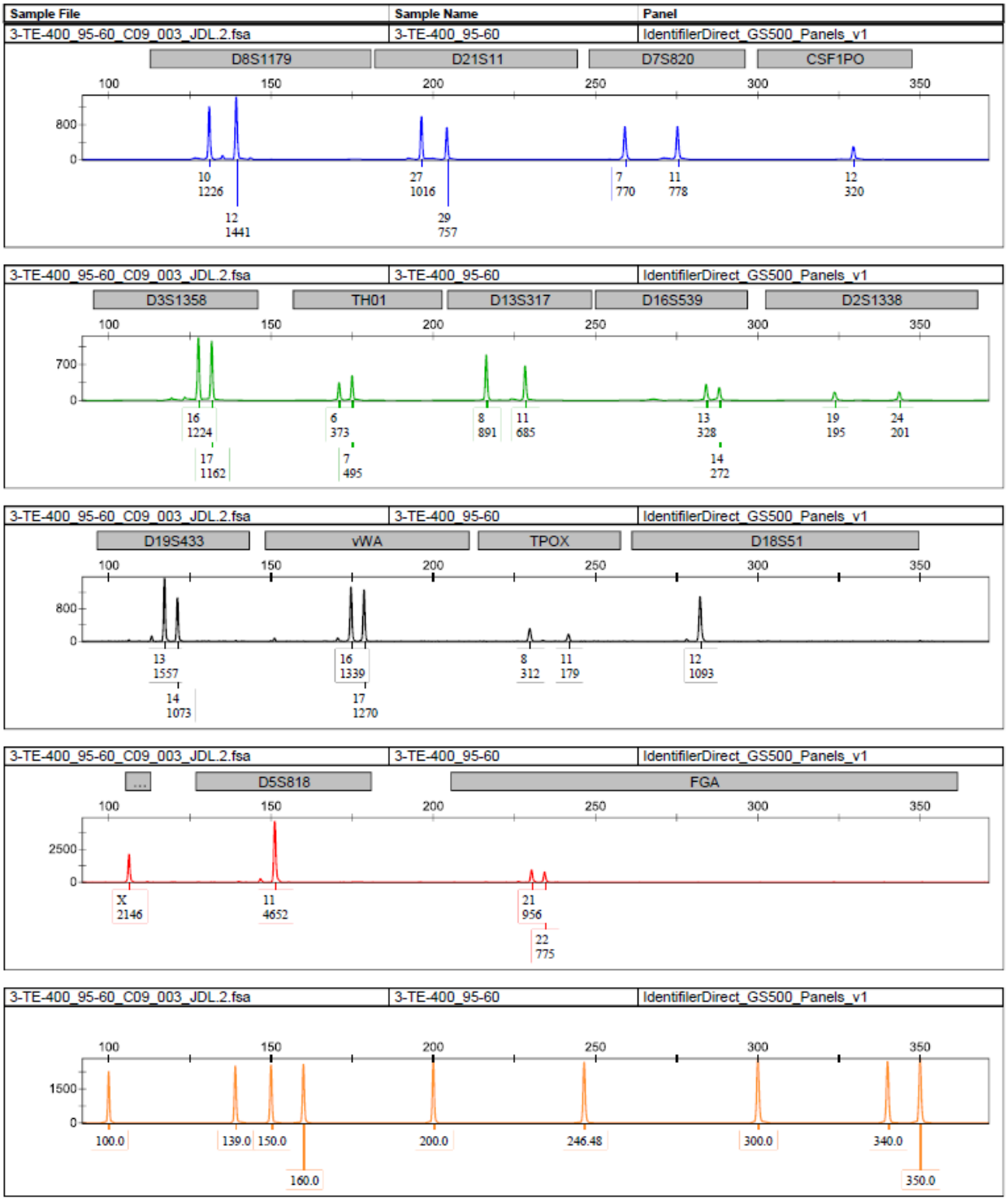
All four samples produced a full DNA profile. One of the samples had peak heights above the detection limit of the 3130, which caused artifacts to be observed. However, this same sample injected on a 3730 DNA analyzer would more than likely produce a clean DNA profile. This method will be explored further in future studies.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 18	1	0.101	0.253	OK	-	1552	84.40%
				OK	-	1363	85.10%
	2	0.017	0.042	OK	-	518	80.33%
				OK	-	518	80.13%
	3	0.168	0.420	OK	-	976	82.39%
				OK	-	982	82.32%
	4	undetected	-	Unexplained peak	Yes	6168	92.50%
				Unexplained peak	Yes	6016	92.44%
Average:		**0.095**	**0.238**			**2262**	**84.95%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 18, Sample #3, Injection 2 of 2 (10 seconds)



Method 19

In methods 3 and 4, it was expected that the DTT present was inhibiting the amplification. The SEB concentration used is 2% which should be tolerable according to Applied Biosystems™. To verify this theory, method 19 used a lysing reagent of SEB with no DTT. None of the samples had peaks present, indicating a complete amplification failure. This proves the SEB is causing inhibition in the amplification.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 19	1	undetected	-	No data	-	-	-
				No data	-	-	-
	2	undetected	-	No data	-	-	-
				No data	-	-	-
	3	undetected	-	No data	-	-	-
				No data	-	-	-
	4	undetected	-	No data	-	-	-
				No data	-	-	-
Average:		-	-			-	-

Method 20

Method 20 used the same lysing combinations as in method 19, but slightly increased the volume of lysing reagent. None of the samples had peaks present, indicating a complete amplification failure. This proves the SEB is causing inhibition in the amplification.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 20	1	undetected	-	No data	-	-	-
				No data	-	-	-
	2	undetected	-	No data	-	-	-
				No data	-	-	-
	3	undetected	-	No data	-	-	-
				No data	-	-	-
	4	undetected	-	No data	-	-	-
				No data	-	-	-
Average:			-			-	-

Method 21

Method 20 used the same lysing combinations as in method 19, but with a full volume of lysing reagent. None of the samples had peaks present, indicating a complete amplification failure. This proves the SEB is causing inhibition in the amplification.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 21	1	undetected	-	No data	-	-	-
				No data	-	-	-
	2	undetected	-	No data	-	-	-
				No data	-	-	-
	3	undetected	-	No data	-	-	-
				No data	-	-	-
	4	undetected	-	No data	-	-	-
				No data	-	-	-
Average:			**-**			**-**	**-**

Study 3

Study 2 demonstrated that samples lysed in T.E.$^{-4}$ could produce a full DNA profile. Acceptable results were obtained for 200ul, 300ul, and 400ul volumes (methods 13, 16, and 18 respectively). Although good results were obtained, the variance in the peak heights between samples was broader than desired. It is believed this is due to the varying amounts of DNA present when a whole swab head (large size) is used.

When analyzing FTA® cards, a standard 1.2mm size punch is used, which creates a more narrow range of DNA that can be amplified (due to a limited surface area of the punch taken). This in turn will produce a more consistent range of peak heights among all samples. For swabs, this same principle can be applied by either taking a punch of the swab head (not explored in this research study), or by using a swab with a smaller size head. The smaller swab head will have a smaller surface area (similar to a 1.2mm FTA® punch) and, in theory, should narrow the range of DNA that is lysed.

In study 3, two different sizes of swabs heads were used (small and medium). Reagent volumes of 200 through 400µl were used to continue to identify which produces a higher DNA yield (see Table 4). All samples were amplified using 2.5µl of sample lysate, and were injected on the 3130 Genetic analyzer twice at 10 seconds.

Table 4: Lysing methods used for Study 3

Method	Swab Head Size	Lysing Solution	Lysis Solution Volume (µl)	Lysing Temp. (°C)	Lysing Time (minutes)
22	Medium	T.E.$^{-4}$	200	95	60
23	Medium	T.E.$^{-4}$	300	95	60
24	Medium	T.E.$^{-4}$	400	95	60
25	Small	T.E.$^{-4}$	200	95	60
26	Small	T.E.$^{-4}$	300	95	60
27	Small	T.E.$^{-4}$	400	95	60

Method 22

One sample produced a full DNA profile, and the remaining three samples had dropout. This is to be expected when evaluating the quantitation results. The large swab head used in method 13 (lysed under the same conditions as method 22) yielded a significantly higher amount of amplifiable DNA (0.235ng/µl average verses 0.016ng/µl average). While the reduced surface area of the medium sized swab head was expected to produce a lower quantitation result, it was not expected to be this drastic. In addition, the reduced surface area of the medium size swab head did little to reduce the broad range of amplifiable DNA observed between samples.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 22	1	0.005	0.011	Dropout x15 loci	-	113	-
				Dropout x16 loci	-	106	-
	2	0.020	0.050	Dropout x1 locus	-	347	76.78%
				Dropout x1 locus	-	293	78.30%
	3	0.007	0.018	Dropout x14 loci	-	165	-
				Dropout x14 loci	-	167	-
	4	0.032	0.080	OK	-	341	83.75%
				Dropout x1 locus	-	345	83.58%
Average:		**0.016**	**0.040**			**235**	**80.60%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 23

One sample produced a full DNA profile, and the remaining three samples had dropout. This is to be expected when evaluating the quantitation results. The large swab head used in method 16 (lysed under the same conditions as method 23) yielded a significantly higher amount of amplifiable DNA (0.128ng/µl average verses 0.028ng/µl average). While the reduced surface area of the medium sized swab head was expected to produce a lower quantitation result, it was not expected to be this drastic. In addition, the reduced surface area of the medium size swab head did little to reduce the broad range of amplifiable DNA observed between samples.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 23	1	0.004	0.011	Dropout x13 loci	-	132	84.33%
				Dropout x13 loci	-	124	80.79%
	2	0.052	0.130	OK	-	317	81.56%
				Dropout x2 loci	-	286	80.52%
	3	0.028	0.070	Dropout x3 loci	-	270	82.52%
				Dropout x4 loci	-	262	83.46%
	4	0.029	0.073	Dropout x3 loci	-	308	76.68%
				Dropout x3 loci	-	301	76.34%
Average:		**0.028**	**0.071**			**250**	**80.78%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 24

Three samples had dropout and one sample showed no data present. This is to be expected when evaluating the quantitation results. The large swab head used in method 18 (lysed under the same conditions as method 24) yielded a significantly higher amount of amplifiable DNA (0.095ng/µl average verses 0.037ng/µl average). While the reduced surface area of the medium sized swab head was expected to produce a lower quantitation result, it was not expected to be this drastic. In addition, the reduced surface area of the medium size swab head did little to reduce the broad range of amplifiable DNA observed between samples.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 24	1	0.005	0.013	No data	-	-	-
				No data	-	-	-
	2	0.088	0.220	Dropout x9 loci	-	196	76.86%
				Dropout x10 loci	-	192	75.43%
	3	0.012	0.029	Dropout x14 loci	-	144	-
				Dropout x14 loci	-	147	-
	4	0.041	0.103	Dropout x1 locus	-	327	83.45%
				Dropout x1 locus	-	306	83.56%
Average:		**0.037**	**0.091**			**219**	**79.83%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 25

All four samples had dropout. This is to be expected when evaluating the quantitation results. The large swab head used in method 13 and the medium swab head used in method 22 (lysed under the same conditions as method 25) both yielded a higher amount of amplifiable DNA (0.235ng/µl average verses 0.016ng/µl average verses 0.006ng/µl average). Similar to the medium sized swab head, while the reduced surface area of the small sized swab head was expected to produce a lower quantitation result, it was not expected to be this drastic. In addition, the reduced surface area of the small size swab head did little to reduce the broad range of amplifiable DNA observed between samples.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 25	1	0.003	0.008	Dropout x12 loci	-	132	91.10%
				Dropout x13 loci	-	125	89.08%
	2	0.009	0.022	Dropout x5 loci	-	300	88.66%
				Dropout x5 loci	-	284	88.08%
	3	0.006	0.014	Dropout x15 loci	-	159	-
				Dropout x15 loci	-	132	-
	4	0.008	0.021	Dropout x7 loci	-	156	81.66%
				Dropout x6 loci	-	150	74.34%
Average:		**0.006**	**0.016**			**180**	**85.49%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 26

One sample produced a full DNA profile, and the remaining three samples had dropout. This is to be expected when evaluating the quantitation results. The large swab head used in method 16 and the medium swab head used in method 23 (lysed under the same conditions as method 26) both yielded a higher amount of amplifiable DNA (0.128ng/µl average verses 0.028ng/µl average verses 0.032ng/µl average). Similar to the medium sized swab head, while the reduced surface area of the small sized swab head was expected to produce a lower quantitation result, it was not expected to be this drastic. In addition, the reduced surface area of the small size swab head did little to reduce the broad range of amplifiable DNA observed between samples.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 26	1	0.006	0.014	Dropout x7 loci	-	165	80.98%
				Dropout x8 loci	-	153	86.56%
	2	0.092	0.230	OK	-	628	82.72%
				Dropout x1 locus	-	547	83.94%
	3	0.017	0.042	Dropout x14 loci	-	163	-
				Dropout x14 loci	-	164	-
	4	0.012	0.031	Dropout x5 loci	-	203	81.00%
				Dropout x5 loci	-	193	81.25%
Average:		**0.032**	**0.079**			**277**	**82.74%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 27

Two samples had dropout and two samples had no data present. This is to be expected when evaluating the quantitation results. The large swab head used in method 18 and the medium swab head used in method 24 (lysed under the same conditions as method 27) both yielded a higher amount of amplifiable DNA (0.095ng/µl average verses 0.037ng/µl average verses 0.020ng/µl average). Similar to the medium sized swab head, while the reduced surface area of the small sized swab head was expected to produce a lower quantitation result, it was not expected to be this drastic. In addition, the reduced surface area of the small size swab head did little to reduce the broad range of amplifiable DNA observed between samples.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 27	1	0.027	0.067	No data (peaks below threshold)	-	-	-
				No data (peaks below threshold)	-	-	-
	2	0.026	0.064	Dropout x15 loci	-	116	-
				Dropout x15 loci	-	124	-
	3	0.014	0.036	No data	-	-	-
				No data	-	-	-
	4	0.014	0.034	Dropout x10 loci	-	147	72.88%
				Dropout x10 loci	-	146	79.60%
Average:		**0.020**	**0.050**			**133**	**76.24%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Study 4

In study 4 we evaluated the effects of decreasing the amount of DNA template used during the amplification step. Personal experience has demonstrated that sometimes using less DNA template can greatly help reduce preferential amplification, thus producing cleaner, more balanced profiles. 9 different lysing methods were used for study 4 (see Table 5). Four samples were analyzed for each method tested. All samples were amplified using 1.0µl of sample lysate (instead of the 2.5µl of sample lysate used in studies 1, 2 and 3) and were injected on the 3130 Genetic analyzer twice at 10 seconds.

Table 5: Lysing methods used for Study 4

Method	Swab Head Size	Lysing Solution	Lysis Solution Volume (µl)	Lysing Temp. (°C)	Lysing Time (minutes)
13b	Large	T.E.$^{-4}$	200	95	60
16b	Large	T.E.$^{-4}$	300	95	60
18b	Large	T.E.$^{-4}$	400	95	60
22b	Medium	T.E.$^{-4}$	200	95	60
23b	Medium	T.E.$^{-4}$	300	95	60
24b	Medium	T.E.$^{-4}$	400	95	60
25b	Small	T.E.$^{-4}$	200	95	60
26b	Small	T.E.$^{-4}$	300	95	60
27b	Small	T.E.$^{-4}$	400	95	60

Method 13b

Sample lysate from method 13 was used for this study. Although an amplification target range of 0.235ng average was used in this study, only 1 sample produced a full DNA profile. These results are not as good as the results from method 13 which used 2.5µl of sample lysate for amplification.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 13b	1	0.318	0.318	Dropout x5 loci	-	373	85.73%
				Dropout x6 loci	-	334	87.09%
	2	0.040	0.039	No data (peaks below threshold)	-	-	-
				No data (peaks below threshold)	-	-	-
	3	0.415	0.415	Unexplained peak	Yes	891	88.33%
				OK	-	797	89.41%
	4	0.168	0.168	Dropout x4 loci	-	615	81.53%
				Dropout x4 loci	-	614	80.59%
Average:		**0.235**	**0.235**			**604**	**85.45%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 16b

Sample lysate from method 16 was used for this study. Although an amplification target range of 0.128ng average was used in this study, all four samples had dropout. These results are not as good as the results from method 16 which used 2.5µl of sample lysate for amplification.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 16b	1	0.119	0.119	Dropout x12 loci	-	203	72.68%
				Dropout x13 loci	-	201	78.09%
	2	0.160	0.160	Dropout x7 loci	-	284	76.38%
				Dropout x6 loci	-	286	83.79%
	3	0.184	0.184	Dropout x5 loci	-	297	79.42%
				Dropout x6 loci	-	276	88.41%
	4	0.051	0.051	Dropout x15 loci	-	135	-
				Dropout x15 loci	-	112	-
Average:		**0.128**	**0.128**			**224**	**79.80%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 18b

Sample lysate from method 18 was used for this study. 1 sample produced a full DNA profile, but the peak heights were off scale, thus producing artifacts. 2 samples had dropout and one sample had peaks present but all were below threshold. These results are not as good as the results from method 18 which used 2.5µl of sample lysate for amplification.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 18b	1	0.101	0.10	Dropout x8 loci	-	318	82.50%
				Dropout x8 loci	-	300	85.15%
	2	0.017	0.017	No data (peaks below threshold)	-	-	-
				No data (peaks below threshold)	-	-	-
	3	0.168	0.168	Dropout x11 loci	-	225	71.16%
				Dropout x11 loci	-	237	71.94%
	4	undetected	-	Unexplained peak	Yes	4497	89.46%
				Bad peak morphology	Yes	4271	89.72%
Average:		**0.095**	**0.095**			**1641**	**81.66%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 22b

Sample lysate from method 22 was used for this study. All four samples had dropout. These results are very similar to the results from method 22 which used 2.5µl of sample lysate for amplification.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 22b	1	0.005	0.005	Dropout x13 loci	-	119	81.36%
				Dropout x15 loci	-	118	78.10%
	2	0.020	0.020	Dropout x3 loci	-	273	88.94%
				Dropout x3 loci	-	246	86.67%
	3	0.007	0.007	Dropout x7 loci	-	170	82.02%
				Dropout x10 loci	-	146	87.93%
	4	0.032	0.032	Dropout x5 loci	-	276	81.95%
				Dropout x5 loci	-	243	81.87%
Average:		**0.016**	**0.016**			**199**	**83.61%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 23b

Sample lysate from method 23 was used for this study. 1 sample produced a full DNA profile, while the other three samples had dropout. These results are very similar to the results from method 23 which used 2.5µl of sample lysate for amplification.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 23b	1	0.004	0.004	Dropout x15 loci	-	134	-
				Dropout x15 loci	-	120	-
	2	0.052	0.052	OK	-	516	83.61%
				OK	-	450	82.40%
	3	0.028	0.028	Dropout x8 loci	-	183	80.49%
				Dropout x9 loci	-	170	88.72%
	4	0.029	0.029	Dropout x4 loci	-	228	73.57%
				Dropout x5 loci	-	212	71.43%
Average:		**0.028**	**0.028**			**252**	**80.04%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 24b

Sample lysate from method 24 was used for this study. 3 samples had dropout and 1 sample had peaks present but all were below the detection threshold. These results are very similar to the results from method 24 which used 2.5µl of sample lysate for amplification.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 24b	1	0.005	0.005	No data (peaks below threshold)	-	-	-
				No data (peaks below threshold)	-	-	-
	2	0.088	0.088	Dropout x4 loci	-	183	74.08%
				Dropout x3 loci	-	169	77.53%
	3	0.012	0.012	Dropout x14 loci	-	167	-
				Dropout x14 loci	-	150	-
	4	0.041	0.041	Dropout x5 loci	-	226	76.27%
				Dropout x5 loci	-	225	75.73%
Average:		**0.037**	**0.037**			**187**	**75.90%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 25b

Sample lysate from method 25 was used for this study. 3 samples had dropout and 1 sample had peaks present but all were below the detection threshold. These results are very similar to the results from method 25 which used 2.5µl of sample lysate for amplification.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 25b	1	0.003	0.003	No data (peaks below threshold)	-	-	-
				No data (peaks below threshold)	-	-	-
	2	0.009	0.009	Dropout x4 loci	-	334	82.14%
				Dropout x3 loci	-	327	82.39%
	3	0.006	0.006	No data (peaks below threshold)	-	-	-
				No data (peaks below threshold)	-	-	-
	4	0.008	0.008	Dropout x13 loci	-	125	-
				Dropout x15 loci	-	123	-
Average:		**0.006**	**0.006**			**227**	**82.27%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 26b

Sample lysate from method 26 was used for this study. 1 sample produced a full DNA profile, while the other three samples had dropout. These results are not as good as the results from method 26 which used 2.5µl of sample lysate for amplification.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 26b	1	0.006	0.006	Dropout x15 loci	-	113	-
				No data (peaks below threshold)	-	-	-
	2	0.092	0.092	OK	-	627	79.70%
				OK	-	629	81.34%
	3	0.017	0.017	Dropout x14 loci	-	125	-
				Dropout x14 loci	-	108	-
	4	0.012	0.012	Dropout x14 loci	-	161	-
				Dropout x14 loci	-	164	-
Average:		**0.032**	**0.032**			**275**	**80.52%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 27b

Sample lysate from method 27 was used for this study. 2 samples had dropout, and 2 samples had peaks present but were below the detection threshold. These results are very similar to the results from method 27 which used 2.5µl of sample lysate for amplification.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 27b	1	0.027	0.027	No data	-	-	-
				No data	-	-	-
	2	0.026	0.026	Dropout x15 loci	-	139	-
				Dropout x15 loci	-	122	-
	3	0.014	0.014	No data	-	-	-
				No data	-	-	-
	4	0.014	0.014	Dropout x14 loci	-	142	-
				Dropout x14 loci	-	122	-
Average:		**0.020**	**0.020**			**131**	**-**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Study 5

Study 4 demonstrated that using a decreased amount of DNA template during amplification did not produce the desired results. In study 5 we evaluated the effects of increasing the amount of DNA template used during the amplification step. Quantitation results multiple samples previously analyzed showed small amounts of amplifiable DNA present. By increasing the amount of DNA template used during amplification, the intent behind study 5 is to increase the amount of DNA amplified without causing preferential amplification effects or artifacts. Six different lysing methods were used for study 5 (see Table 6). Four samples previously lysed were analyzed for each method tested. All samples were amplified using 5.0µl of sample lysate, and were injected on the 3130 Genetic analyzer twice at 10 seconds.

Table 6: Lysing methods used for Study 5

Method	Swab Head Size	Lysing Solution	Lysis Solution Volume (µl)	Lysing Temp. (°C)	Lysing Time (minutes)
22c	Medium	T.E.$^{-4}$	200	95	60
23c	Medium	T.E.$^{-4}$	300	95	60
24c	Medium	T.E.$^{-4}$	400	95	60
25c	Small	T.E.$^{-4}$	200	95	60
26c	Small	T.E.$^{-4}$	300	95	60
27c	Small	T.E.$^{-4}$	400	95	60

Study 5 demonstrated that increasing the amount of DNA template during amplification will increase the amount of DNA detected, thus producing a more complete profile. However, it was clear that targeting an amplification range greater than ~0.3ng will significantly increase the chances of achieving the desired results (full DNA profile with no artifacts). This may have been better demonstrated by using 5.0µl of sample lysate on the samples previously extracted in methods 13, 16, and 18 (best methods tested thus far). However, due to an oversight by the laboratory, these methods were not tested in this research study.

Method 22c

Sample lysate from method 22 was used for this study. One sample produced a full DNA profile, while the other three samples all had dropout. These results were similar to the results from method 22 which used 2.5µl of sample lysate for amplification. This is likely attributed to the amplification target volume still being less than desired (0.080ng average).

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 22c	1	0.005	0.023	Bad injection	-	-	-
				Dropout x13 loci	-	155	72.08%
	2	0.020	0.100	OK	-	592	86.40%
				OK	-	537	86.65%
	3	0.007	0.037	Dropout x6 loci	-	242	81.06%
				Dropout x6 loci	-	237	81.61%
	4	0.032	0.160	Dropout x2 loci	-	356	80.66%
				Dropout x2 loci	-	347	81.82%
Average:		**0.016**	**0.080**			**352**	**81.47%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 23c

Sample lysate from method 23 was used for this study. One sample produced a full DNA profile, while the other three samples all had dropout. These results were similar to the results from method 23 which used 2.5µl of sample lysate for amplification. This is likely attributed to the amplification target volume still being less than desired (0.141ng average).

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 23c	1	0.004	0.021	Dropout x14 loci	-	125	85.27%
				Dropout x14 loci	-	124	82.84%
	2	0.052	0.259	OK	-	482	85.49%
				OK	-	476	85.93%
	3	0.028	0.139	Dropout x3 loci	-	392	90.81%
				Dropout x3 loci	-	405	90.88%
	4	0.029	0.146	Dropout x1 locus	-	472	77.11%
				Dropout x1 locus	-	448	77.01%
Average:		**0.028**	**0.141**			**366**	**84.42%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 24c

Sample lysate from method 24 was used for this study. One sample produced a full DNA profile, two samples had dropout, and one sample had peaks present but all were below the detection threshold. These results were slightly better than the results from method 24 which used 2.5µl of sample lysate for amplification.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 24c	1	0.005	0.025	No data (peaks below threshold)	-	-	-
				No data (peaks below threshold)	-	-	-
	2	0.088	0.440	Dropout x5 loci	-	220	79.23%
				Dropout x5 loci	-	212	78.32%
	3	0.012	0.059	Dropout x14 loci	-	118	-
				Dropout x14 loci	-	125	-
	4	0.041	0.207	OK	-	454	82.68%
				OK	-	427	82.25%
Average:		**0.037**	**0.183**			**259**	**80.62%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 25c

Sample lysate from method 25 was used for this study. All four samples had dropout. These results were similar to the results from method 25 which used 2.5µl of sample lysate for amplification. This is likely attributed to the amplification target volume still being less than desired (0.032ng average).

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 25c	1	0.003	0.017	Dropout x11 loci	-	146	70.90%
				Dropout x13 loci	-	135	53.24%
	2	0.009	0.044	Dropout x4 loci	-	219	84.00%
				Dropout x5 loci	-	204	86.00%
	3	0.006	0.028	Dropout x12 loci	-	170	88.76%
				Dropout x12 loci	-	170	62.40%
	4	0.008	0.041	Dropout x6 loci	-	204	65.93%
				Dropout x6 loci	-	196	82.10%
Average:		**0.006**	**0.032**			**181**	**74.17%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 26c

Sample lysate from method 26 was used for this study. One sample produced a full DNA profile, while the other three samples all had dropout. These results were similar to the results from method 26 which used 2.5µl of sample lysate for amplification. This is likely attributed to the amplification target volume still being less than desired (0.159ng average).

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 26c	1	0.006	0.028	Dropout x6 loci	-	169	88.17%
				Dropout x7 loci	-	163	87.06%
	2	0.092	0.461	OK	-	599	88.48%
				OK	-	557	88.38%
	3	0.017	0.084	Dropout x8 loci	-	180	83.23%
				Dropout x8 loci	-	163	78.45%
	4	0.012	0.062	Dropout x5 loci	-	188	80.88%
				Dropout x5 loci	-	197	80.45%
Average:		**0.032**	**0.159**			**277**	**84.39%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 27c

Sample lysate from method 27 was used for this study. Three samples had dropout and one sample had peaks present, but all were below the detection threshold. These results were similar to the results from method 27 which used 2.5µl of sample lysate for amplification. This is likely attributed to the amplification target volume still being less than desired (0.100ng average).

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 27c	1	0.027	0.133	No data (peaks below threshold)	-	-	-
				No data (peaks below threshold)	-	-	-
	2	0.026	0.129	Dropout x13 loci	-	148	90.32%
				Dropout x13 loci	-	140	93.75%
	3	0.014	0.072	Dropout x15 loci	-	180	-
				Dropout x15 loci	-	177	-
	4	0.014	0.068	Dropout x8 loci	-	166	74.76%
				Dropout x10 loci	-	153	84.10%
Average:		**0.020**	**0.100**			**161**	**85.73%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
1	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
2	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
3	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Study 6

Thus far, we have explored using different lysing temperatures, different lysing times, various lysing reagents, various sizes of swab heads, and amplifying various amounts of template DNA. At this point in the research study, no additional new methods were attempted. After calculating and comparing data from studies 1 though 5, the best 3 methods were selected. Study 6 will test 10 new samples lysed under the same conditions as methods 13, 16, and 18 (see Table 7). By testing 10 samples instead of the previous 4, data from a larger sampling pool can be examined and compared. All samples were amplified using 2.5µl of sample lysate, and were injected on the 3130 Genetic analyzer twice at 10 seconds.

Table 7: Lysing methods used for Study 6

Method	Swab Head Size	Lysing Solution	Lysis Solution Volume (µl)	Lysing Temp. (°C)	Lysing Time (minutes)
13	Large	$T.E.^{-4}$	200	95	60
16	Large	$T.E.^{-4}$	300	95	60
18	Large	$T.E.^{-4}$	400	95	60

Method 13 (x10 samples)

From the 10 samples analyzed, full DNA profiles were obtained from 6 samples, with the other 4 samples containing dropout. 2 of the samples exhibited off scale data on the 3130, which caused bad peak morphology. If these samples had been injected on the 3730 DNA Analyzer (which has a much higher off scale limit) the peak morphology could have been much better. It is also believed that if these samples were amplified using a higher template volume (perhaps 5.0µl of sample lysate) and injected on a 3730 DNA Analyzer, better results may have been obtained. Additional research in these areas is highly encouraged.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 13	1	0.080	0.200	Dropout x12 loci	-	167	92.11%
				Dropout x12 loci	-	163	89.86%
	2	0.034	0.085	Dropout x3 loci	-	285	79.84%
				Dropout x3 loci	-	273	80.99%
	3	0.047	0.119	Dropout x5 loci	-	371	67.99%
				Dropout x5 loci	-	375	67.32%
	4	2.910	7.28	Bad peak morphology	Yes	4687	81.13%
				Bad peak morphology	Yes	5378	85.93%
	5	0.734	1.84	OK	-	1019	88.68%
				OK	-	955	88.20%
	6	0.089	0.222	Dropout x6 loci	-	308	88.89%
				Dropout x6 loci	-	305	86.06%
	7	0.293	0.733	OK	-	1618	86.69%
				OK	-	1595	86.43%
	8	0.680	1.700	Bad peak morphology	Yes	5470	90.85%
				Bad peak morphology	Yes	5399	90.10%
	9	0.149	0.373	OK	-	1239	81.72%
				OK	-	1245	81.51%
	10	0.076	0.190	OK	Yes	4108	91.58%
				OK	Yes	3999	91.76%
Average:		**0.509**	**1.273**			**1948**	**84.88%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
1	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
2	12, 13	28, 31	8, 9	10, 11	15, 18	9.3, 9.3	9, 11	12, 13	16, 18	14, 16	17, 20	11, 12	14, 16	X, X	11, 12	20, 25
2	12, 13	28, 31	8, 9	10, 11	15, 18	9.3, 9.3	9, 11	12, 13	16, 18	14, 16	17, 20	11, 12	14, 16	X, X	11, 12	20, 25
3	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
3	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	14, 14	28, 28	10, 12	10, 11	15, 17	7, 9.3	8, 12	9, 12	20, 21	14, 14.2	17, 18	8, 8	12, 14	X, Y	10, 11	22, 24
4	14, 14	28, 28	10, 12	10, 11	15, 17	7, 9.3	8, 12	9, 12	20, 21	14, 14.2	17, 18	8, 8	12, 14	X, Y	10, 11	22, 24
5	13, 15	30, 31	10, 11	9, 11	15, 17	6, 6	12, 12	12, 13	18, 22	13, 14	14, 14	8, 11	13, 17	X, Y	10, 11	22, 24
5	13, 15	30, 31	10, 11	9, 11	15, 17	6, 6	12, 12	12, 13	18, 22	13, 14	14, 14	8, 11	13, 17	X, Y	10, 11	22, 24
6	10, 13	30, 31.2	8, 8	12, 12	16, 16	6, 9	10, 11	12, 13	20, 24	13, 15	16, 16	8, 11	14, 15	X, X	12, 12	18, 21
6	10, 13	30, 31.2	8, 8	12, 12	16, 16	6, 9	10, 11	12, 13	20, 24	13, 15	16, 16	8, 11	14, 15	X, X	12, 12	18, 21
7	10, 13	28, 32.2	8, 10	10, 12	15, 18	6, 9.3	8, 12	11, 13	17, 19	14, 15	17, 17	8, 12	14, 18	X, X	9, 11	20, 21
7	10, 13	28, 32.2	8, 10	10, 12	15, 18	6, 9.3	8, 12	11, 13	17, 19	14, 15	17, 17	8, 12	14, 18	X, X	9, 11	20, 21
8	9, 14	32, 35	9, 9	8, 11	16, 18	7, 9.3	10, 11	9, 13	19, 22	11, 12	17, 21	8, 9	15, 17	X, X	13, 13	23, 24
8	9, 14	32, 35	9, 9	8, 11	16, 18	7, 9.3	10, 11	9, 13	19, 22	11, 12	17, 21	8, 9	15, 17	X, X	13, 13	23, 24
9	10, 13	28, 30	8, 11	12, 13	16, 16	6, 9.3	10, 12	11, 12	20, 22	14, 15	15, 16	10, 11	15, 19	X, X	11, 12	18, 21
9	10, 13	28, 30	8, 11	12, 13	16, 16	6, 9.3	10, 12	11, 12	20, 22	14, 15	15, 16	10, 11	15, 19	X, X	11, 12	18, 21
10	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
10	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 16 (x10 samples)

From the 10 samples analyzed, full DNA profiles were obtained from 7 samples, with 2 samples showing dropout and 1 sample with peaks present, but all below the peak detection threshold. 1 of the samples exhibited off scale data on the 3130, which caused bad peak morphology. If this sample had been injected on the 3730 DNA Analyzer (which has a much higher off scale limit) the peak morphology could have been much better. It is also believed that if these samples were amplified using a higher template volume (perhaps 5.0µl of sample lysate) and injected on a 3730 DNA Analyzer, better results may have been obtained. Additional research in these areas is highly encouraged.

Based on these results, method 16 proved to be the best combination of all 42 previous methods tested, and was used in Study 7 for the side-by-side comparison to FTA® samples.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 16	1	0.336	0.840	OK	-	836	86.04%
				Dropout x1 locus	-	803	86.96%
	2	0.018	0.046	Dropout x7 loci	-	210	79.27%
				Dropout x7 loci	-	203	79.18%
	3	0.047	0.117	Dropout x11 loci	-	210	82.89%
				Dropout x11 loci	-	207	87.04%
	4	2.070	5.175	Bad peak morphology	Yes	5706	87.30%
				Bad peak morphology	Yes	5821	87.39%
	5	0.967	2.418	OK	-	3155	89.54%
				OK	-	2906	89.28%
	6	0.008	0.020	No data (peaks below threshold)	-	-	-
				No data (peaks below threshold)	-	-	-
	7	0.118	0.295	OK	-	764	79.43%
				OK	-	804	79.27%
	8	0.216	0.540	OK	-	2786	89.45%
				OK	-	2675	89.18%
	9	0.063	0.158	OK	-	767	87.21%
				OK	-	759	87.65%
	10	0.036	0.089	OK	-	2805	80.99%
				OK	-	2911	81.23%
Average:		**0.388**	**0.970**			**1907**	**84.91%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
1	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
2	12, 13	28, 31	8, 9	10, 11	15, 18	9.3, 9.3	9, 11	12, 13	16, 18	14, 16	17, 20	11, 12	14, 16	X, X	11, 12	20, 25
2	12, 13	28, 31	8, 9	10, 11	15, 18	9.3, 9.3	9, 11	12, 13	16, 18	14, 16	17, 20	11, 12	14, 16	X, X	11, 12	20, 25
3	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
3	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	14, 14	28, 28	10, 12	10, 11	15, 17	7, 9.3	8, 12	9, 12	20, 21	14, 14.2	17, 18	8, 8	12, 14	X, Y	10, 11	22, 24
4	14, 14	28, 28	10, 12	10, 11	15, 17	7, 9.3	8, 12	9, 12	20, 21	14, 14.2	17, 18	8, 8	12, 14	X, Y	10, 11	22, 24
5	13, 15	30, 31	10, 11	9, 11	15, 17	6, 6	12, 12	12, 13	18, 22	13, 14	14, 14	8, 11	13, 17	X, Y	10, 11	22, 24
5	13, 15	30, 31	10, 11	9, 11	15, 17	6, 6	12, 12	12, 13	18, 22	13, 14	14, 14	8, 11	13, 17	X, Y	10, 11	22, 24
6	10, 13	30, 31.2	8, 8	12, 12	16, 16	6, 9	10, 11	12, 13	20, 24	13, 15	16, 16	8, 11	14, 15	X, X	12, 12	18, 21
6	10, 13	30, 31.2	8, 8	12, 12	16, 16	6, 9	10, 11	12, 13	20, 24	13, 15	16, 16	8, 11	14, 15	X, X	12, 12	18, 21
7	10, 13	28, 32.2	8, 10	10, 12	15, 18	6, 9.3	8, 12	11, 13	17, 19	14, 15	17, 17	8, 12	14, 18	X, X	9, 11	20, 21
7	10, 13	28, 32.2	8, 10	10, 12	15, 18	6, 9.3	8, 12	11, 13	17, 19	14, 15	17, 17	8, 12	14, 18	X, X	9, 11	20, 21
8	9, 14	32, 35	9, 9	8, 11	16, 18	7, 9.3	10, 11	9, 13	19, 22	11, 12	17, 21	8, 9	15, 17	X, X	13, 13	23, 24
8	9, 14	32, 35	9, 9	8, 11	16, 18	7, 9.3	10, 11	9, 13	19, 22	11, 12	17, 21	8, 9	15, 17	X, X	13, 13	23, 24
9	10, 13	28, 30	8, 11	12, 13	16, 16	6, 9.3	10, 12	11, 12	20, 22	14, 15	15, 16	10, 11	15, 19	X, X	11, 12	18, 21
9	10, 13	28, 30	8, 11	12, 13	16, 16	6, 9.3	10, 12	11, 12	20, 22	14, 15	15, 16	10, 11	15, 19	X, X	11, 12	18, 21
10	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
10	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 18 (x10 samples)

From the 10 samples analyzed, full DNA profiles were obtained from 4 samples, with the other 6 samples containing dropout. 1 of the samples exhibited off scale data on the 3130, which caused bad peak morphology. If this sample had been injected on the 3730 DNA Analyzer (which has a much higher off scale limit) the peak morphology could have been much better. It is also believed that if these samples were amplified using a higher template volume (perhaps 5.0µl of sample lysate) and injected on a 3730 DNA Analyzer, better results may have been obtained. Additional research in these areas is highly encouraged.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Artifacts	Average RFU	Average PHR
Method 18	1	0.366	0.915	Dropout x1 locus	-	855	82.52%
				Dropout x1 locus	-	825	83.30%
	2	0.066	0.165	Dropout x4 loci	-	327	75.20%
				Dropout x4 loci	-	334	74.75%
	3	0.088	0.221	Dropout x2 loci	-	931	80.77%
				Dropout x2 loci	-	924	81.20%
	4	1.560	3.900	Dropout x3 loci	Yes	3801	77.35%
				Dropout x3 loci	Yes	3697	77.16%
	5	0.667	1.668	OK	Yes	6001	93.80%
				OK	Yes	5850	93.83%
	6	0.008	0.020	Dropout x10 loci	-	196	66.04%
				Dropout x8 loci	-	195	82.40%
	7	0.522	1.305	OK	-	1555	86.99%
				OK	-	1504	86.83%
	8	0.125	0.313	OK	-	2086	87.88%
				OK	-	1937	88.11%
	9	0.064	0.160	Bad peak morphology	Yes	5243	88.91%
				Bad peak morphology	Yes	5067	89.19%
	10	0.037	0.092	Dropout x1 locus	-	1793	81.82%
				Dropout x1 locus	-	1762	80.95%
Average:						**2244**	**82.95%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
1	10, 12	27, 29	7, 11	12, 12	16, 17	6, 7	8, 11	13, 14	19, 24	13, 14	16, 17	8, 11	12, 12	X, X	11, 11	21, 22
2	12, 13	28, 31	8, 9	10, 11	15, 18	9.3, 9.3	9, 11	12, 13	16, 18	14, 16	17, 20	11, 12	14, 16	X, X	11, 12	20, 25
2	12, 13	28, 31	8, 9	10, 11	15, 18	9.3, 9.3	9, 11	12, 13	16, 18	14, 16	17, 20	11, 12	14, 16	X, X	11, 12	20, 25
3	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
3	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
4	14, 14	28, 28	10, 12	10, 11	15, 17	7, 9.3	8, 12	9, 12	20, 21	14, 14.2	17, 18	8, 8	12, 14	X, Y	10, 11	22, 24
4	14, 14	28, 28	10, 12	10, 11	15, 17	7, 9.3	8, 12	9, 12	20, 21	14, 14.2	17, 18	8, 8	12, 14	X, Y	10, 11	22, 24
5	13, 15	30, 31	10, 11	9, 11	15, 17	6, 6	12, 12	12, 13	18, 22	13, 14	14, 14	8, 11	13, 17	X, Y	10, 11	22, 24
5	13, 15	30, 31	10, 11	9, 11	15, 17	6, 6	12, 12	12, 13	18, 22	13, 14	14, 14	8, 11	13, 17	X, Y	10, 11	22, 24
6	10, 13	30, 31.2	8, 8	12, 12	16, 16	6, 9	10, 11	12, 13	20, 24	13, 15	16, 16	8, 11	14, 15	X, X	12, 12	18, 21
6	10, 13	30, 31.2	8, 8	12, 12	16, 16	6, 9	10, 11	12, 13	20, 24	13, 15	16, 16	8, 11	14, 15	X, X	12, 12	18, 21
7	10, 13	28, 32.2	8, 10	10, 12	15, 18	6, 9.3	8, 12	11, 13	17, 19	14, 15	17, 17	8, 12	14, 18	X, X	9, 11	20, 21
7	10, 13	28, 32.2	8, 10	10, 12	15, 18	6, 9.3	8, 12	11, 13	17, 19	14, 15	17, 17	8, 12	14, 18	X, X	9, 11	20, 21
8	9, 14	32, 35	9, 9	8, 11	16, 18	7, 9.3	10, 11	9, 13	19, 22	11, 12	17, 21	8, 9	15, 17	X, X	13, 13	23, 24
8	9, 14	32, 35	9, 9	8, 11	16, 18	7, 9.3	10, 11	9, 13	19, 22	11, 12	17, 21	8, 9	15, 17	X, X	13, 13	23, 24
9	10, 13	28, 30	8, 11	12, 13	16, 16	6, 9.3	10, 12	11, 12	20, 22	14, 15	15, 16	10, 11	15, 19	X, X	11, 12	18, 21
9	10, 13	28, 30	8, 11	12, 13	16, 16	6, 9.3	10, 12	11, 12	20, 22	14, 15	15, 16	10, 11	15, 19	X, X	11, 12	18, 21
10	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
10	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Study 7

The data from study 6 was carefully examined and compared. Taking all factors into account (full profile obtained, artifacts present, overall peak heights and peak height ratios), it was decided that method 16 gave the best overall data. Study 7 will test 24 new samples lysed under the same conditions as methods 16.

24 new buccal swabs were lysed under the same conditions as method 16 (see table 3). 2.5µl of sample lysate was combined with 25µl of the Identifiler® Direct Reaction Mix in each sample well. The plate was amplified at 28 cycles on a single well 9700 thermalcycler following the recommended PCR cycling conditions as outlined in Table 1. The samples were prepared for genetic analysis by combining 1µl of amplified DNA template to 9µl of a formamide/size standard mixture, and injected on the 3130 Genetic Analyzer and the 3730 DNA Analyzer once at 5 and once at 10 seconds. The same genetic analysis plate was used for both instrument types to eliminate any variables that may be caused during sample setup.

In addition to the buccal samples, 24 new FTA® cards were analyzed for comparison purposes to the swab samples. Following the Identifiler® Direct kit procedures, a single 1.2mm punch was taken from each FTA® sample and placed into a 96-well PCR plate. 25µl of the Identifiler® Direct Reaction Mix was added to the sample wells, and the plate was amplified at 28 cycles on a single well 9700 thermalcycler following the recommended PCR cycling conditions as outlined in Table 1. The samples were prepared for genetic analysis by combining 1µl of amplified DNA template to 9µl of a formamide/size standard mixture, and injected on the 3130 Genetic Analyzer and the 3730 DNA Analyzer once at 5 and once at 10 seconds. The same genetic analysis plate was used for both instrument types to eliminate any variables caused by sample setup.

To simulate an actual collection facility and to remove any potential sample collection bias, the 24 known samples (swabs and FTA® cards) were collected by the donor themselves. Instructions were given to the group of donors on how to collect the two types of buccal samples, but no hands-on influence was provided.

Method 16 (x24 samples) **3130 Genetic Analyzer**

As previously mentioned, these samples were injected at both 5 and 10 seconds. This is similar to how samples are analyzed in the OSBI's CODIS Unit. If the data from the 5 second injection produces a good DNA profile, the 10 second injection is not analyzed. In contrast, if the data from the 5 second injection does not produce a good DNA profile, the 10 second injection can be used. The results below only depict the better of the two injections.

Method 16 (x24 samples) 3130 Genetic Analyzer	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Injection Time (seconds)	Artifacts	Average RFU	Average PHR
	1	0.075	0.189	OK	5		261	82.56%
	2	0.206	0.515	Dropout x 1 locus	10		732	83.44%
	3	0.660	1.650	OK	5	Yes	3091	86.81%
	4	0.403	1.008	OK	10		2115	89.32%
	5	0.196	0.490	Dropout x 2 loci	10		446	83.89%
	6	0.481	1.203	OK	5		1848	88.27%
	7	0.289	0.723	OK	10		2963	90.77%
	8	0.050	0.126	Dropout x 4 loci	10		295	81.18%
	9	1.770	4.425	OK	5		2769	87.04%
	10	0.046	0.115	OK	5		703	89.08%
	11	0.262	0.655	OK	5		1820	87.72%
	12	0.122	0.305	Dropout x 4 loci	10		490	85.32%
	13	0.735	1.838	OK	5		506	85.98%
	14	0.496	1.240	OK	5		743	80.33%
	15	1.000	2.500	OK	5	Yes	3725	90.99%
	16	0.020	0.050	No data	10		-	-
	17	1.580	3.950	OK	5	Yes	3987	88.38%
	18	0.178	0.445	Dropout x 3 loci	10		754	84.94%
	19	0.162	0.405	Dropout x 1 locus	10		921	77.09%
	20	0.997	2.493	OK	5	Yes	2852	88.26%
	21	0.719	1.798	OK	5		889	88.93%
	22	0.360	0.900	OK	5		374	79.40%
	23	0.088	0.221	Dropout x 5 loci	10		315	78.84%
	24	0.058	0.145	Dropout x 4 loci	10		397	80.10%
Average:		**0.456**	**1.141**				**1435**	**85.16%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 13	29, 30	10, 11	10, 12	17, 17	9.3, 9.3	10, 13	11, 12	18, 19	13, 14	16, 16	8, 8	12, 16	X, Y	11, 13	20, 21
2	10, 14	28, 32.2	10, 12	10, 10	15, 19	6, 9	11, 12	11, 11	17, 17	13, 14.2	15, 16	10, 11	12, 13	X, X	11, 13	20, 26
3	12, 14	29, 33.2	8, 11	10, 11	14, 16	6, 9.3	11, 12	12, 13	17, 24	13, 14	16, 17	8, 8	14, 15	X, X	11, 12	21, 24
4	11, 13	30, 30.2	10, 11	11, 12	14, 14	6, 9.3	11, 14	12, 13	17, 24	13, 14	17, 19	8, 8	14, 17	X, Y	11, 12	21, 22.2
5	13, 13	29, 33.2	8, 10	9, 11	14, 16	6, 7	8, 12	9, 12	19, 22	14.1, 14.2	18, 19	8, 9	14, 15	X, X	11, 12	19, 24
6	11, 13	32.2, 33.2	9, 11	10, 11	15, 16	6, 9.3	8, 11	11, 13	19, 22	14, 15	14, 18	10, 11	15, 17	X, Y	11, 11	22, 23
7	10, 12	28, 30	9, 11	10, 11	16, 17	8, 9.3	11, 12	11, 12	24, 26	13, 15	16, 19	8, 11	14, 15	X, X	9, 10	22, 23
8	11, 13	30, 31	9, 9	11, 12	15, 16	9, 9.3	11, 13	10, 12	16, 18	14, 14	18, 19	8, 8	12, 16	X, X	11, 12	22, 23.2
9	14, 15	28, 31	9, 9	11, 11	15, 16	6, 9.3	10, 11	10, 11	17, 18	13, 15	19, 19	8, 8	11, 15	X, Y	11, 12	21, 21
10	11, 13	30, 33	10, 12	11, 12	17, 18	6, 8	11, 11	11, 12	19, 20	14, 15	16, 18	8, 8	13, 17	X, X	10, 11	21, 25
11	12, 13	31, 31	11, 11	12, 13	17, 18	6, 9.3	10, 14	9, 11	24, 25	14, 14	15, 19	8, 11	15, 17	X, X	9, 12	19, 21
12	11, 13	29, 31.2	10, 10	11, 12	16, 17	6, 7	8, 12	9, 13	19, 24	13, 14	14, 18	8, 11	17, 23	X, X	10, 11	21, 21
13	14, 16	31, 32.2	10, 12	11, 12	16, 18	6, 8	11, 11	11, 13	17, 19	14, 15	14, 19	8, 8	12, 12	X, X	12, 12	21, 23
14	12, 12	30.2, 32.2	8, 12	12, 12	14, 15	9.3, 10	9, 11	11, 13	17, 26	12, 14.2	13, 16	8, 11	12, 16	X, Y	12, 12	22, 22
15	14, 14	28, 28	10, 12	10, 11	15, 17	7, 9.3	8, 12	9, 12	20, 21	14, 14.2	17, 18	8, 8	12, 14	X, Y	10, 11	22, 24
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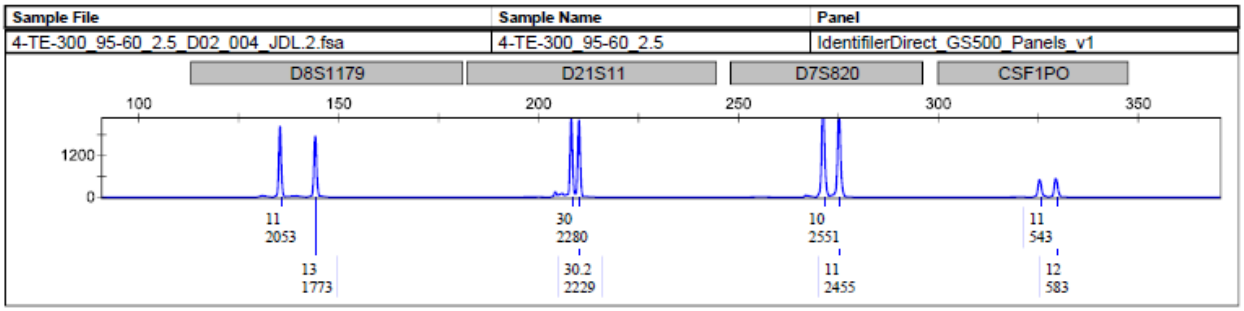
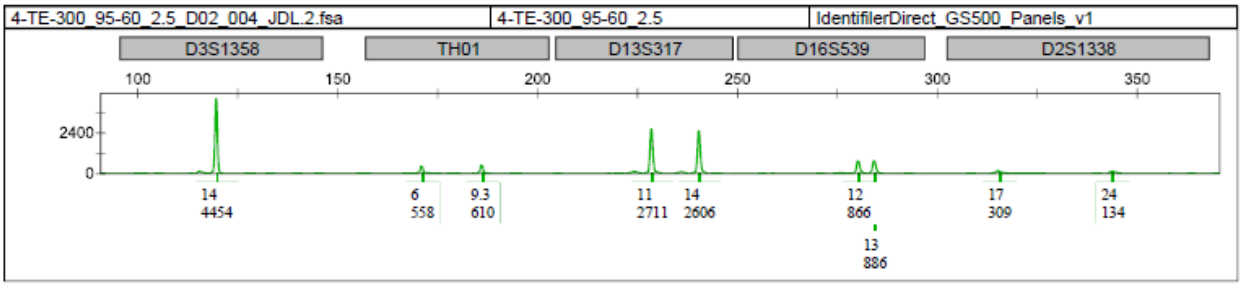
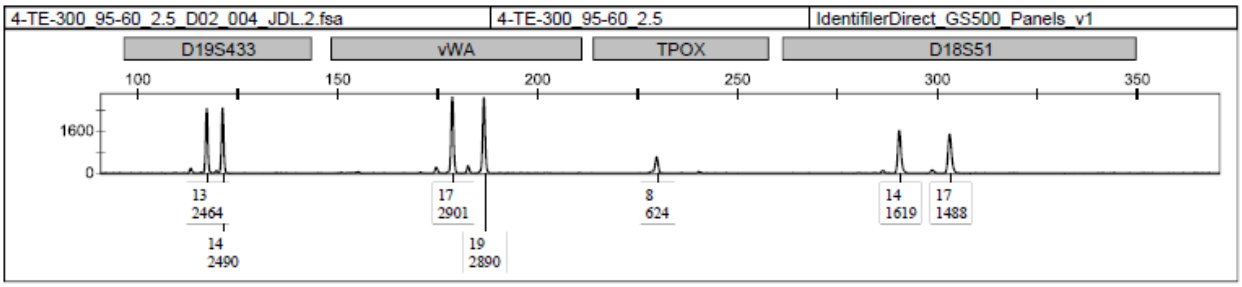
24	12, 13	28, 31	9, 12	11, 11	15, 15	7, 9.3	9, 12	12, 12	16, 19	14, 16	16, 20	9, 12	14, 19	X, Y	11, 12	20, 22

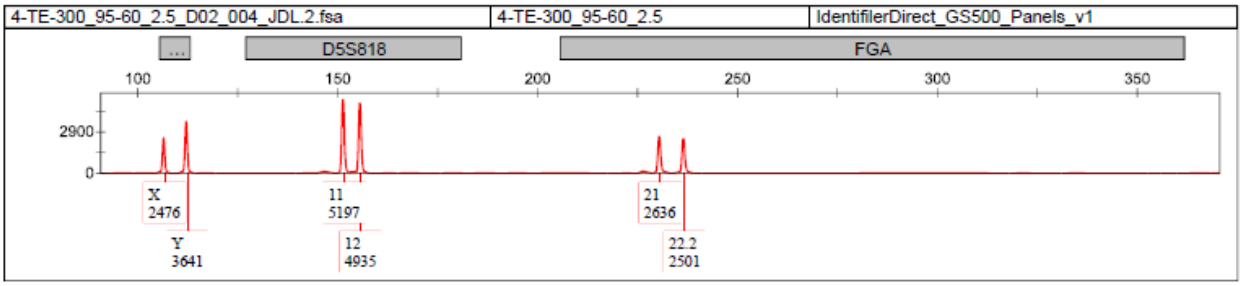
Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

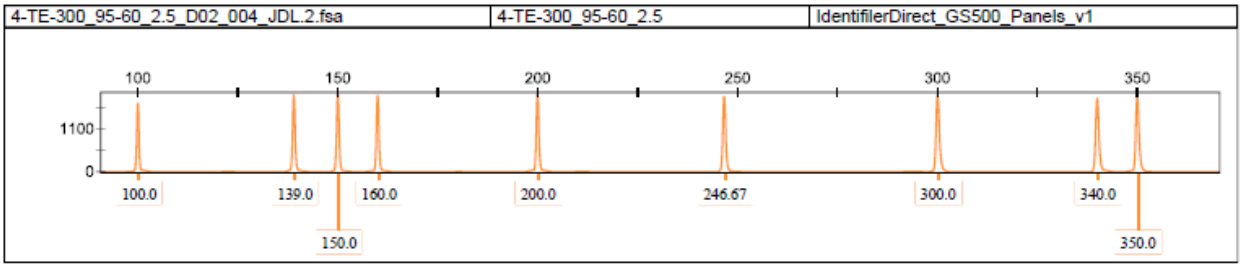
Method 16 (x24 samples), 3130 Genetic Analyzer, Sample #4, Injection 2 of 2 (10 seconds)











FTA® (x24 samples) **3130 Genetic Analyzer**

Quantitation cannot be performed using this method, so the average DNA yield and average amplification target range cannot be determined. These samples were injected at both 5 and 10 seconds. The results below only depict the better of the two injections.

	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Injection Time (seconds)	Artifacts	Average RFU	Average PHR
FTA (x24 samples) 3130 Genetic Analyzer	**1**	n/a	Unknown	OK	5		1206	88.72%
	2	n/a	Unknown	OK	5	Yes	4181	89.03%
	3	n/a	Unknown	Bad peak morphology	5	Yes	6556	89.94%
	4	n/a	Unknown	OK	5	Yes	3350	90.06%
	5	n/a	Unknown	OK	5		1399	91.16%
	6	n/a	Unknown	OK	5		3092	91.57%
	7	n/a	Unknown	OK	5	Yes	2900	90.09%
	8	n/a	Unknown	OK	5		1412	86.00%
	9	n/a	Unknown	OK	5		1235	88.50%
	10	n/a	Unknown	OK	5	Yes	2064	85.41%
	11	n/a	Unknown	OK	5	Yes	3399	84.39%
	12	n/a	Unknown	OK	5		1610	83.09%
	13	n/a	Unknown	OK	5		1208	83.98%
	14	n/a	Unknown	OK	5		1870	90.16%
	15	n/a	Unknown	OK	5	Yes	2889	86.39%
	16	n/a	Unknown	OK	5		2601	88.36%
	17	n/a	Unknown	Bad peak morphology	5	Yes	4980	82.50%
	18	n/a	Unknown	OK	5	Yes	4406	87.98%
	19	n/a	Unknown	Unexplained peak	5	Yes	2955	84.84%
	20	n/a	Unknown	OK	5	Yes	2988	86.56%
	21	n/a	Unknown	OK	5	Yes	4320	88.34%
	22	n/a	Unknown	OK	5		1592	84.93%
	23	n/a	Unknown	OK	5	Yes	1918	83.42%
	24	n/a	Unknown	OK	5	Yes	3927	87.02%
Average:							**2836**	**87.19%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 13	29, 30	10, 11	10, 12	17, 17	9.3, 9.3	10, 13	11, 12	18, 19	13, 14	16, 16	8, 8	12, 16	X, Y	11, 13	20, 21
2	10, 14	28, 32.2	10, 12	10, 10	15, 19	6, 9	11, 12	11, 11	17, 17	13, 14.2	15, 16	10, 11	12, 13	X, X	11, 13	20, 26
3	12, 14	29, 33.2	8, 11	10, 11	14, 16	6, 9.3	11, 12	12, 13	17, 24	13, 14	16, 17	8, 8	14, 15	X, X	11, 12	21, 24
4	11, 13	30, 30.2	10, 11	11, 12	14, 14	6, 9.3	11, 14	12, 13	17, 24	13, 14	17, 19	8, 8	14, 17	X, Y	11, 12	21, 22.2
5	13, 13	29, 33.2	8, 10	9, 11	14, 16	6, 7	8, 12	9, 12	19, 22	14.1, 14.2	18, 19	8, 9	14, 15	X, X	11, 12	19, 24
6	11, 13	32.2, 33.2	9, 11	10, 11	15, 16	6, 9.3	8, 11	11, 13	19, 22	14, 15	14, 18	10, 11	15, 17	X, Y	11, 11	22, 23
7	10, 12	28, 30	9, 11	10, 11	16, 17	8, 9.3	11, 12	11, 12	24, 26	13, 15	16, 19	8, 11	14, 15	X, X	9, 10	22, 23
8	11, 13	30, 31	9, 9	11, 12	15, 16	9, 9.3	11, 13	10, 12	16, 18	14, 14	18, 19	8, 8	12, 16	X, X	11, 12	22, 23.2
9	14, 15	28, 31	9, 9	11, 11	15, 16	6, 9.3	10, 11	10, 11	17, 18	13, 15	19, 19	8, 8	11, 15	X, Y	11, 12	21, 21
10	11, 13	30, 33	10, 12	11, 12	17, 18	6, 8	11, 11	11, 12	19, 20	14, 15	16, 18	8, 8	13, 17	X, X	10, 11	21, 25
11	12, 13	31, 31	11, 11	12, 13	17, 18	6, 9.3	10, 14	9, 11	24, 25	14, 14	15, 19	8, 11	15, 17	X, X	9, 12	19, 21
12	11, 13	29, 31.2	10, 10	11, 12	16, 17	6, 7	8, 12	9, 13	19, 24	13, 14	14, 18	8, 11	17, 23	X, X	10, 11	21, 21
13	14, 16	31, 32.2	10, 12	11, 12	16, 18	6, 8	11, 11	11, 13	17, 19	14, 15	14, 19	8, 8	12, 12	X, X	12, 12	21, 23
14	12, 12	30.2, 32.2	8, 12	12, 12	14, 15	9.3, 10	9, 11	11, 13	17, 26	12, 14.2	13, 16	8, 11	12, 16	X, Y	12, 12	22, 22
15	14, 14	28, 28	10, 12	10, 11	15, 17	7, 9.3	8, 12	9, 12	20, 21	14, 14.2	17, 18	8, 8	12, 14	X, Y	10, 11	22, 24
16	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
17	10, 13	28, 32.2	8, 10	10, 12	15, 18	6, 9.3	8, 12	11, 13	17, 19	14, 15	17, 17	8, 12	14, 18	X, X	9, 11	20, 21
18	11, 12	28, 30	11, 12	10, 11	14, 15	9, 9.3	11, 12	11, 11	20, 23	14, 15	14, 18	8, 11	12, 13	X, Y	10, 13	25, 25
19	12, 15	28, 31	8, 8	11, 12	17, 17	6, 9.3	11, 11	12, 13	17, 17	13, 13	17, 17	8, 8	14, 16	X, X	10, 11	22, 23
20	13, 13	27, 29	9, 11	10, 12	14, 16	7, 9.3	11, 14	9, 11	19, 23	12, 14	16, 17	8, 9	15, 17	X, Y	11, 11	22, 22
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22	12, 13	28, 28	11, 12	12, 12	16, 18	6, 7	8, 12	12, 12	23, 23	13, 16	15, 15	8, 8	13, 16	X, X	12, 13	21, 23
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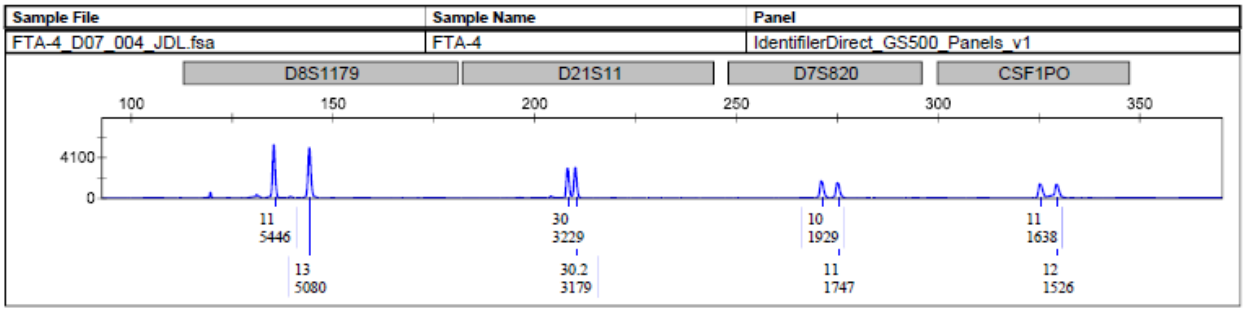
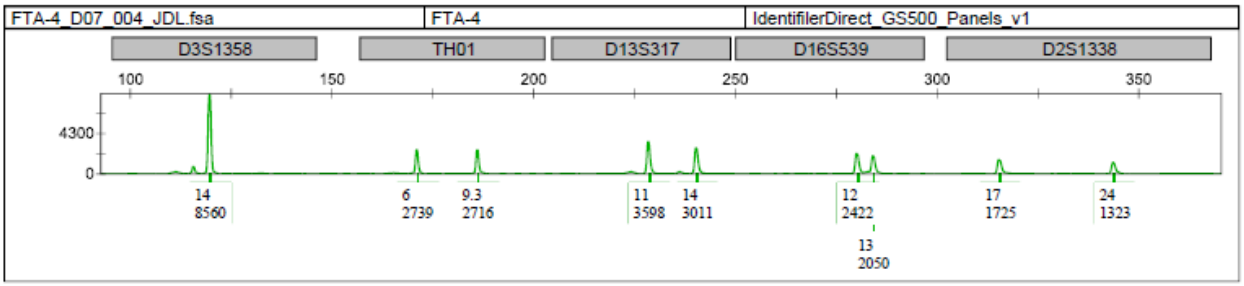
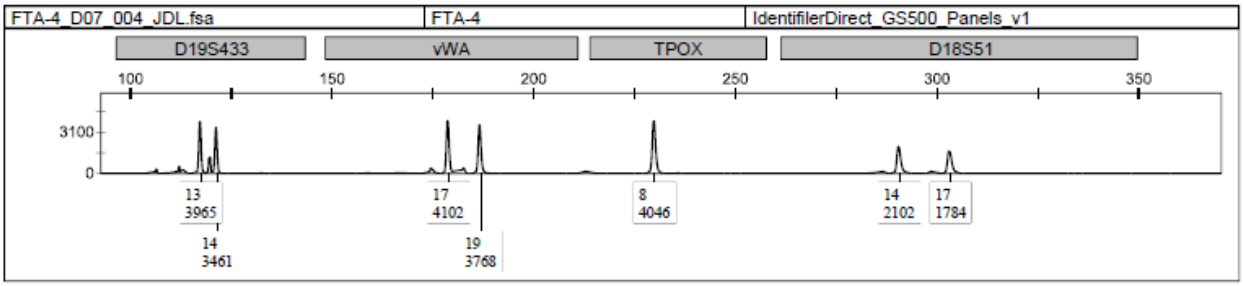
24	12, 13	28, 31	9, 12	11, 11	15, 15	7, 9.3	9, 12	12, 12	16, 19	14, 16	16, 20	9, 12	14, 19	X, Y	11, 12	20, 22

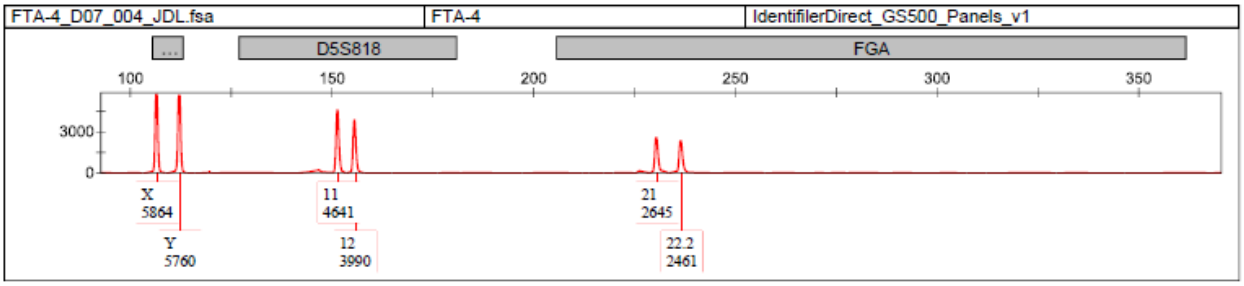
Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

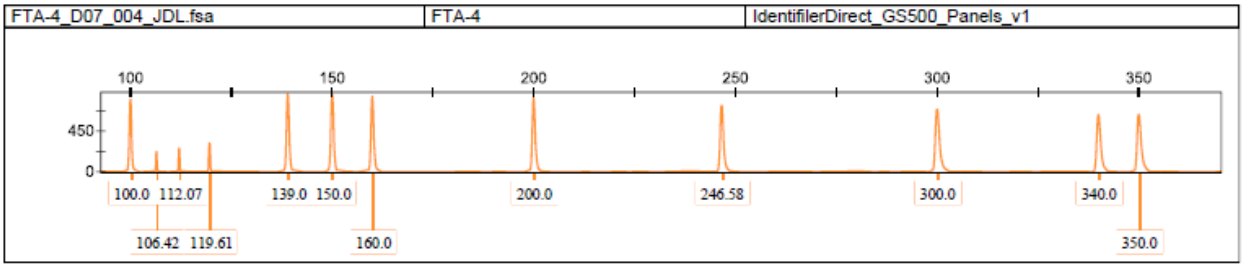
FTA (x24 samples), 3130 Genetic Analyzer, Sample #4, Injection 1 of 2 (5 seconds)











Method 16 (x24 samples) **3730 DNA Analyzer**

These samples were injected at both 5 and 10 seconds. The results below only depict the better of the two injections.

Method 16 (x24 samples) 3730 DNA Analyzer	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Injection Time (seconds)	Artifacts	Average RFU	Average PHR
	1	0.075	0.189	Dropout x 1	10		299	80.35%
	2	0.206	0.515	OK	10		656	84.05%
	3	0.660	1.650	OK	5		1548	86.18%
	4	0.403	1.008	Dropout x 1	10		1250	92.73%
	5	0.196	0.490	Dropout x 5	10		266	84.93%
	6	0.481	1.203	OK	5		1614	88.81%
	7	0.289	0.723	OK	5		1008	89.38%
	8	0.050	0.126	Dropout x 8	10		170	83.03%
	9	1.770	4.425	OK	5		1792	86.39%
	10	0.046	0.115	Dropout x 1	10		532	92.49%
	11	0.262	0.655	OK	5		812	87.37%
	12	0.122	0.305	Dropout x 6	10		278	87.34%
	13	0.735	1.838	OK	10		848	85.72%
	14	0.496	1.240	OK	10		637	82.38%
	15	1.000	2.500	OK	5		1607	91.50%
	16	0.020	0.050	No Data	10		-	-
	17	1.580	3.950	OK	5		1932	87.12%
	18	0.178	0.445	Dropout x 5	10		407	86.06%
	19	0.162	0.405	Dropout x 4	10		549	77.23%
	20	0.997	2.493	OK	5	Yes	1938	89.08%
	21	0.719	1.798	OK	10		718	87.84%
	22	0.360	0.900	OK	10		314	81.80%
	23	0.088	0.221	Dropout x 9	10		211	85.04%
	24	0.058	0.145	Dropout x 9	10		214	89.73%
Average:		**0.456**	**1.141**				**852**	**86.37%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
1	13, 13	29, 30	10, 11	10, 12	17, 17	9.3, 9.3	10, 13	11, 12	18, 19	13, 14	16, 16	8, 8	12, 16	X, Y	11, 13	20, 21
2	10, 14	28, 32.2	10, 12	10, 10	15, 19	6, 9	11, 12	11, 11	17, 17	13, 14.2	15, 16	10, 11	12, 13	X, X	11, 13	20, 26
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16	13, 15	28, 31.2	10, 11	11, 13	16, 16	9.3, 9.3	11, 12	11, 12	19, 22	14, 15	15, 17	10, 11	15, 19	X, Y	11, 12	20, 21
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18	11, 12	28, 30	11, 12	10, 11	14, 15	9, 9.3	11, 12	11, 11	20, 23	14, 15	14, 18	8, 11	12, 13	X, Y	10, 13	25, 25
19	12, 15	28, 31	8, 8	11, 12	17, 17	6, 9.3	11, 11	12, 13	17, 17	13, 13	17, 17	8, 8	14, 16	X, X	10, 11	22, 23
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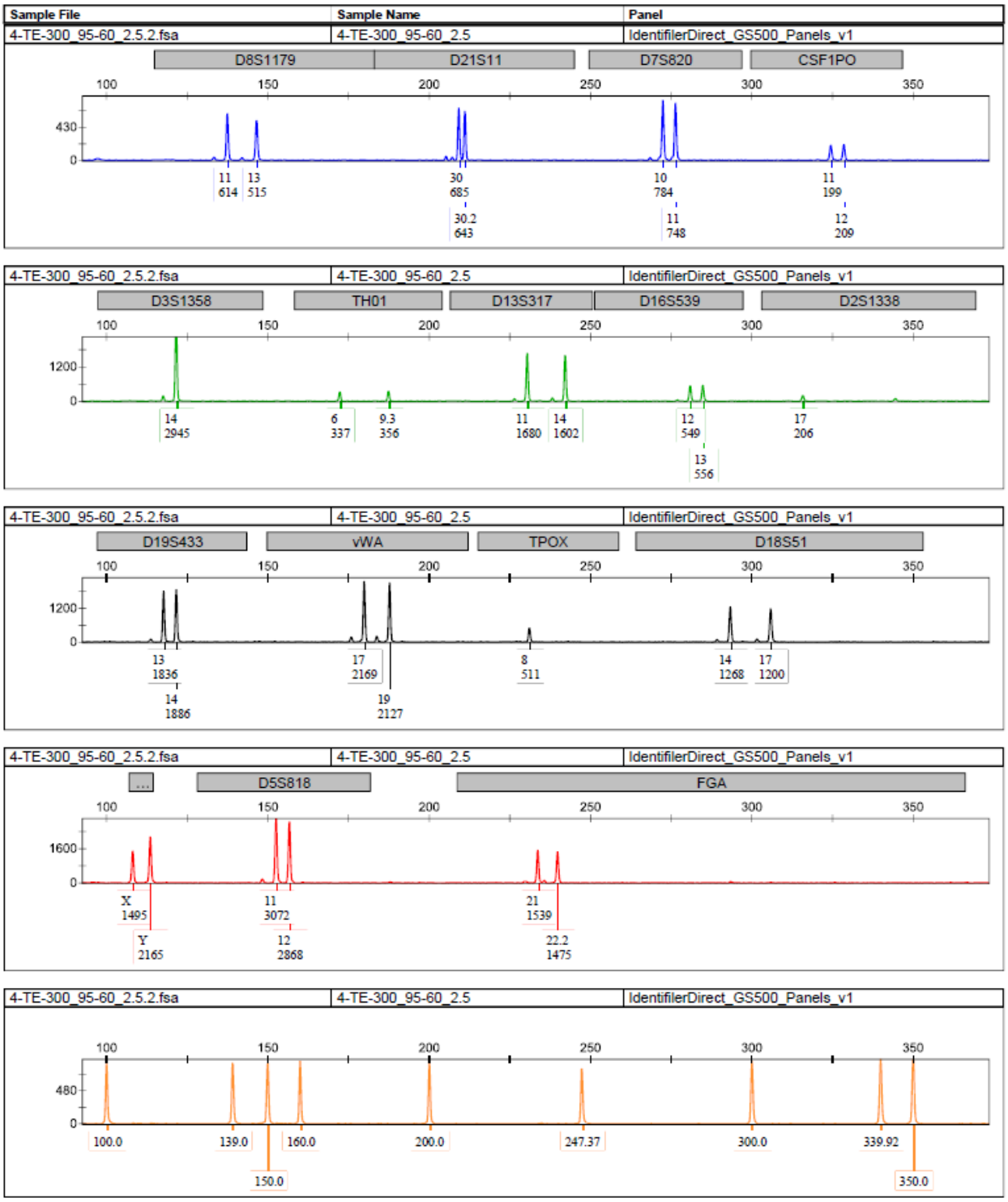
Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

Method 16 (x24 samples), 3730 DNA Analyzer, Sample #4, Injection 2 of 2 (10 seconds)



FTA® (x24 samples) **3730 DNA Analyzer**

Quantitation cannot be performed using this method, so the average DNA yield and average amplification target range cannot be determined. These samples were injected at both 5 and 10 seconds. The results below only depict the better of the two injections.

FTA (x24 samples) 3730 DNA Analyzer	Sample #	Quant Yield (ng/µl)	Amp target (ng)	Results	Injection Time (seconds)	Artifacts	Average RFU	Average PHR
	1	n/a	Unknown	OK	5		1068	88.97%
	2	n/a	Unknown	OK	5		2893	89.33%
	3	n/a	Unknown	OK	5		2954	86.51%
	4	n/a	Unknown	OK	5		2147	90.72%
	5	n/a	Unknown	OK	5		1075	92.22%
	6	n/a	Unknown	OK	5		1821	90.95%
	7	n/a	Unknown	OK	5		1108	88.72%
	8	n/a	Unknown	OK	5		1289	86.05%
	9	n/a	Unknown	OK	5		924	89.39%
	10	n/a	Unknown	OK	5		827	85.53%
	11	n/a	Unknown	OK	5		1797	87.07%
	12	n/a	Unknown	OK	5		1096	84.08%
	13	n/a	Unknown	OK	5		740	84.22%
	14	n/a	Unknown	OK	5		681	91.08%
	15	n/a	Unknown	OK	5		3608	88.12%
	16	n/a	Unknown	OK	5		2290	87.72%
	17	n/a	Unknown	OK	5		3034	81.07%
	18	n/a	Unknown	OK	5		3073	87.55%
	19	n/a	Unknown	OK	5		2715	84.63%
	20	n/a	Unknown	OK	5		2433	87.52%
	21	n/a	Unknown	OK	5		2024	86.59%
	22	n/a	Unknown	OK	5		1900	86.85%
	23	n/a	Unknown	OK	5		1911	84.46%
	24	n/a	Unknown	OK	10		3603	86.26%
Average:							**1959**	**87.32%**

	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Amel	D5	FGA
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2	10, 14	28, 32.2	10, 12	10, 10	15, 19	6, 9	11, 12	11, 11	17, 17	13, 14.2	15, 16	10, 11	12, 13	X, X	11, 13	20, 26
3	12, 14	29, 33.2	8, 11	10, 11	14, 16	6, 9.3	11, 12	12, 13	17, 24	13, 14	16, 17	8, 8	14, 15	X, X	11, 12	21, 24
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6	11, 13	32.2, 33.2	9, 11	10, 11	15, 16	6, 9.3	8, 11	11, 13	19, 22	14, 15	14, 18	10, 11	15, 17	X, Y	11, 11	22, 23
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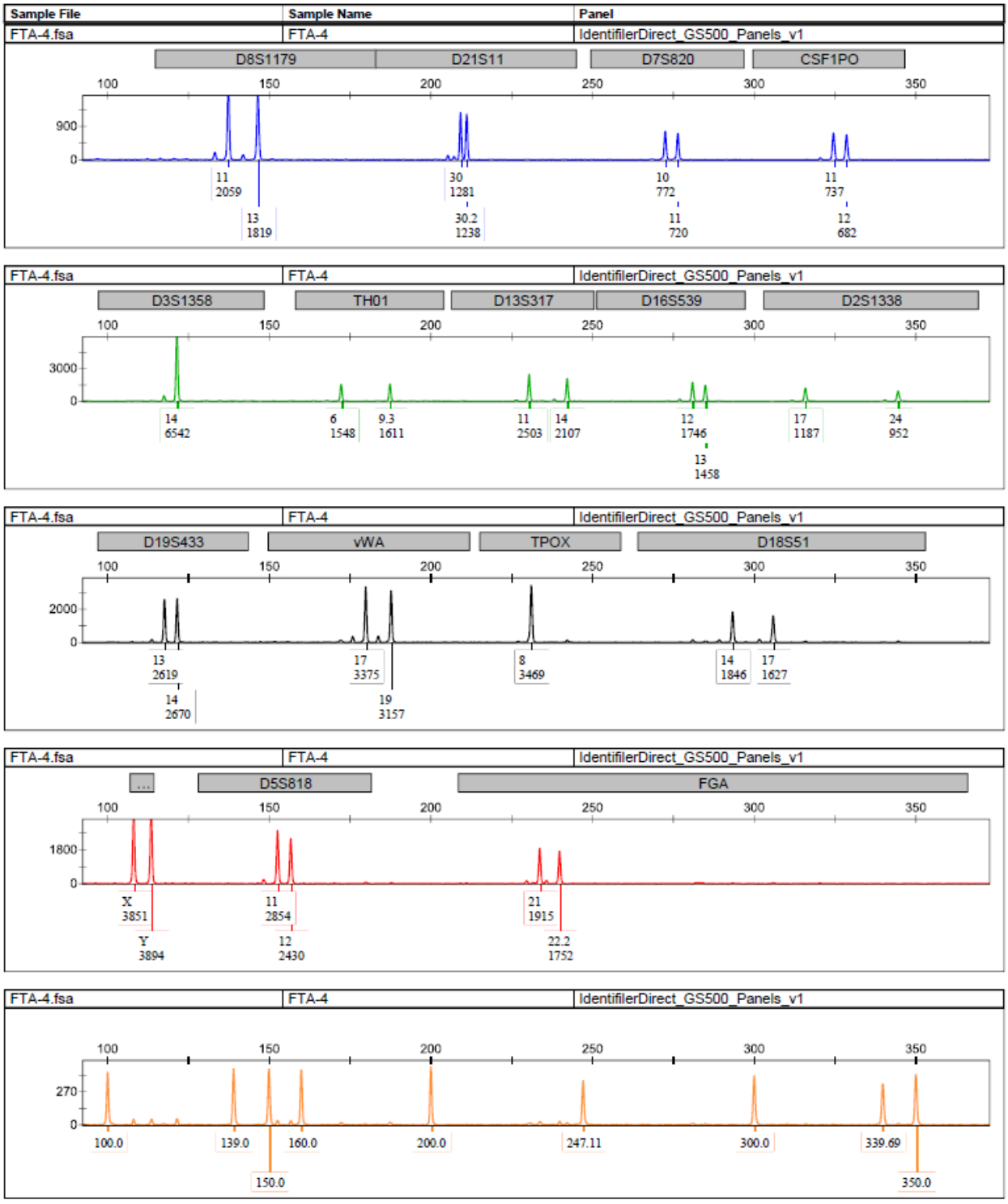
24	12, 13	28, 31	9, 12	11, 11	15, 15	7, 9.3	9, 12	12, 12	16, 19	14, 16	16, 20	9, 12	14, 19	X, Y	11, 12	20, 22

Note: This table lists the expected profile for each sample. Red font indicates the alleles that had dropped out.

FTA (x24 samples), 3730 DNA Analyzer, Sample #4, Injection 1 of 2 (5 seconds)



Conclusions

In study 1 we explored using different lysing reagents, different volumes for the lysing reagents, different lysing temperatures, and different lysing times. Although none of the ten method tested produced the desired results, the study did eliminated several different techniques and narrowed the scope of the research for future studies conducted. Lysing reagents that contained guanine, stain extraction buffer (2%), and dithiotreitol all proved to inhibit the PCR amplification. Water proved not to be a good lysing reagent regardless of the lysing temperature and the lysing time. Buccal swabs lysed with T.E.$^{-4}$ did show some potential, but DNA peaks observed were very low and no full DNA profiles were obtained. The conclusion drawn from study 1 was that direct amplification from a swab was possible, but the optimal lysing technique had not been obtained.

In study 2 we continued to explore different lysing techniques by modifying the lysing reagents, lysing reagent volumes, lysing temperatures, and lysing times. As with study 1, results obtained from study 2 eliminated several more techniques, and narrowed the scope of the research even further. Methods 13, 16, and 18 produced full profiles but had varying ranges in peak heights, peak height ratios, artifacts, and amount of preferential amplification. This is expected to be caused by the varying amounts of DNA that were present on the large swab heads. The conclusion drawn from study 2 was that samples lysed in T.E.$^{-4}$ could produce a full DNA profile, but the optimal lysing technique had not been obtained. Data also showed that, for maximum yield, 95°C appears to be the optimal lysing temperature and 60 minutes appears to be the optimum lysing time.

In study 3, two different sizes of swabs heads were used (small and medium). When analyzing FTA® cards, a standard 1.2mm size punch is used, which creates a more narrow range of DNA that can be amplified (due to a limited surface area of the punch taken). This in turn will produce a more consistent range of peak heights among all samples. For swabs, this same principle was applied by using a swab with a smaller size head. The smaller swab head will have a smaller surface area (similar to a 1.2mm FTA® punch) and should narrow the range of DNA

that is lysed. For all methods tested, all samples did have DNA peaks present, but the majority of them were below the peak detection threshold. 2.5ul of lysate is not a sufficient amplification volume for these medium and small swabs. This was further demonstrated when the samples were quantitated. It does appear that these swabs could give full profiles if a larger volume of lysate is used during the amplification (5.0ul, 7.5ul, 10ul, etc). This will only be known if additional studies are performed.

In study 4 we evaluated the effects of decreasing the amount of DNA template used during the amplification step. It has been proven that sometimes using less DNA template can greatly help reduce preferential amplification, thus producing cleaner, more balanced profiles. From the nine different methods analyzed, very few samples had any useable data, as the majority of the peaks observed were below threshold. 1.0ul of lysate is not a sufficient amplification volume for the large, medium or small swabs. This was further demonstrated when the samples were quantitated.

In study 5 we evaluated the effects of increasing the amount of DNA template used during the amplification step. Quantitation results for a lot of samples tested showed small amounts of DNA present. By increasing the amount of DNA template used during amplification, the intent behind study 5 was to increase the amount of DNA amplified without causing preferential amplification effects. From the six different methods analyzed, all samples did have DNA peaks present, but the majority of them were below the peak detection threshold. However, increasing the amount of template DNA that is amplified did increase the peak heights of most samples without causing artifacts and preferential amplification effects. It does appear that these swabs could give full profiles if a larger volume of lysate is used during the amplification (5.0ul, 7.5ul, 10ul, etc). This will only be known if additional studies are performed. The three best methods from studies 1 through 4 were not analyzed using 5.0ul of template DNA, an oversight by the laboratory. It is expected that this combination may have produced data comparable to the FTA® cards, but this will only be known if additional studies are performed.

For study 6, no additional new methods were attempted. After calculating and comparing data from studies 1 though 5, the best 3 methods were selected. Study 6 tested a larger number of samples lysed under the same conditions as methods 13, 16, and 18 (see Table 7). The data from study 6 was carefully examined and compared. Taking all factors into account, it was decided that method 16 gave the best overall data. This method was used for the final comparison against FTA® cards.

For study 7, a side-by-side comparison was made between buccal samples lysed using method 16, and FTA® cards amplified according to the Identifiler® Direct recommended procedures. Results of this comparison are seen in Table 9 (see appendix 1 through 4 for electropherograms).

Table 9: Study 7 side-by-side comparison

Sample Type	Genetic Analyzer	Average RFU (5 seconds)	Average RFU (10 seconds)	Average PHR (5 seconds)	Average PHR (10 seconds)	% Pass	% Artifacts
Swabs	3130	1257	2039	85.34%	85.64%	63%	17%
FTA	3130	2836	4420	87.19%	88.42%	88%	58%
Swabs	3730	725	1327	86.69%	86.34%	58%	4%
FTA	3730	1892	3483	87.31%	87.64%	100%	0%

For both the 3130 and 3730 Genetic Analyzers, the FTA® samples had a better pass rate, with 100% of the samples tested providing a full DNA profile (CODIS uploadable) on the first analysis attempt. For both buccal swab and FTA® samples, the best overall results were obtained on the 3730 DNA Analyzer. The 3730 has an off-scale detection limit of 30,000 rfu, while the 3130 is ~8,200 rfu. This larger off-scale limit allows for more variation in peak heights between samples, something commonly observed when taking any sample to direct amplification.

Although the swab samples only had a 58% pass rate on the 3730, the majority of samples that did not pass were due to allelic dropout. This can be expected since the overall

average rfu value for these samples was less than half the FTA® samples. When examining the quantitation results for the method 16 samples, the average amplification target range was 0.321ng, much lower than the typical recommended range of 0.5-1.0ng. By increasing the amplification template volume to 5.0µl, the peak heights for the buccal samples would increase, and an increased number of samples should produce a full DNA profile. This would undoubtedly produce a more consistent result between the buccal swabs and the FTA® samples. However, this can only be proven through additional research, which time simply did not allow for in this research study.

Recommended Additional Research

42 different lysing methods were attempted in this research project. Although none of these methods tested on swabs produced better results than FTA® cards, useable data was obtained. We highly encourage additional research studies be conducted to find the exact combination of lysing reagents, lysing temperatures and lysing times to allow direct amplification from swabs. Recommendations for further research include:

1. Amplify methods 13, 16, and/or 18 using an increased volume of sample lysate (5.0µl, 7.5µl, 10.0µl), and inject them at 5 and 10 seconds on a 3130 Genetic Analyzer and a 3730 DNA Analyzer. This should increase the peak heights observed to a comparable level as the FTA® cards.
2. Take a 1.2mm punch from the side or tip of a buccal swab and go straight to amplification (similar to using a 1.2mm FTA® punch). No pre-lysing of the swab. The initial step of 95°C for 11 minutes on the thermalcycler may also lyse open cells allowing them to be amplified. One could also increase this initial step to 30 minutes to see what the effects are.
3. Add lysing reagents to the swab and allow the swab to dry (similar concept as with lysing reagents on FTA® cards). Take a punch of the swab directly to amplification.
4. Take a 1.2mm punch from the side or tip of a buccal swab, add small amounts of lysing reagents, add amplification reagents, place onto thermalcycler. Use the first step of 95°C for 11 minutes to lyse the sample at the same time the polymerase is being

activated. Can also extend this initial step to 30 minutes to see what the effects are. If the lysing reagents used are mild enough, they may not interfere with the amplification reagents during the amplification process.

5. Explore the use of using half the amount of amplification reagents (1/2 reaction study) for both buccal swabs and FTA® cards.

In a recent DNA conference it was announced that Applied Biosystem's has been conducting research on a technique that would allow the Identifiler® Direct kit to be used on buccal swabs. According to the information presented at this conference, a technique has been successfully validated and is due to be released to the forensic community in the very near future. However, until this technique is tested it remains unclear if a better method for direct amplification of buccal swabs exists.

Conclusion

Any forensic laboratory, regardless of size, can increase their efficiency and prepare to handle an increased number of sample submissions without creating a backlog. In doing so, laboratories should not solely look at purchasing more equipment and hiring more analysts, but rather re-evaluate their current procedures being used in an effort to eliminate inefficiencies.

Two key weaknesses in the current DNA analysis methods have been identified. If improved, the cost of analysis per sample and the time it takes to process these samples with drastically decrease. Whether a CODIS database sample or a known sample in a criminal case, these new methodologies provide a more efficient and cost effective means of performing DNA analysis on known reference samples. This will have a significant impact on the overall backlog of criminal cases.

References

[1] Applied Biosystems™ Product Bulletin "AmpFℓSTR® Identifiler® Direct PCR Amplification Kit", September 2009

[2] National Institute of Justice Special Report "Making Sense of DNA Backlogs", June 2010

[3] www.Whatman® .com

[4] www.Bode™tech.com

Appendix 1

Protocol for Swab Samples (Method 16)

Lysis Step:

1. Take an entire swab head and place it into a 96-well plate (Promega's Slickprep 96 device) or a 1.5µl microcentrifuge tube.
2. Dispense 300µl of T.E.$^{-4}$ to each sample well/tube.
3. If using a 96-well plate, seal the device with an adhesive cover.
4. Incubate the 96-well plate or sample tube(s) in a water bath at ~95°C.
5. After ~60 minutes, remove the 96-well plate or sample tube(s) from the water bath.
6. If using a Slickprep 96 device, insert the u-shaped collar so the device is in the spin position.
7. Centrifuge the 96-well plate/tube(s) at ~1,500g for ~5 minutes to remove any liquid from the swab.

Amplification Step:

1. Dispense 25µl of Identifiler® Direct PCR reagents to a 96-well amplification plate or individual amplification tubes.
2. Add 2.5µl (or amount validated internally by your laboratory) of the sample lysate to each appropriate well/tube.
3. If using a 96-well plate, seal the device with an adhesive cover.
4. Amplify samples on a 9700 thermalcycler using the following PCR cycling conditions:

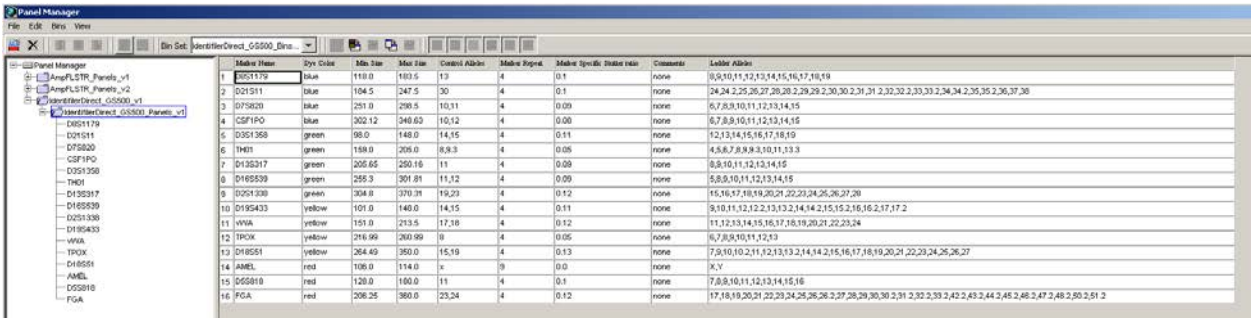
Initial Incubation Step	Cycle (26, 27, or 28 cycles)			Final Extension	Final Hold
	Denature	Anneal	Extend		
Hold	Cycle			Hold	Hold
95°C	94°C	59°C	72°C	60°C	4°C
11 minutes	20 seconds	2 minutes	1 minute	25 minutes	∞

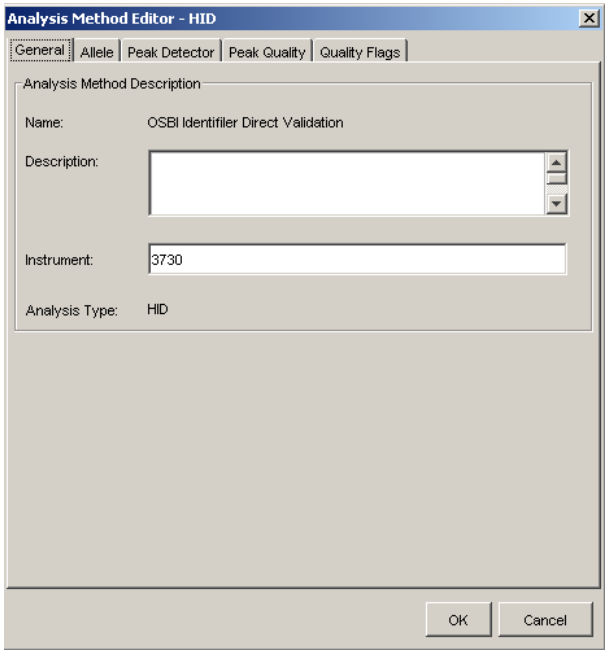
Genetic Analysis:

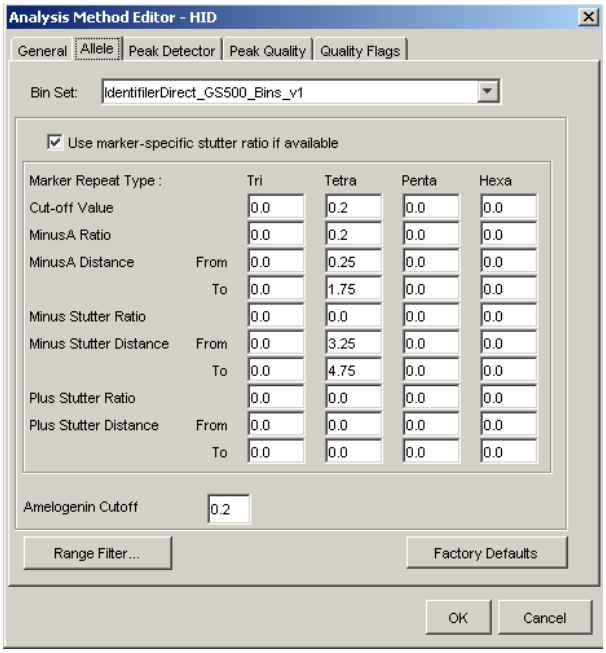
1. Prepare a mixture of deionized formamide and GS-500 LIZ size standard according to manufacturer's recommendations.
2. Dispense 9µl of the formamide/LIZ solution into the appropriate wells of the optical plate.
3. Add 1.0µl of PCR product or Identifiler® Direct allelic ladder to the appropriate sample wells.
4. Cover the optical plate with a 96-well plate septa.
5. Inject samples on either a 3130 Genetic Analyzer or a 3730 DNA Analyzer following manufacturer's recommendations.

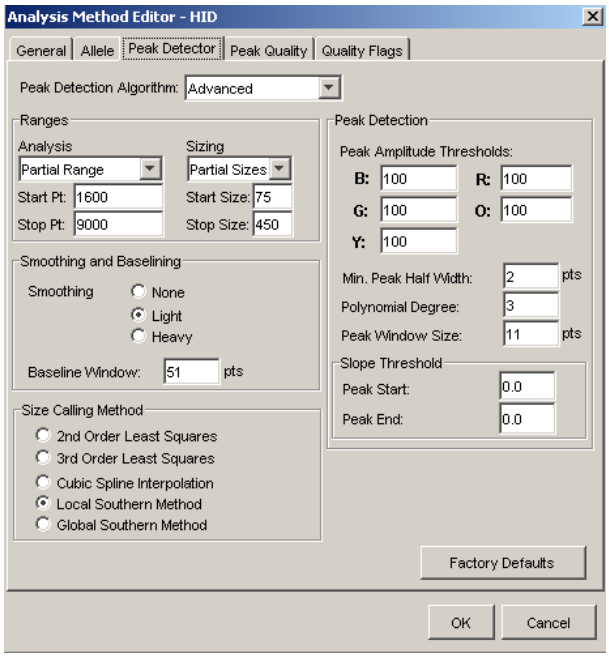
Appendix 2

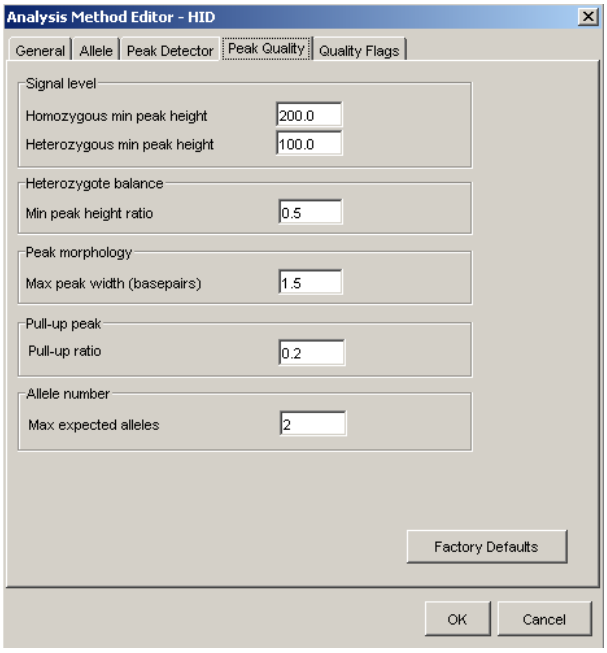
GeneMapper ID v3.2 Settings:

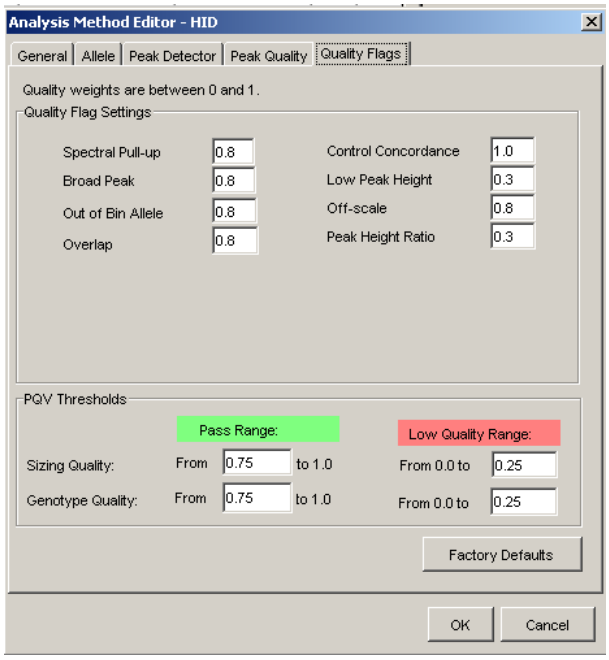


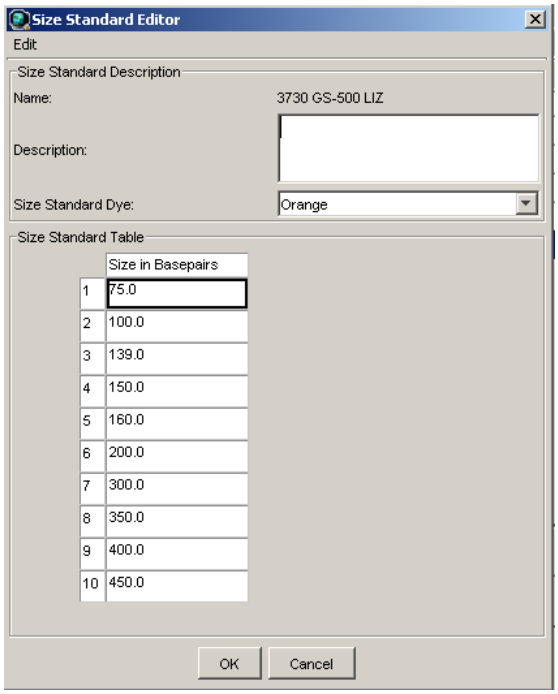












Appendix 3

Chemical Formulation for Stain Extraction Buffer:

Stain Extraction Buffer					
Components	*Quantity*	*Actual Quantity*	*Supplier*	*Lot #*	*Expiration*
1 M Tris-HCl	5 mL				
0.5 M EDTA	10 mL				
Dithiothreitol (DTT)	3.01 g				
5 M NaCl	10 mL				
20% SDS	50 mL				
Ultrapure DI H_2O	~500 mL		OSBI		
Sodium Hydroxide					

Preparation

Add Tris-HCl, EDTA, DTT, and NaCl to ~half of the ultrapure deionized water. Add sodium hydroxide until the pH of the solution is 8.0 ± 0.2. Add to this mixture the 20% SDS. Add a sufficient quantity of ultrapure deionized water to the solution to achieve the desired final volume. Aliquot the buffer.

Storage

The aliquoted Stain Extraction Buffer can be stored frozen, refrigerated, or at room temperature.

Appendix 4

Chemical Formulation for ProK:

Proteinase K					
Components	*Quantity*	*Actual Quantity*	*Supplier*	*Lot #*	*Expiration*
Proteinase K	500 mg				
Ultrapure DI H_2O	25 mL		OSBI		

Preparation

Dissolve Proteinase K in ultrapure deionized water. Aliquot the solution. Do not UV sterilize Proteinase K.

Storage

The aliquoted Proteinase K shall be stored frozen. Discard tube and unused enzyme after initial use. Do not reuse leftover enzyme.

Appendix 5

Chemical Formulation for T.E.$^{-4}$:

T.E.$^{-4}$					
Components	*Quantity*	*Actual Quantity*	*Supplier*	*Lot #*	*Expiration*
1 M Tris-HCl	10 mL				
0.5 M EDTA	200 µL				
Ultrapure DI H_2O	~1.0 L		OSBI		N/A

Preparation

To approximately ¾ of total volume of ultrapure deionized water, add Tris-HCl (pH 8.0) and EDTA. Add a sufficient quantity of ultrapure deionized water to the solution to achieve the desired final volume. Autoclave before storage.

Storage

The stock TE^{-4} reagent and aliquots should be stored at room temperature.