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DNA analysis for human identification is a multi-step process culminating in the generation of a DNA profile unique to the contributor of the biological sample. In order for human identification by way of DNA analysis to be successful, comparisons of a known sample to an unknown sample must be made. Processing of the known or reference samples should be efficient, reliable and reproducible. The Buccal DNA Collector™ (Bode Technology Group, Lorton, VA) used in conjunction with the AmpFLSTR® Identifiler® Direct PCR Amplification Kit (Applied Biosystems, Foster City, CA) has been shown to be an effective method for processing single source reference samples. This technique can be reliably applied to the processing of samples for DNA databasing, paternity or reference samples for forensic casework.

# OPTIMIZATION AND VALIDATION OF THE BODE™ BUCCAL DNA COLLECTOR™ WITH THE AMPFLSTR® IDENTIFILER® DIRECT PCR AMPLIFICATION KIT FOR SINGLE-SOURCE REFERENCE SAMPLES

Presented to the Graduate Council of the

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By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLISTRATIONS

# INTRODUCTION

DNA analysis has proven to be a useful tool for the forensic community. This type of testing is particularly helpful for human identification, kinship analysis, paternity testing and DNA databasing. The process of obtaining a DNA profile involves the analysis of genetic polymorphisms that have been identified within the human genome. DNA profiles are obtained from reference samples provided by known individuals and evidentiary samples originating from an unknown contributor. The DNA profiles are then compared in order to determine the source of the unknown evidentiary sample. In order to obtain a DNA profile, a step-wise process is conducted beginning with the collection of a biological sample. Once collected, the DNA contained within the sample is extracted, the DNA is purified away from potential PCR inhibitors, quantified to determine the amount of sample recovered, an optimum amount of DNA is then added to the PCR amplification reaction to identify the polymorphic variations within the DNA, the different length amplified products are then separated by capillary electrophoresis, and finally the results are analyzed by one or more computer programs. The end result of this time-consuming and labor-intensive process is a genetic profile unique to every person, with the possible exception of identical twins. For the purposes of human identification, DNA analysis requires the collection of appropriate reference samples for comparison to missing persons or unidentified decedent samples. The reference samples must under go the same step wise laborious and costly process to generate a DNA profile.

The types of samples that are routinely encountered in forensic casework are highly variable. However, the types of samples collected for use as reference samples are typically either a blood or buccal sample. Reference samples are generally collected by either swabbing the inside of the cheek for buccal cells, or by phlembotomy or a finger stick for the collection of

blood. The profiles derived from these samples are almost always from a single source and of known origin. The purpose of this project was to develop a method that would decrease the time and labor of processing single source reference samples by eliminating the purification and quantification steps of DNA analysis. The hypothesis is that by utilizing a novel sample collection device in conjunction with a new PCR STR amplification kit the purification and quantification steps of DNA analysis can be eliminated and still produce reliable and consistent genetic profiles.

This project tested the use of the Buccal DNA Collector™ (Bode Technology Group, Lorton, VA), as the collection device, in conjunction with the AmpFLSTR® Identifiler® Direct PCR Amplification Kit (Applied Biosystems, Foster City, CA). In the development of the AmpFLSTR® Identifiler® Direct PCR Amplification Kit, Applied Biosystems was obtaining DNA profiles from both buccal samples and bloodstains collected on FTA® Paper (Whatman Inc., Clifton, NJ) (7). FTA® Paper consists of 100% cotton paper with a proprietary solution that has been impregnated into the paper. A biological sample such as either buccal cells or a small amount of blood is directly applied to the FTA paper. Upon contact the buccal or white blood cells lyse and the DNA compacted within the nucleus is released and entangles, becoming trapped within the matrix of the paper (4). In addition, the reagents within the FTA treated paper prevent the bound DNA from degrading and allows for storage at room temperature. A small sample punch is taken and that punch is washed several times in order to remove PCR inhibitors, such as the heme found within the red blood cells as well as the chemicals that are impregnated in the paper (5). This washing step can take 2 to 3 hours. Once this is complete the sample is ready for amplification. Since FTA® Paper contains a number of different chemicals, the FTA paper cannot be directly placed within the mouth, and secondary transfer step is required to for

the application of buccal cells onto the FTA Paper. This can be problematic because collection of a buccal sample and then transfer to the FTA® Paper is inefficient a substantial amount of the collected sample could be lost.

The Buccal DNA Collector™ is a collection device that could be used to obtain buccal and blood samples directly on cotton paper without any need for a secondary transfer step. It consists of a plastic handle and a collection paper which is comprised of untreated 100% cotton paper. Since the collection paper is not treated, the cells will not efficiently lyse upon initial contact with the paper. However during the drying process the cells will break down and the DNA will ultimately entrap. Similar to FTA® Paper, a small 1.2 sample punch is taken from the Bode collection device. However, with the Buccal DNA Collector™ the sample punch is added to the Bode PunchPrep™ Solution, and is then incubated at 70°C for 20 minutes. This additional incubation step is presumed to increase the efficiency of cell lysis and the release of DNA from the cells collected on the device. The AmpFLSTR® Identifiler® Direct amplification mix can then be added directly to the sample plate (2). The Buccal DNA Collector™ was the collection device chosen for this project for the following reasons: the collection paper is untreated, therefore a direct buccal sample can be obtained; also, the extraction step is a 20 minute incubation as opposed to a 2 to 3 hour washing step.

The AmpFLSTR® Identifiler® Direct PCR Amplification Kit was the amplification system chosen to work in conjunction with the Buccal DNA Collector™. The design of the AmpFLSTR® Identifiler® Direct PCR Amplification Kit was developed in order to eliminate the need for DNA purification and quantification from buccal or blood single source reference samples. This kit consists of two separate components. The primers, which include the 13 CORE CODIS loci, CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820,

D8S1179, D13S317, D16S539, D18S51, D21S11, in addition to D2S1338 and D19S433 (7). This primer set also includes degenerate primers for D8S1170, vWA, and D16S539. The second component is the PCR reaction mix, which contains a proprietary buffer system, dNTPS, a carrier protein to help overcome inhibition, and the Taq polymerase. The PCR buffering system and cycling parameters of the AmpFLSTR® Identifiler® Direct PCR Amplification Kit were optimized to overcome the inhibitors that are usually removed during the purification step (6). In 2004, the University of Granada (UGR), Spain, Genetic Identification Laboratory suggested the development of worldwide DNA registries consisting of parents with a missing or abducted child, homeless children found living on the street or in shelters, and children found in orphanages or illegal adoption centers. From this idea the UGR in collaboration with the University of North Texas Center for Human Identification (UNTCHI) launched the DNA-ProKids (Program for Kids Identification with DNA Systems) program. DNA-ProKids is an international humanitarian effort to help identify missing children in order to reunite abducted and homeless children with their parents, and to provide law enforcement agencies a scientific methodology to help deter the human trafficking of children (1). In order to facilitate the collection and processing of samples for the DNA-ProKids program and to increase the utilization of the program throughout the word a simple and efficient process for obtaining DNA profiles must be developed and validated. The use of the Bode Buccal DNA Collector ™ in conjunction with the AmpFLSTR® Identifiler® Direct PCR Amplification Kit could provide the required collection system and an efficient and effective method of processing the reference samples. This collection device together with the new AmpFLSTR® Identifiler® Direct PCR amplification kit could simplify the process and save significant amounts of time when processing single source reference samples. This is vital for the expansion of DNA databasing.

The more samples that can be processed and loaded into DNA databases, the greater the potential for making identifications, as well as reuniting families and ultimately saving lives.

# MATERIALS AND METHODS

This study consisted of a series of experiments designed to generate data in order to optimize the various steps within the entire process. Once each step in the process had been optimized the overall protocol was tested to assess the ability to obtain reliable DNA profiles from single source reference samples. The validation of the optimized protocol is required by National Standards prior to implementation and analysis of actual casework type samples.

## *Samples*

The samples used in this study were obtained from 100 anonymous volunteer donors under UNTHSC IRB protocol number 2010-012. Each of these volunteer donors provided both a buccal swab sample as well as a blood sample. The samples were collected on the Buccal DNA Collector™ by self-collection of buccal cells from inside of the cheek and by blood applied to the collector from a finger stick. The protocols for collection of these two samples types are in Appendix C and Appendix D. These 100 paired samples were used for both the optimization and validation portions of this project.

## *Sample preparation*

All samples for the optimization experiments and the validation portion of the project were processed in high throughput configuration utilized a 96 well plate. A 2 µL of the Bode PunchPrep™ Solution (formula is proprietary) was added to each sample well. A 1.2 mm punch was taken from the collection device using a Harris Micro-Punch (Ted Pella, Inc. Redding, CA) and added directly to the 2 µL to the Bode PunchPrep™ Solution. The sample plate underwent a 20 minute incubation at 70°C. While conducting the optimization experiment, static electricity

was observed. Static electricity can be problematic as the punched disc may "jump" out of the well and into a non-designated well. The protocols were modified by adding the Bode PunchPrep™ Solution FIRST, then punching the disc directly into the appropriate well location. By changing these two steps, a decrease in static electricity was observed. Also, evaporation of the Bode PunchPrep™ Solution was observed. Therefore, when processing a full plate of samples, dispense the Bode PunchPrep™ Solution into a maximum of four columns, add the punched sample disc to the appropriate wells, and then seal the columns. These steps should be repeated until the plate is complete.

*Amplification set-up*

During the incubation period, the master mix for the amplification was made. The necessary volumes of reagents were calculated by taking the number of samples to be run and adding a 10% pipetting error. The master mix is made by a 1:1 mixture of the AmpFLSTR® Identifiler® Direct PCR reaction mix and the AmpFLSTR® Identifiler® Direct primers. Once the incubation is complete, 25 µL of the master mix is dispensed directly into each sample well.

*Amplification*

The thermocycler parameters are dependent on the sample type being amplified (*i.e.* buccal samples versus bloodstains). Once the amplification master mix is added to each sample well, the plate is then placed on a GeneAmp® PCR System 9700 (Applied Biosystems). The thermocycling program was selected for the appropriate sample type (See Appendix E).

*Capillary Electrophoresis*

Capillary electrophoresis was performed on an ABI PRISM® 3130*xl* Genetic Analyzer (Applied Biosystems). Before the amplified product could be analyzed, it had to be prepared for capillary electrophoresis. This was done by preparing a master mix of Hi-Di Formamide and GeneScan 500 LIZ Size Standard (Applied Biosystems). For each sample, the master mix contained 8.7 µL of Hi-Di Formamide and 0.3 µL of the GeneScan 500 LIZ Size Standard was added. The total volume of the master mix was calculated based upon the total number of samples and then increased by an additional 10% to account for any potential pipetting error. A new 96 well plate was used and 9 µL of the master mix was added to every well that would be used to run amplified product or allelic ladder. Once the master mix was dispensed, 1µL of amplified product or allelic ladder was added to the appropriated sample well. The allelic ladder used was the AmpFLSTR® Identifiler® Direct Allelic Ladder (Applied Biosysems).

*Data analysis*

Data analysis and interpretation was performed using GeneMapper®*ID* v3.2.1 (Applied Biosystems) analysis software. This software was used in two functions. It was used to interpret the data generated from the genetic analyzer and make the appropriate allele calls, generate peak heights and peak areas for all samples for statistical analysis. The second function of the software is as an expert system. The software requires the user or laboratory to establish a defined set of criteria that includes thresholds of detection for each allele, an acceptable peak height for heterozygotes and homozygotes, and an acceptable peak height ratio. If the data do not meet this defined set of criteria, then the expert system will fire a rule. The rule firings are an indication to the analyst that closer inspection of the data may be needed. These defined sets of criteria for the

lab that this project was conducted in are as follows. The detection threshold for all alleles was set at 50 relative florescence units (RFU). With respect to peak height, the detection threshold was set at 200 RFUs for homozygotes and 100 RFUs for heterozygotes. The acceptable peak height ratio for this lab is anything greater than or equal to 60%.

*Statistical Analysis*

Statistical analyses were performed, using Microsoft Excel and the Data Analysis ToolPak add-in for Microsoft Excel, in order to determine any variation between sample sets, loci and the dyes of the STR amplification kit. These analyses included an ANOVA test, paired t-test, and a Chi-squared test. These statistical tests were performed with respect to peak height ratio and peak area. Peak height (PH), peak area (PA), peak height ratio (PHR), and peak area ratio (PAR) were calculated for each locus. The average PH, average PHR, average PA, and average PAR were calculated per locus, per dye color and per sample set.

## RESULTS

Several parameters were evaluated to produce an optimized protocol using the Buccal DNA Collector™ in conjunction with the AmpFLSTR® Identifiler® Direct PCR Amplification Kit for the use of buccal samples and bloodstains: 1) An evaluation of two different Harris MicroPunch (Ted Pella, Inc. Redding, CA) devices (1.0mm and 1.2mm) for buccal samples. Only the 1.2mm was used for the bloodstains. Since blood has a consistently higher amount of cells for a 1.2 mm punch size, different cycle numbers for PCR amplification of both buccal samples and bloodstains were evaluated; 2) Two different in-house incubation solutions were evaluated; 3) Varying amounts of Bode PunchPrep™ Solution for incubation were evaluated; 4) A map of the Buccal DNA Collector™ was designed to evaluate the quantity of DNA for each of its five regions for buccal sample collection (Figure 1). Since bloodstains are visible, a punch from the center of the stain was taken.

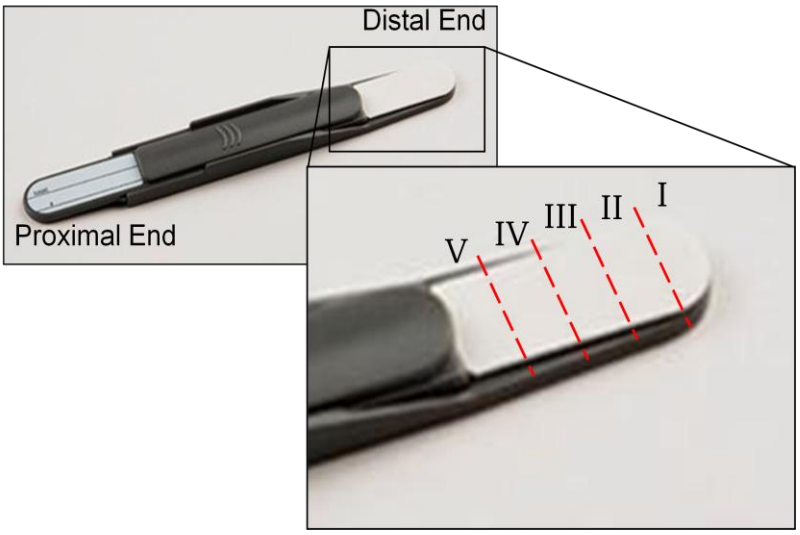



**Figure 1 Region Map of the Buccal DNA™ Collector.** The Buccal DNA Collector™ was mapped into five different regions. Discs were punched from the center of each region. (Photograph courtesy of Pam Curtis)

The optimized protocols were implemented for the validation portion of this project. A total of 200 samples were processed. These samples consisted of 100 paired buccal and blood samples. The buccal samples were processed separately from the bloodstains due to the different cycle numbers.

Procedures for 1.0mm and 1.2mm punch sizes were optimized for buccal samples and a 1.2mm punch size procedure was optimized for bloodstains. The cycle numbers that exhibited increased locus and allele dropout, low peak height, increased pull-up, and increased stutter include 28 cycles for the 1.0mm punch size for buccal samples, 27 cycles for the 1.2mm punch size for buccal samples, and 26 cycles for bloodstains. The cycle number that consistently produced a balanced profile was chosen for each punch size and sample type (Table 1).

| Sample | Buccal | | Blood |
|---|---|---|---|
| Punch Size | 1.0mm | 1.2mm | 1.2mm |
| Cycle Number | 26 | 25 | 24 |
| | 27 | 26 | 25 |
| | 28 | 27 | 26 |

**Table 1 Evaluation of Cycle Number.** Optimization experiments were performed to evaluate the best cycle number for each sample type and each sample punch size. The chosen cycle numbers are highlighted. These cycle numbers produced complete and balanced profiles.

Evaluation of two in-house solutions was performed in addition to the Bode PunchPrep™ Solution. A solution comprised of 0.1% SDS in 1X PBS Buffer (made fresh in-house) and a solution comprised of 0.1% SDS in $TE^{-4}$ Buffer (made in-house) were the incubations solutions evaluated. The results indicated that either one of these solutions would produce profiles with acceptable RFU levels. The Bode PunchPrep™ Solution produced a higher signal intensity and therefore was determined to be the optimal incubation solution. Results for evaluation of these solutions are shown in Figure 2 below. Figure 2 is representative of all the samples from this experiment.

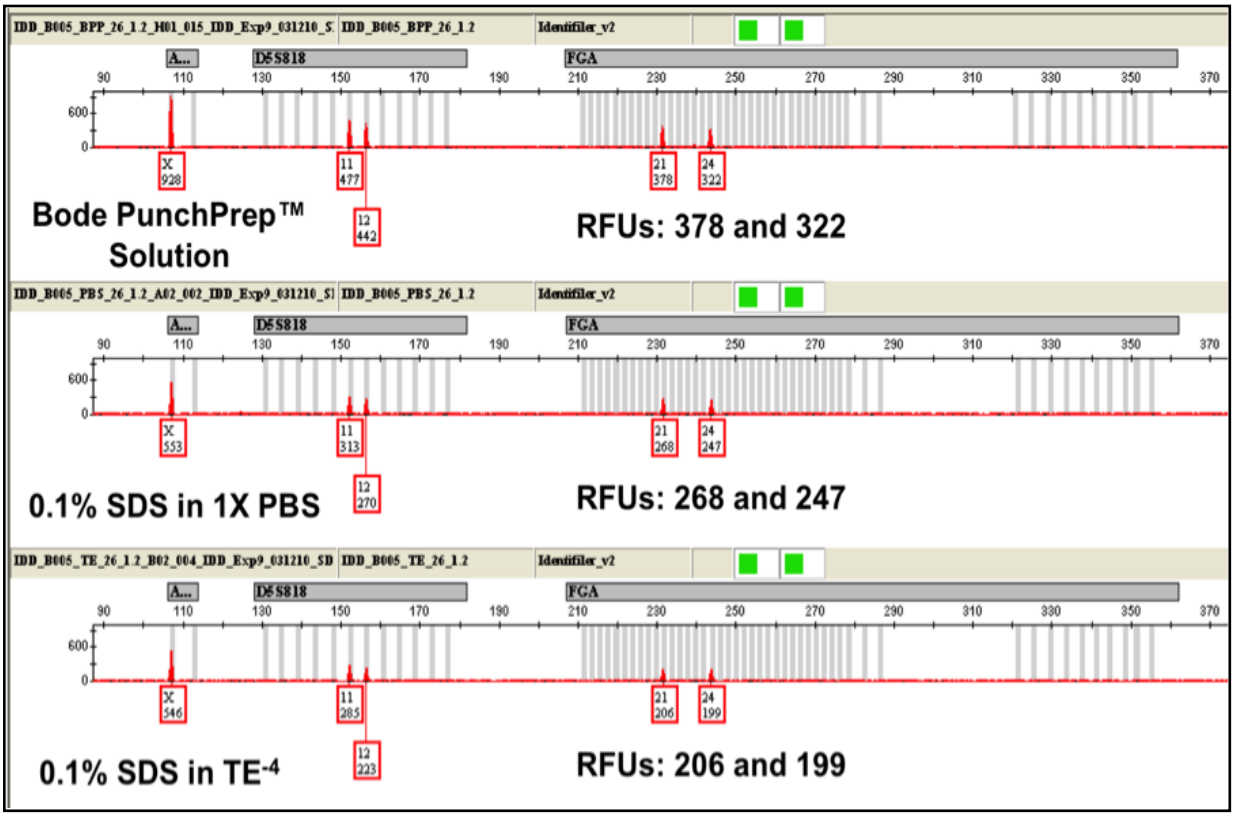



**Figure 2 Electropharogram of Amelogenin, D5S818, and FGA for In-house Incubation Solution Evaluation.** The RFU levels are labeled for the FGA locus. As shown, both in-house solutions produced acceptable RFU levels; however the Bode PunchPrep™ Solution produced the highest signal intensity. Relative fluorescence units (RFU) are labeled for the FGA locus.

Incubation experiments were conducted using 0µL, 2µL (manufacturer's recommended volume), and 4µL of the Bode PunchPrep™ Solution. With 0µL of the Bode PunchPrep™ Solution, locus dropout and low signal were observed. With 4µL of Bode PunchPrep™ Solution, decreased signal intensity was observed; this decrease in signal was possibly due to dilution of the amplification reaction mix. A balanced profile was obtained using the 2µL volume of Bode PunchPrep™ Solution (Figure 3). Figure 3 is representative of all samples processed for this experiment.

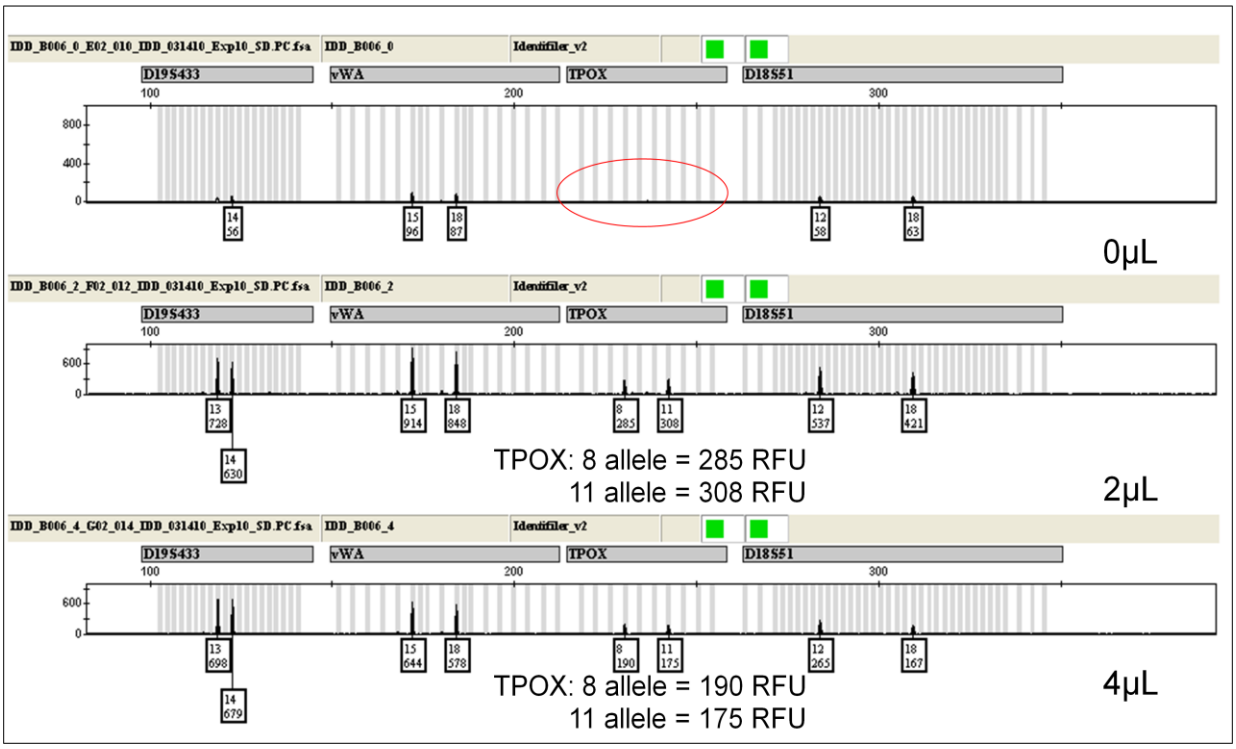



**Figure 3 Electropharogram of D19S433, vWA, TPOX, and D18S51 for Incubation Solution Volume.** The same buccal sample was incubated in 0µL, 2µL, and 4µL of the Bode PunchPrep™ Solution. Locus dropout was observed at the TPOX locus for the 0µL incubation volume (red elliptical). The relative fluorescence units (RFU) at the TPOX locus for 2µL and 4µL are labeled. The manufacturer's recommendation of 2µL was found to be the optimal Bode PunchPrep™ Solution volume.

The collection end or the distal end of the collection device was mapped into five different regions. A decrease in signal was observed in the most proximal regions of the Buccal DNA Collector™ (*i.e.*, Regions III, IV, and V). Full profiles were obtained from all five regions, but consistent achievement of full profiles and high signal intensity is best obtained with punched discs from Regions I and II (Figures 4 and 5). Figure 4 and Figure 5 representative of all the samples that were tested for this experiment.

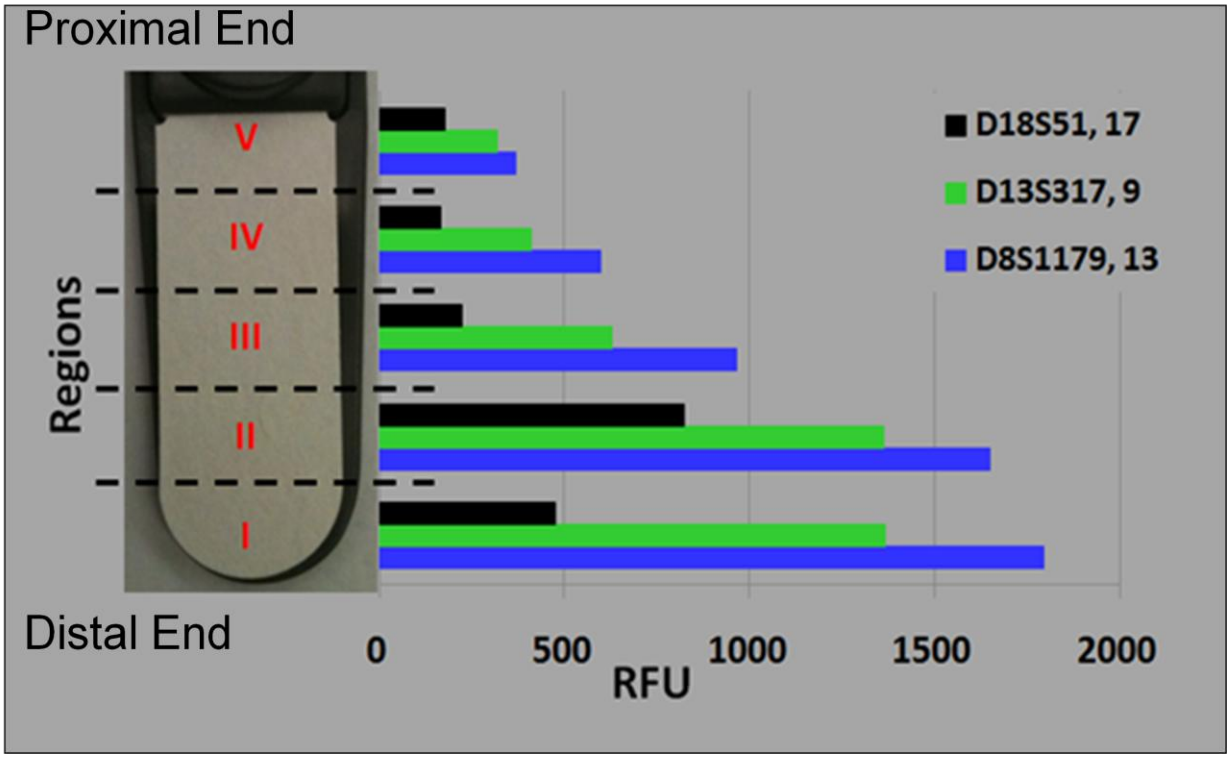



**Figure 4 Bar Graph of Region Data.** This bar graph shows the decreasing signal (RFU) obtained from each of the five regions of the Buccal DNA Collector™ for one sample. Regions I and II yielded the highest RFUs when compared to the other regions. (Photograph courtesy of Pam Curtis).

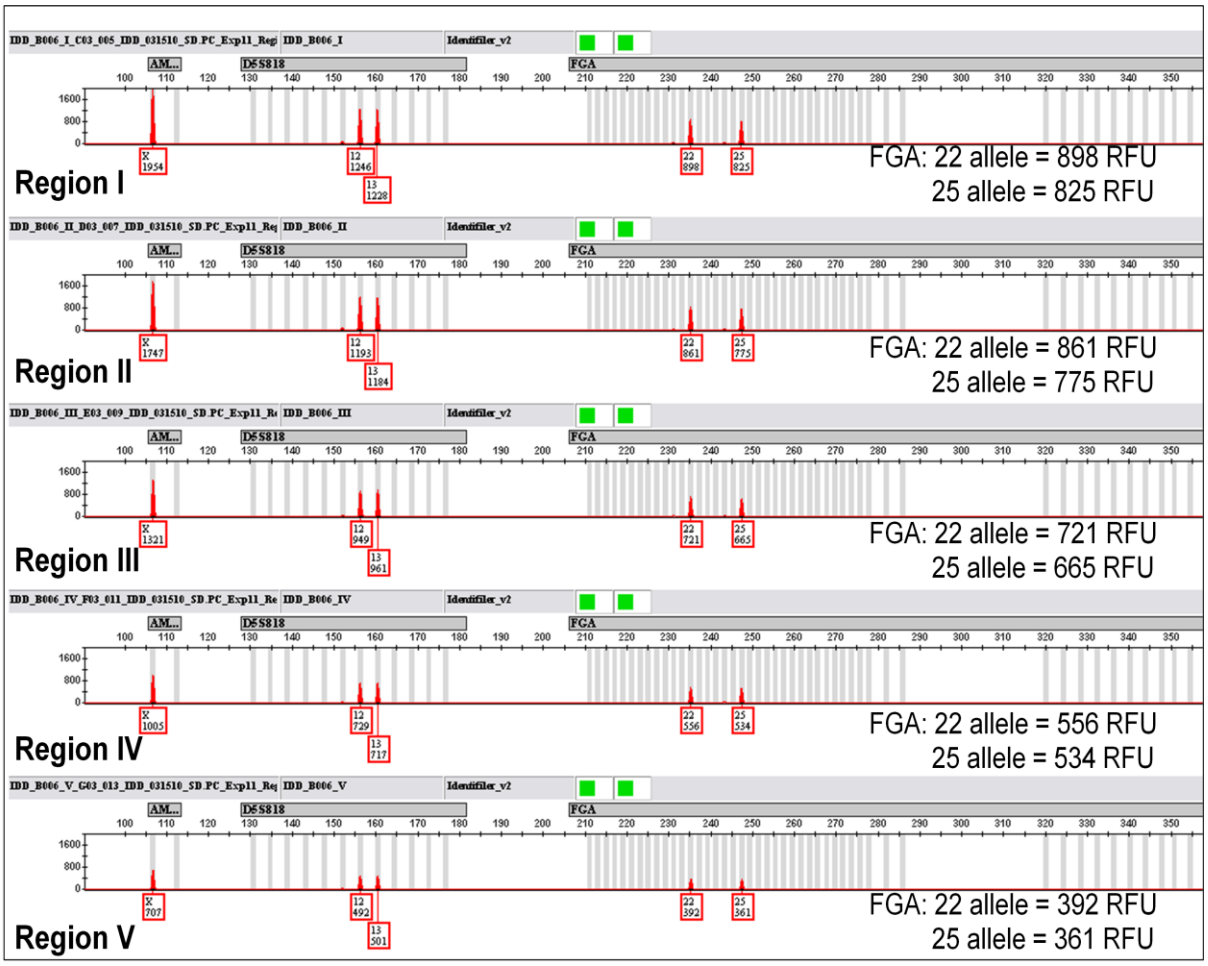



**Figure 5 Electropharogram of Amelogenin, D5S818, and FGA for the Different Regions.**The same buccal sample was punched in the center of each of the five regions of the Buccal DNA Collector™. Regions I and II displayed a higher signal intensity. As the regions move closer to the proximal end of the collector, signal intensity decreases. The RFU levels are labeled for the FGA locus.

Optimized protocols were used for the validation of the Buccal DNA Collector™ and AmpFLSTR® Identifiler® Direct PCR Amplification Kit for both buccal samples and bloodstains. Concordance between 99 of the 100 paired buccal samples and bloodstains following the optimized protocols was reached (Figures 6, 6A and Table 2). Allele and locus dropout were observed in four of 100 buccal samples on the first amplification. Three of the four samples were recovered upon re-amplification. The one sample that did not produce a profile

was due to poor sample collection. A SALIgAE® test (Abacus Diagnostics, West Hills, CA) which tests for the presence of amylase found in saliva was performed to see if there was any saliva present on the sample that gave no DNA results. This test was negative for the presence of saliva (data not shown). No dropout was observed for the bloodstains.

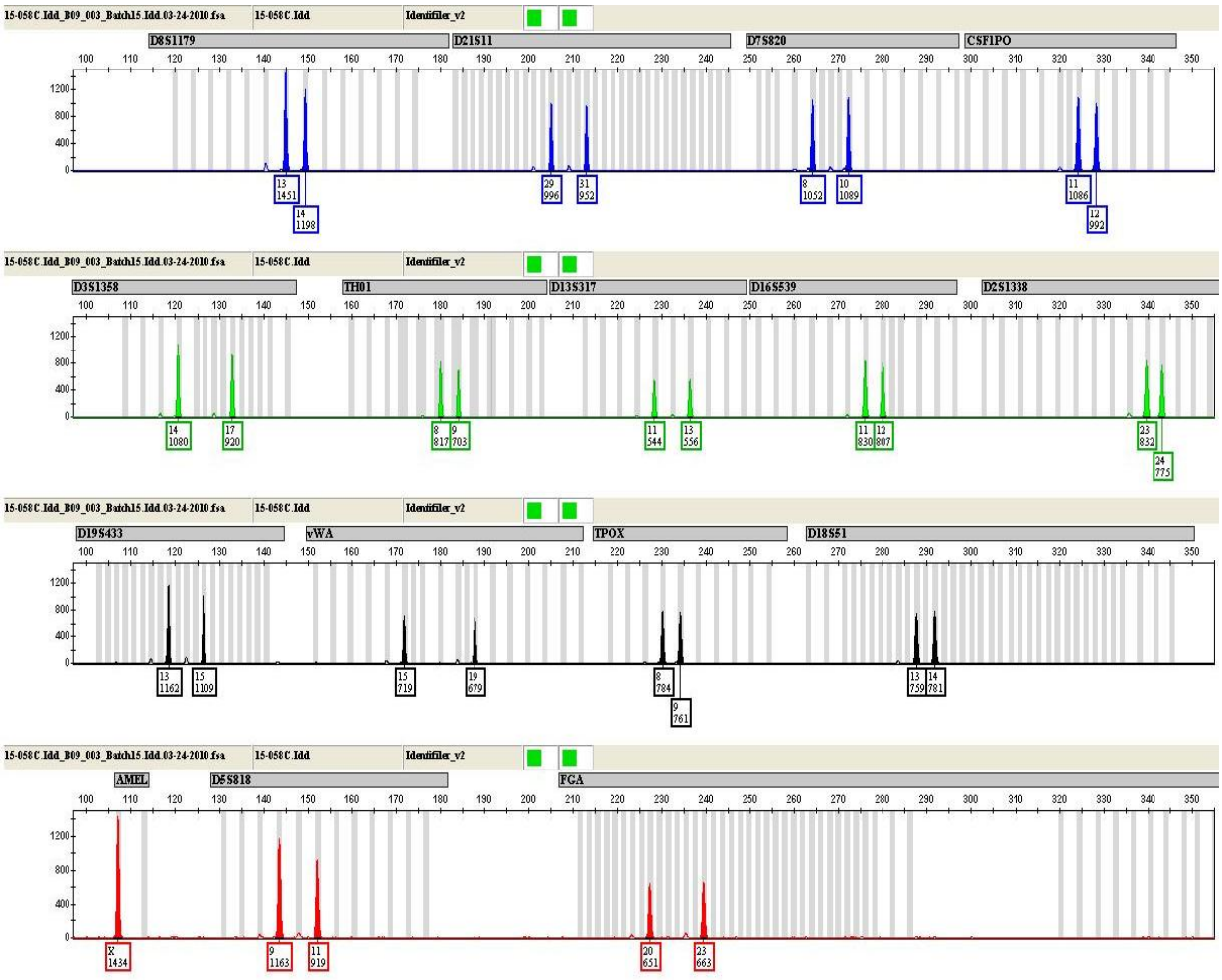



**Figure 6 Electropharogram of a Buccal Sample Processed with the Optimized Protocol.** Shown here is a DNA profile for one of the buccal samples processed with the optimized protocol. It is the same profile as that shown in Figure 6A below.

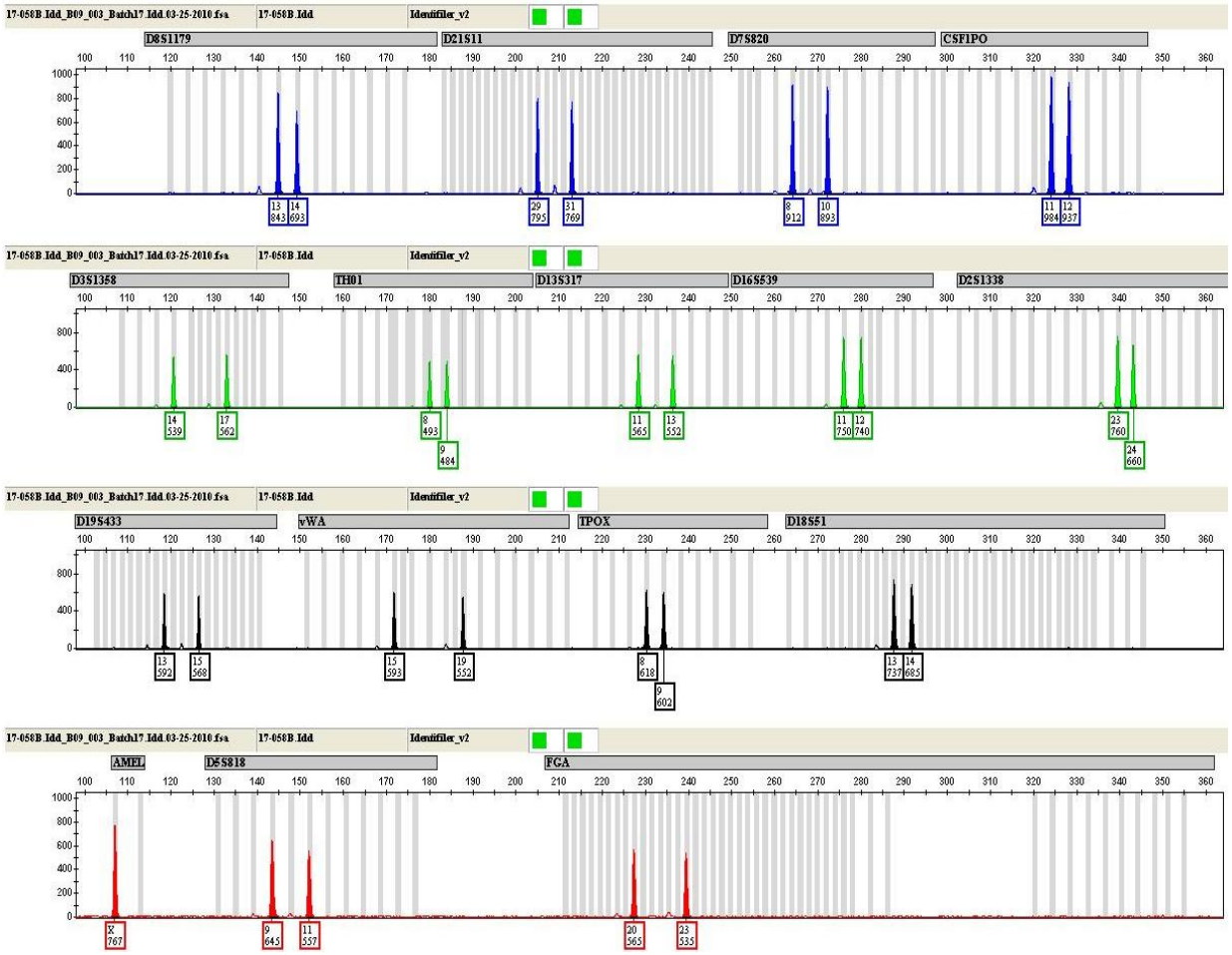



**Figure 6A Electropharogram of a Blood Sample Processed with the Optimized Protocol.** Shown is the profile obtained from one of the blood samples and it is concordant with Figure 6. It is the same profile as that shown in Figure 5 above.

| Sample # = 200 / Data | Buccal (N=100) | Blood (N=100) |
|---|---|---|
| % Full Profiles Obtained | 96% | 100% |
| Avg. PHR (Range) | 91% (58% - 100%) | 92% (64% - 100%) |
| # of Samples with Rule Firings | 21 | 12 |
| Rules Fired | PHR, LPH, AN | BIN, PHR, LPH, AN |

**Table 2 Concordance.** The average peak height ratio (PHR) was calculated for all samples across all heterozygote loci. The PHR range for each sample set is in parentheses. The rules fired were PHR (Peak Height Ratio, ≥60%), LPH (Low Peak Height, ≥200 RFU for homozygotes and ≥100 RFU for heterozygotes), AN (Allele Number, N=2), and BIN (Out of Bin Allele).The BIN rule fires when a peak is not in a designated bin. These observed rule firings are due to sample collection and the effects of the PCR process.

Table 2 shows concordance from the first amplification. As previously stated, three additional profiles were recovered upon re-amplification. This makes the percentage of full profiles obtained from buccal samples 99%. The rules fired for the buccal samples are shown in Table 2. These rules are fired when the data does not meet a defined criteria set by the lab. The PHR (peak height ratio) and AN (allele number) are fired when a stutter peak is called as an allele. Stutter is a known, reproducible artifact of PCR amplification. The LPH (low peak height) rule will fire when the allele peak heights do not meet the defined criteria. This rule also fires when stutter is called as an allele.

Statistical analyses were performed to determine any variation between the sample sets. Table 3 and Table 4 included the average peak height (PH), peak height ratio (PHR), standard deviation of the peak height and peak height ratio as well as the variances for peak height and peak height ratio for buccal samples and bloodstains respectively. This data was calculated for each locus and across the sample set. See Tables 3 through 8. Comparison statistics were calculated to determine any variation between the sample sets. These analyses include an Analysis of Variance (ANOVA), a paired t-test, and a Chi-squared test (Table 9). For complete ANOVA and t-test tables, refer to Appendix A and Appendix B.

| Buccal Samples | Avg. PH | Avg. PHR | Std. Dev. PH | Std. Dev. PHR | Var. PH | Var. PHR |
|---|---|---|---|---|---|---|
| D8S1179 | 1208.1050 | 89.91% | 821.5598 | 0.0717 | 674960.4611 | 0.0051 |
| D21S11 | 955.9553 | 92.67% | 569.7170 | 0.0423 | 324577.4474 | 0.0018 |
| D7S820 | 1035.9576 | 90.44% | 669.0273 | 0.1194 | 447597.5287 | 0.0142 |
| CSF1PO | 658.1471 | 91.63% | 464.7100 | 0.0566 | 215955.3924 | 0.0032 |
| D3S1358 | 842.2667 | 90.92% | 470.6553 | 0.0555 | 221516.4407 | 0.0031 |
| TH01 | 423.4364 | 92.89% | 298.2533 | 0.0530 | 88955.0035 | 0.0028 |
| D13S317 | 656.0341 | 91.94% | 401.7388 | 0.1147 | 161394.0445 | 0.0132 |
| D16S539 | 627.5864 | 91.75% | 396.0777 | 0.0443 | 156877.5732 | 0.0020 |
| D2S1338 | 486.9371 | 87.58% | 346.3457 | 0.0824 | 119955.3581 | 0.0068 |
| D19S433 | 762.1761 | 92.39% | 427.9381 | 0.0454 | 183131.0259 | 0.0021 |
| vWA | 823.32745 | 91.69% | 529.0963 | 0.0530 | 279942.8804 | 0.0028 |
| TPOX | 566.2500 | 92.74% | 407.8887 | 0.0460 | 166373.1821 | 0.0021 |
| D18S51 | 652.7765 | 89.25% | 479.9003 | 0.0818 | 230304.3318 | 0.0067 |
| AMEL | 1085.2883 | 91.21% | 608.4427 | 0.0368 | 370202.4980 | 0.0014 |
| D5S818 | 987.4326 | 90.83% | 595.6073 | 0.0555 | 354748.0661 | 0.0031 |
| FGA | 613.8811 | 91.53% | 362.7258 | 0.0434 | 131569.9967 | 0.0019 |
| Across Sample set | 759.5700 | 91.06% | 605.0629 | 0.0703 | 303513.6161 | 0.0049 |

**Table 3 Peak Height and Peak Height Ratio Data by Locus for Buccal Samples.** Data shown is the average peak height (PH), average peak height ration (PHR), standard deviations and variances for each locus across the sample set and the grand average of all loci across the sample set for buccal samples.

| Bloodstains | Avg. PH | Avg. PHR | Std. Dev PH | Std. Dev. PHR | Var. PH | Var. PHR |
|---|---|---|---|---|---|---|
| D8S1179 | 1497.9669 | 90.50% | 655.9405 | 0.0495 | 430257.9656 | 0.0024 |
| D21S11 | 1346.2611 | 93.47% | 624.9039 | 0.0296 | 390504.8979 | 0.0009 |
| D7S820 | 1468.1205 | 92.95% | 855.1120 | 0.0359 | 731216.5551 | 0.0013 |
| CSF1PO | 1522.8580 | 93.31% | 1012.7635 | 0.0275 | 1030774.8999 | 0.0008 |
| D3S1358 | 1056.7091 | 95.67% | 672.4320 | 0.0324 | 452164.7807 | 0.0011 |
| TH01 | 710.2909 | 96.08% | 417.8604 | 0.0304 | 174607.3539 | 0.0009 |
| D13S317 | 1000.6590 | 93.28% | 542.2428 | 0.0318 | 294027.3074 | 0.0010 |
| D16S539 | 1362.5706 | 92.83% | 785.3876 | 0.0303 | 616833.6416 | 0.0009 |
| D2S1338 | 1093.1469 | 90.38% | 622.8180 | 0.0654 | 387902.2738 | 0.0043 |
| D19S433 | 1054.6023 | 94.41% | 483.0830 | 0.0292 | 233369.1895 | 0.0008 |
| vWA | 1174.3059 | 92.38% | 641.8729 | 0.0469 | 412000.8053 | 0.0022 |
| TPOX | 1070.5390 | 93.77% | 609.1853 | 0.0470 | 371106.7207 | 0.0022 |
| D18S51 | 1095.6648 | 91.63% | 580.3563 | 0.0446 | 336813.4813 | 0.0020 |
| AMEL | 1237.1429 | 96.74% | 603.3213 | 0.0329 | 363996.6100 | 0.0011 |
| D5S818 | 1048.6927 | 93.32% | 469.1746 | 0.0428 | 220124.7758 | 0.0018 |
| FGA | 911.8541 | 93.16% | 428.9899 | 0.0354 | 184032.2992 | 0.0013 |
| Across sample set | 1162.1976 | 93.10% | 678.3612 | 0.0426 | 460173.9041 | 0.0018 |

**Table 4 Peak Height and Peak Height Ratio Data by Locus for Bloodstains.** Data shown is the average peak height (PH), average peak height ration (PHR), standard deviations and variances for each locus across the sample set and the grand average of all loci across the sample set for bloodstains.

| Buccal Samples | Avg. PA | Avg. PAR | Std. Dev. PA | Std. Dev. PAR | Var. PA | Var. PAR |
|---|---|---|---|---|---|---|
| D8S1179 | 14071.9399 | 89.33% | 10194.2511 | 0.0737 | 103922756.3865 | 0.0054 |
| D21S11 | 11667.6429 | 89.46% | 7590.5019 | 0.0417 | 57615718.8496 | 0.0017 |
| D7S820 | 14599.0118 | 89.49% | 10855.8894 | 0.0472 | 117850335.2380 | 0.0022 |
| CSF1PO | 10135.4826 | 89.53% | 7588.8887 | 0.0540 | 57591231.2570 | 0.0029 |
| D3S1358 | 10195.7229 | 89.49% | 6150.7083 | 0.0555 | 37831212.9773 | 0.0031 |
| TH01 | 4879.4970 | 89.55% | 3584.6438 | 0.0567 | 12849670.8660 | 0.0032 |
| D13S317 | 8856.6723 | 89.46% | 5571.5825 | 0.0448 | 31042531.9375 | 0.0020 |
| D16S539 | 8996.4356 | 89.48% | 6096.9805 | 0.0476 | 37173170.7288 | 0.0023 |
| D2S1338 | 7592.3146 | 89.40% | 5823.5521 | 0.0753 | 33913758.8948 | 0.0057 |
| D19S433 | 8281.5593 | 89.54% | 5192.2149 | 0.0459 | 26959096.0774 | 0.0021 |
| vWA | 9881.0347 | 89.69% | 6757.2504 | 0.1160 | 45660432.9872 | 0.0135 |
| TPOX | 7782.3871 | 89.76% | 6028.9793 | 0.0563 | 36348591.2388 | 0.0032 |
| D18S51 | 9764.9834 | 89.91% | 7707.2804 | 0.0752 | 59402171.7386 | 0.0057 |
| AMEL | 15885.2432 | 89.99% | 9934.3731 | 0.0379 | 98691769.7857 | 0.0014 |
| D5S818 | 14274.4833 | 90.04% | 9583.0131 | 0.0504 | 91834139.7372 | 0.0025 |
| FGA | 9330.3369 | 90.17% | 5954.3658 | 0.0432 | 35454472.4074 | 0.0019 |
| Across Sample Set | 10276.2441 | 91.79% | 7774.9881 | 0.0630 | 60450439.7867 | 0.0040 |

**Table 5 Peak Area and Peak Area Ratio Data by Locus for Buccal Samples.** Data shown is the average peak area (PA), average peak area ration (PAR), standard deviations and variances for each locus across the sample set and the grad average of all loci across the sample set for buccal samples.

| Bloodstains | Avg. PA | Avg. PAR | Std. Dev. PA | Std. Dev. PAR | Var. PA | Var. PAR |
|---|---|---|---|---|---|---|
| D8S1179 | 17710.5549 | 91.95% | 7554.5497 | 0.0436 | 57071221.5854 | 0.0019 |
| D21S11 | 16654.5549 | 91.99% | 7380.0289 | 0.0276 | 54464826.9666 | 0.0008 |
| D7S820 | 20010.6471 | 92.15% | 11472.7275 | 0.0274 | 131623475.6972 | 0.0007 |
| CSF1PO | 23089.3779 | 92.08% | 14964.6231 | 0.0283 | 223939944.2248 | 0.0008 |
| D3S1358 | 12980.1455 | 92.06% | 8122.6784 | 0.0353 | 65977903.8812 | 0.0012 |
| TH01 | 8190.3653 | 92.12% | 4786.9267 | 0.0300 | 22914666.9441 | 0.0009 |
| D13S317 | 13261.4294 | 92.11% | 6969.2814 | 0.0298 | 48570882.9510 | 0.0009 |
| D16S539 | 19660.7222 | 92.12% | 10898.2187 | 0.0303 | 118771171.2826 | 0.0009 |
| D2S1338 | 16996.4022 | 92.22% | 9613.7473 | 0.0555 | 92424137.5789 | 0.0031 |
| D19S433 | 11282.0670 | 92.28% | 5014.0558 | 0.0794 | 25140755.5011 | 0.0063 |
| vWA | 14114.2151 | 92.35% | 6575.5531 | 0.0403 | 43237898.8131 | 0.0016 |
| TPOX | 14961.0129 | 92.41% | 9281.0681 | 0.0458 | 86138225.3765 | 0.0021 |
| D18S51 | 16228.5635 | 92.44% | 8048.4427 | 0.0357 | 64777429.5473 | 0.0013 |
| AMEL | 18081.6667 | 92.53% | 8743.1289 | 0.0185 | 76442302.5697 | 0.0003 |
| D5S818 | 15259.9056 | 92.61% | 6721.9580 | 0.0391 | 45184719.4491 | 0.0015 |
| FGA | 13557.0963 | 92.63% | 5744.7399 | 0.0332 | 33002036.0229 | 0.0011 |
| Across Sample Set | 15702.4774 | 94.10% | 9243.3877 | 0.0424 | 85440215.5329 | 0.0018 |

**Table 6 Peak Area and Peak Area Ratio Data by Locus for Bloodstains.** Data shown is the average peak area (PA), average peak area ration (PAR), standard deviations and variances for each locus across the sample set and the grad average of all loci across the sample set for bloodstains.

| | Buccal Samples | | Bloodstains | |
|---|---|---|---|---|
| | Avg. PH | Avg. PHR | Avg. PH | Avg. PHR |
| Blue_6-FAM | 964.54 | 91.16% | 1458.80 | 91.64% |
| Green_VIC | 607.25 | 91.02% | 1044.68 | 92.33% |
| Yellow_NED | 701.13 | 91.52% | 1098.78 | 91.57% |
| Red_PET | 893.88 | 90.86% | 1065.90 | 90.11% |

**Table 7 Peak Height and Peak Height Ratio Data by Dye Color**. Shown is the average peak height (PH) and average peak height ratio (PHR) across the different dyes for each samples set.

| | Buccal Samples | | Bloodstains | |
|---|---|---|---|---|
| | Avg. PA | Avg. PAR | Avg. PA | Avg. PAR |
| Blue_6-FAM | 12618.5193 | 89.45% | 19366.2837 | 92.04% |
| Green_VIC | 8104.1285 | 89.48% | 14217.8129 | 92.13% |
| Yellow_NED | 8927.4911 | 89.73% | 14146.4646 | 92.37% |
| Red_PET | 13163.3545 | 90.07% | 15632.8895 | 92.59% |

**Table 8 Peak Area and Peak Area Ratio Data by Dye Color**. Shown is the average peak area (PA) and average peak area ratio (PAR) across the different dyes for each samples set.

| | p-value (ANOVA) | p-value (paired t-test) | Chi-Squared Value |
|---|---|---|---|
| PH-Locus | 1.6886E-06 | 1.6886E-06 | 1.0000000 |
| PH-Dye | 1.3005E-02 | 5.5599E-02 | 0.9994266 |
| PHR-Locus | 4.9623E-01 | 4.9623E-01 | 1.0000000 |
| PHR-Dye | 5.6958E-01 | 7.6380E-01 | 1.0000000 |
| PA-Locus | 8.4967E-06 | 1.7500E-05 | 1.0000000 |
| PA-Dye | 1.2145E-02 | 5.2375E-02 | 0.9992549 |
| PAR-Locus | 9.6919E-24 | 4.8411E-22 | 1.0000000 |
| PAR-Dye | 3.4751E-06 | 2.6325E-04 | 0.9999998 |

**Table 9 Summary of Inferential Data Analysis.** Summary table with the p-values for all ANOVA, t-tests, and chi-squared tests performed. ($\alpha = 0.05$)

# DISCUSSION

Optimization of the protocols for each sample type was a vital part of this study. The parameters that are in place for each step of processing the samples were carefully evaluated to ascertain the most optimal results. The reasoning behind optimization of cycle number is that if the cycle number is too low, a full profile may not be obtained. If the cycle number is too high, then increase stutter and pull-up can occur and this can cause rule firings that would slow down the analyst.

Optimization of the incubation solution volume was two-fold. First, it was thought that the incubation solution may not be a necessary step. Second, 2µL is a very small volume and just barely completely submerged the sample disc. It was hypothesized that increasing the incubation solution volume would more completely submerge the sample disc. The problem with increasing the incubation solution volume is that as the incubation solution volume increases, so does the dilution factor of the amplification mix. Dilution of the amplification mix can cause the sample to not completely amplify. The manufacturer of the Bode PunchPrep™ Solution recommends the 2µL solution volume.

The instructions for using the Buccal DNA Collector™ for collection of buccal samples recommend dragging the collector "firmly toward the lips and out of mouth… similar to the "popping" of the cheek with a finger..."(2, Appendix D). It was hypothesized that varying amounts of DNA maybe deposited during buccal sample collection. This is the reason that the collection device was mapped into the five different regions.

The optimization experiments further increased the efficiency of sample processing with the Buccal DNA Collector™ in conjunction with the AMPFLSTR® Identifiler® Direct PCR Amplification Kit. The processing of single source reference samples should not be time

consuming or laborious. DNA analysis from extraction to data analysis can take up to a week to complete. Elimination of DNA purification and quantification does decrease the time and labor spent on sample processing. The method tested in this project allows for a full plate of samples (86 samples to a plate plus 10 controls) to be processed and analyzed in less than a day.

Using GeneMapper®*ID* v3.2.1 (Applied Biosystems) analysis software as an expert system also further decreases time and labor spent on sample processing and analysis. As mentioned previously, the software will fire a rule when the data do not meet a defined criteria. Using the software in this manner allows the analyst to only look at the profiles that fire rules. This can save time for the analyst by looking at a few profiles that need closer inspection as opposed to hundreds of profiles.

There is no transfer between sample tubes with this method. Decreasing the amount of sample transfer is advantageous for three reasons. First, there is less chance to introduce contamination into the sample. Second, there is less opportunity to loose portions of the sample during transfer. Lastly, there is less chance of sample switching or mislabeling when processing large amounts of samples.

# CONCLUSIONS

In conclusion, optimized protocols for the different sample types were developed using the Bode Technology Buccal DNA Collector™ in conjunction with the AmpFLSTR® Identifiler® Direct PCR Amplification Kit to obtain DNA profiles for reference samples. Evaluation of paired buccal samples and bloodstains showed that the protocols are effective in producing full, balanced DNA profiles. Poor sample collection may be attributed to the observed allele and locus dropout in the buccal sample that did not give any results. Also, differences in an individual's diet can produce different PCR inhibitors, which could affect the amplification process (8,9).

The AmpFLSTR® Identifiler® Direct PCR Amplification Kit system allows for direct amplification which decreases sample processing time. While the sample punching must be done manually, the dispensing of the necessary reagents can be easily automated which results in more time savings. Saving time during the processing of samples not only reduces cost, but also allows for more reference samples to be processed. Increasing the number of reference samples processed leads to the potential for making more positive identifications.

The statistical analyses showed that there was a statistically significant difference between the loci. This is to be expected given the nature of DNA. There was also a statistically significant difference between the dyes within each sample set. Again, this is expected given the chemistry of the amplification kit. All statistical test performed indicated that there was no statistically significant difference between the samples sets. This was the expected result given that the samples were paired samples. The statistical analysis was performed after the first amplification, and again after the second amplification. Incomplete profiles were not included in

the analysis. The descriptive statistical tables (*i.e.* Average PH, Average PHR, etc.) including the additional profiles obtained from the second amplification are in Appendix B.

An effective and efficient method for processing reference samples was obtained through these experiments. The Bode Technology DNA Collector™ coupled with the AmpFLSTR® Identifiler® Direct PCR Amplification Kit using the optimized protocols established with these experiments is an ideal collection and processing method for any type of single-source or reference sample (*i.e.* suspect reference samples, paternity samples and samples for DNA databasing). The optimized protocols used in the validation portion of this project produced 99% full profiles for the buccal samples after two amplifications and 100% full profiles for the blood samples after only one amplification. No off scale data was seen in any of the profiles generated from the paired samples.

APPENDIX A

The following tables and graphs are the ANOVA and paired t-test tables that were generated during the statistical analysis portion of this study. The ANOVA and the paired t-test were performed for peak height, peak height ration, peak area, and peak area ratio with respect to each locus and dye color.

**Table A ANOVA for Peak Height (PH) with Respect to Locus.**

ANOVA: Two-Factor Without Replication (α = 0.05)

| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *St. Dev.* |
|---|---|---|---|---|---|
| D8S1179 | 2 | 2706.0718 | 1353.0359 | 42009.9543 | 204.9633 |
| D21S11 | 2 | 2302.2164 | 1151.1082 | 76169.3103 | 275.9879 |
| D7S820 | 2 | 2504.0781 | 1252.0390 | 93382.3887 | 305.5853 |
| CSF1PO | 2 | 2181.0050 | 1090.5025 | 373862.4957 | 611.4430 |
| D3S1358 | 2 | 1898.9758 | 949.4879 | 22992.7767 | 151.6337 |
| TH01 | 2 | 1133.7273 | 566.8636 | 41142.7651 | 202.8368 |
| D13S317 | 2 | 1656.6931 | 828.3465 | 59383.1500 | 243.6866 |
| D16S539 | 2 | 1990.1570 | 995.0785 | 270100.8374 | 519.7123 |
| D2S1338 | 2 | 1580.0840 | 790.0420 | 183745.1304 | 428.6550 |
| D19S433 | 2 | 1816.7784 | 908.3892 | 42756.5226 | 206.7765 |
| vWA | 2 | 1997.6334 | 998.8167 | 61592.9176 | 248.1792 |
| TPOX | 2 | 1636.7890 | 818.3945 | 127153.6781 | 356.5861 |
| D18S51 | 2 | 1748.4413 | 874.2207 | 98074.9950 | 313.1693 |
| AMEL | 2 | 2322.4311 | 1161.2156 | 11529.9050 | 107.3774 |
| D5S818 | 2 | 2031.1676 | 1015.5838 | 2192.4036 | 46.8231 |
| FGA | 2 | 1525.7351 | 762.8676 | 44393.9463 | 210.6987 |
| Buccal | 16 | 12380.6000 | 773.7875 | 51689.2947 | 227.3528 |
| Blood | 16 | 18651.3844 | 1165.7115 | 50811.3592 | 225.4138 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Loci | 1215862.158 | 15 | 81057.47721 | 3.780105827 | 0.007163642 | 2.403447072 |
| Sample Sets | 1228835.526 | 1 | 1228835.526 | 57.30659889 | 1.68859E-06 | 4.543077123 |
| Error | 321647.6505 | 15 | 21443.1767 | | | |
| Total | 2766345.335 | 31 | | | | |

**Table A1 Paired t-test Data for Peak Height with Respect to Locus.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 773.7875 | 1165.7115 |
| Variance | 51689.2947 | 50811.3592 |
| Observations | 16 | 16 |
| Pearson Correlation | 0.5816 | |
| Hypothesized Mean Difference | 0 | |
| df | 15 | |
| t Stat | -7.5701 | |
| P(T<=t) one-tail | 8.4429E-07 | |
| t Critical one-tail | 1.7531 | |
| P(T<=t) two-tail | 1.6886E-06 | |
| t Critical two-tail | 2.1314 | |

**Table B ANOVA for Peak Height Data with Respect to Dye Color.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *St. Dev.* | |
| Blue_6-FAM | 2 | 2423.3428 | 1211.6714 | 122146.6613 | 349.4949 | |
| Green_VIC | 2 | 1651.9274 | 825.9637 | 95669.5035 | 309.3049 | |
| Yellow_NED | 2 | 1799.9105 | 899.9553 | 79060.9451 | 281.1778 | |
| Red_PET | 2 | 1959.7780 | 979.8890 | 14794.6041 | 121.6331 | |
| Buccal | 4 | 3166.8073 | 791.7018 | 27512.8700 | 165.8700 | |
| Blood | 4 | 4668.1514 | 1167.0379 | 38329.2208 | 195.7785 | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Dyes | 167608.8201 | 3 | 55869.6067 | 5.6024 | 0.0954 | 9.2766 |
| Sample Sets | 281754.2618 | 1 | 281754.2618 | 28.2532 | 0.0130 | 10.1280 |
| Error | 29917.45227 | 3 | 9972.4841 | | | |
| Total | 479280.5341 | 7 | | | | |

**Table B1 Paired t-test for Peak Height Data with Respect to Dye Color.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 734.0887 | 1069.7832 |
| Variance | 21353.6662 | 743.1052 |
| Observations | 3 | 3 |
| Pearson Correlation | 0.2018 | |
| Hypothesized Mean Difference | 0 | |
| df | 2 | |
| t Stat | -4.0621 | |
| P(T<=t) one-tail | 0.0278 | |
| t Critical one-tail | 2.9200 | |
| P(T<=t) two-tail | 0.0556 | |
| t Critical two-tail | 4.3027 | |

**Table C ANOVA for Peak Height Ratio Data with Respect to Locus.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *St. Dev.* | |
| D8S1179 | 2 | 1.8041 | 0.9020 | 0.0000 | 0.0042 | |
| D21S11 | 2 | 1.8504 | 0.9252 | 0.0000 | 0.0021 | |
| D7S820 | 2 | 1.8080 | 0.9040 | 0.0000 | 0.0005 | |
| CSF1PO | 2 | 1.8493 | 0.9247 | 0.0001 | 0.0119 | |
| D3S1358 | 2 | 1.8658 | 0.9329 | 0.0011 | 0.0336 | |
| TH01 | 2 | 1.8496 | 0.9248 | 0.0000 | 0.0058 | |
| D13S317 | 2 | 1.8403 | 0.9201 | 0.0000 | 0.0011 | |
| D16S539 | 2 | 1.8322 | 0.9161 | 0.0000 | 0.0020 | |
| D2S1338 | 2 | 1.7796 | 0.8898 | 0.0004 | 0.0197 | |
| D19S433 | 2 | 1.8450 | 0.9225 | 0.0000 | 0.0021 | |
| vWA | 2 | 1.8047 | 0.9024 | 0.0004 | 0.0206 | |
| TPOX | 2 | 1.8651 | 0.9325 | 0.0001 | 0.0073 | |
| D18S51 | 2 | 1.8087 | 0.9044 | 0.0003 | 0.0168 | |
| AMEL | 2 | 1.7720 | 0.8860 | 0.0014 | 0.0369 | |
| D5S818 | 2 | 1.8204 | 0.9102 | 0.0003 | 0.0168 | |
| FGA | 2 | 1.8365 | 0.9183 | 0.0000 | 0.0042 | |
| Buccal | 16 | 14.5836 | 0.9115 | 0.0002 | 0.0144 | |
| Blood | 16 | 14.6482 | 0.9155 | 0.0005 | 0.0216 | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Loci | 0.0061 | 15 | 0.0004 | 1.5145 | 0.2154 | 2.4034 |
| Sample Sets | 0.0001 | 1 | 0.0001 | 0.4864 | 0.4962 | 4.5431 |
| Error | 0.0040 | 15 | 0.0003 | | | |
| Total | 0.0102 | 31 | | | | |

**Table C1 Paired t-test for Peak Height Ratio Data with Respect to Locus.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 0.9115 | 0.9155 |
| Variance | 0.0002 | 0.0005 |
| Observations | 16 | 16 |
| Pearson Correlation | 0.2213 | |
| Hypothesized Mean Difference | 0 | |
| df | 15 | |
| t Stat | -0.6974 | |
| P(T<=t) one-tail | 0.2481 | |
| t Critical one-tail | 1.7531 | |
| P(T<=t) two-tail | 0.4962 | |
| t Critical two-tail | 2.1314 | |

**Table D ANOVA for Peak Height Ratio Data with Respect to Dye Color.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *St. Dev.* | |
| Blue_6-FAM | 2 | 1.8280 | 0.9140 | 1.1360E-05 | 0.0034 | |
| Green_VIC | 2 | 1.8335 | 0.9168 | 8.6939E-05 | 0.0093 | |
| Yellow_NED | 2 | 1.8309 | 0.9154 | 1.2740E-07 | 0.0004 | |
| Red_PET | 2 | 1.8096 | 0.9048 | 2.8009E-05 | 0.0053 | |
| Buccal | 4 | 3.6455 | 0.9114 | 7.9849E-06 | 0.0028 | |
| Blood | 4 | 3.6564 | 0.9141 | 8.7621E-05 | 0.0094 | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Dyes | 0.0002 | 3 | 5.84775E-05 | 1.5750 | 0.3590 | 9.2766 |
| Sample Sets | 1.5051E-05 | 1 | 1.5051E-05 | 0.4054 | 0.5696 | 10.1280 |
| Error | 0.0001 | 3 | 3.71282E-05 | | | |
| Total | 0.0003 | 7 | | | | |

**Table D1 Paired t-test for Peak Height Ratio Data with Respect to Dye Color.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 0.9134 | 0.9113 |
| Variance | 0.0001 | 1.1945E-05 |
| Observations | 3 | 3 |
| Pearson Correlation | 0.4009 | |
| Hypothesized Mean Difference | 0 | |
| df | 2 | |
| t Stat | 0.3438 | |
| P(T<=t) one-tail | 0.3819 | |
| t Critical one-tail | 2.9200 | |
| P(T<=t) two-tail | 0.7638 | |
| t Critical two-tail | 4.3027 | |

**Table E ANOVA for Peak Area Data with Respect to Locus.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *Std. Dev.* | |
| D8S1179 | 2 | 31782.4948 | 15891.2474 | 6619759.7569 | 2572.8894 | |
| D21S11 | 2 | 28322.1978 | 14161.0989 | 12434646.0863 | 3526.2794 | |
| D7S820 | 2 | 34609.6589 | 17304.8294 | 14642897.9015 | 3826.6040 | |
| CSF1PO | 2 | 33224.8605 | 16612.4302 | 83901702.3543 | 9159.7872 | |
| D3S1358 | 2 | 23175.8683 | 11587.9342 | 3876504.5046 | 1968.8841 | |
| TH01 | 2 | 13069.8623 | 6534.9311 | 5480924.3290 | 2341.1374 | |
| D13S317 | 2 | 22118.1017 | 11059.0508 | 9700942.3883 | 3114.6336 | |
| D16S539 | 2 | 28657.1578 | 14328.5789 | 56863504.7636 | 7540.7894 | |
| D2S1338 | 2 | 24588.7168 | 12294.3584 | 44218432.0566 | 6649.6941 | |
| D19S433 | 2 | 19563.6264 | 9781.8132 | 4501523.2801 | 2121.6794 | |
| vWA | 2 | 23995.2498 | 11997.6249 | 8959908.2942 | 2993.3106 | |
| TPOX | 2 | 22743.4000 | 11371.7000 | 25766334.2345 | 5076.0550 | |
| D18S51 | 2 | 25993.5470 | 12996.7735 | 20888933.9224 | 4570.4413 | |
| AMEL | 2 | 33966.9099 | 16983.4550 | 2412137.9275 | 1553.1059 | |
| D5S818 | 2 | 29534.3889 | 14767.1944 | 485528.4780 | 696.7987 | |
| FGA | 2 | 22887.4332 | 11443.7166 | 8932747.3364 | 2988.7702 | |
| | | | | | | |
| Buccal | 16 | 166194.7475 | 10387.1717 | 8919845.5921 | 2986.6111 | |
| Blood | 16 | 252038.7265 | 15752.4204 | 13234488.9814 | 3637.9237 | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Loci | 2.529E+08 | 15 | 1.6861E+07 | 3.1854 | 0.015775965 | 2.4034 |
| Sample Set | 2.303E+08 | 1 | 2.3029E+08 | 43.5055 | 8.4967E-06 | 4.5431 |
| Error | 7.940E+07 | 15 | 5.2933E+06 | | | |
| | | | | | | |
| Total | 5.626E+08 | 31 | | | | |

**Table E1 Paired t-test for Peak Area Data with Respect to Locus.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 1.0142E+04 | 1.5622E+04 |
| Variance | 8.5225E+06 | 1.3888E+07 |
| Observations | 15 | 15 |
| Pearson Correlation | 5.1909E-01 | |
| Hypothesized Mean Difference | 0 | |
| df | 14 | |
| t Stat | -6.3663 | |
| P(T<=t) one-tail | 0.0000 | |
| t Critical one-tail | 1.7613 | |
| P(T<=t) two-tail | 1.7500E-05 | |
| t Critical two-tail | 2.1448 | |

**Table F ANOVA for Peak Area Data with Respect to Dye Color.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *St. Dev.* | |
| Blue_6-FAM | 2 | 31984.8030 | 15992.4015 | 22766162.3940 | 4771.3900 | |
| Green_VIC | 2 | 22321.9414 | 11160.9707 | 18688568.6620 | 4323.0277 | |
| Yellow_NED | 2 | 23073.9558 | 11536.9779 | 13618842.2859 | 3690.3716 | |
| Red_PET | 2 | 28796.2440 | 14398.1220 | 3049301.5614 | 1746.2249 | |
| | | | | | | |
| Buccal | 4 | 42813.4934 | 10703.3733 | 6543040.9048 | 2557.9368 | |
| Blood | 4 | 63363.4508 | 15840.8627 | 5992373.8506 | 2447.9326 | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Dye | 3.2271E+07 | 3 | 1.0757E+07 | 6.0486 | 0.0868 | 9.2766 |
| Sample Set | 5.2788E+07 | 1 | 5.2788E+07 | 29.6822 | 0.0121 | 10.1280 |
| Error | 5.3353E+06 | 3 | 1.7784E+06 | | | |
| | | | | | | |
| Total | 9.0394E+07 | 7 | | | | |

**Table F1 Paired t-test for Peak Area Data with Respect to Dye Color.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 10064.9914 | 14665.7224 |
| Variance | 7369372.0483 | 702831.8538 |
| Observations | 3 | 3 |
| Pearson Correlation | 0.9811 | |
| Hypothesized Mean Difference | 0 | |
| df | 2 | |
| t Stat | -4.1960 | |
| P(T<=t) one-tail | 0.0262 | |
| t Critical one-tail | 2.9200 | |
| P(T<=t) two-tail | 0.0524 | |
| t Critical two-tail | 4.3027 | |

**Table G ANOVA for Peak Area Ration with Respect to Locus.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *St. dev* | |
| D8S1179 | 2 | 1.8128 | 0.9064 | 3.4190E-04 | 0.0265 | |
| D21S11 | 2 | 1.8145 | 0.9072 | 3.1842E-04 | 0.0263 | |
| D7S820 | 2 | 1.8164 | 0.9082 | 3.5250E-04 | 0.0266 | |
| CSF1PO | 2 | 1.8161 | 0.9081 | 3.2650E-04 | 0.0264 | |
| D3S1358 | 2 | 1.8155 | 0.9077 | 3.3198E-04 | 0.0267 | |
| TH01 | 2 | 1.8168 | 0.9084 | 3.3063E-04 | 0.0262 | |
| D13S317 | 2 | 1.8157 | 0.9079 | 3.5343E-04 | 0.0269 | |
| D16S539 | 2 | 1.8160 | 0.9080 | 3.4931E-04 | 0.0265 | |
| D2S1338 | 2 | 1.8162 | 0.9081 | 3.9683E-04 | 0.0268 | |
| D19S433 | 2 | 1.8182 | 0.9091 | 3.7366E-04 | 0.0259 | |
| vWA | 2 | 1.8204 | 0.9102 | 3.5256E-04 | 0.0246 | |
| TPOX | 2 | 1.8217 | 0.9109 | 3.5011E-04 | 0.0238 | |
| D18S51 | 2 | 1.8235 | 0.9117 | 3.2208E-04 | 0.0224 | |
| AMEL | 2 | 1.8252 | 0.9126 | 3.2184E-04 | 0.0212 | |
| D5S818 | 2 | 1.8265 | 0.9132 | 3.3023E-04 | 0.0222 | |
| FGA | 2 | 1.8280 | 0.9140 | 3.0218E-04 | 0.0215 | |
| | | | | | | |
| Buccal | 16 | 14.3429 | 0.8964 | 6.4818E-06 | 0.0025 | |
| Blood | 16 | 14.7605 | 0.9225 | 4.7342E-06 | 0.0008 | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Loci | 0.0002 | 15 | 1.0832E-05 | 28.1919 | 2.7867E-08 | 2.4034 |
| Sample Set | 0.0054 | 1 | 0.0054 | 14180.5452 | 9.6919E-24 | 4.5431 |
| Error | 5.763E-06 | 15 | 3.8422E-07 | | | |
| | | | | | | |
| Total | 0.0056 | 31 | | | | |

**Table G1 Paired t-test for Peak Area Ratio with Respect to Locus.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 0.8966 | 0.9227 |
| Variance | 6.2027E-06 | 4.3550E-06 |
| Observations | 15 | 15 |
| Pearson Correlation | 0.9365 | |
| Hypothesized Mean Difference | 0 | |
| df | 14 | |
| t Stat | -111.3904 | |
| P(T<=t) one-tail | 2.4205E-22 | |
| t Critical one-tail | 1.7613 | |
| P(T<=t) two-tail | 4.8411E-22 | |
| t Critical two-tail | 2.1448 | |

**Table H ANOVA for Peak Area Ratio Data with Respect to Dye Color.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *St. Dev* | |
| Blue_6-FAM | 2 | 1.8149 | 0.9075 | 0.0003 | 0.0265 | |
| Green_VIC | 2 | 1.8160 | 0.9080 | 0.0004 | 0.0266 | |
| Yellow_NED | 2 | 1.8209 | 0.9105 | 0.0003 | 0.0242 | |
| Red_PET | 2 | 1.8266 | 0.9133 | 0.0003 | 0.0217 | |
| | | | | | | |
| Buccal | 4 | 3.5872 | 0.8968 | 8.1754E-06 | 0.0029 | |
| Blood | 4 | 3.6913 | 0.9228 | 6.1188E-06 | 0.0005 | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Dye | 4.233E-05 | 3 | 1.4111E-05 | 76.9620 | 0.0025 | 9.2766 |
| Sample Set | 1.354E-03 | 1 | 1.3535E-03 | 7382.2849 | 3.4751E-06 | 10.1280 |
| Error | 5.500E-07 | 3 | 1.8335E-07 | | | |
| | | | | | | |
| Total | 1.396E-03 | 7 | | | | |

**Table H1 Paired t-test for Peak Area Ratio Data with Respect to Dye Color.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 0.8976 | 0.9236 |
| Variance | 8.8270E-06 | 5.2992E-06 |
| Observations | 3 | 3 |
| Pearson Correlation | 0.9935 | |
| Hypothesized Mean Difference | 0 | |
| df | 2 | |
| t Stat | -61.6216 | |
| P(T<=t) one-tail | 0.0001 | |
| t Critical one-tail | 2.9200 | |
| P(T<=t) two-tail | 2.6325E-04 | |
| t Critical two-tail | 4.3027 | |

APPENDIX B

The tables that are in this appendix are the same as the ones in Appendix A. The only difference is that these tables include the calculations for the three buccal profiles that were recovered and the paired bloodstains that match those buccal samples.

**Table A. Average Peak Height, Peak Height Ratio, Standard Deviation and Variance with Respect to Loci for Buccal Samples.**

| Buccal Samples | Avg. PH | Avg. PHR | Std. Dev. PH | Std. Dev. PHR | Var. PH | Var. PHR |
|---|---|---|---|---|---|---|
| D8S1179 | 1204.7849 | 90.09% | 813.8003 | 0.0718 | 6.6227E+05 | 0.0052 |
| D21S11 | 955.0811 | 92.74% | 566.5206 | 0.0419 | 3.2095E+05 | 0.0018 |
| D7S820 | 1025.7368 | 90.42% | 664.1285 | 0.1177 | 4.4107E+05 | 0.0139 |
| CSF1PO | 656.8276 | 91.68% | 465.8553 | 0.0564 | 2.1702E+05 | 0.0032 |
| D3S1358 | 841.7368 | 90.99% | 464.0442 | 0.0547 | 2.1534E+05 | 0.0030 |
| TH01 | 422.3118 | 92.81% | 293.9756 | 0.0526 | 8.6422E+04 | 0.0028 |
| D13S317 | 661.9061 | 91.84% | 399.4692 | 0.7902 | 1.5958E+05 | 0.0130 |
| D16S539 | 619.2619 | 91.83% | 393.4540 | 0.0439 | 1.5481E+05 | 0.0019 |
| D2S1338 | 481.6722 | 87.69% | 344.0674 | 0.0818 | 1.1838E+05 | 0.0067 |
| D19S433 | 760.0604 | 92.39% | 423.3391 | 0.0449 | 1.7922E+05 | 0.0020 |
| vWA | 825.7045 | 91.84% | 522.6213 | 0.0535 | 2.7313E+05 | 0.0029 |
| TPOX | 560.5961 | 92.84% | 404.3082 | 0.0462 | 1.6347E+05 | 0.0021 |
| D18S51 | 650.1848 | 89.10% | 476.8387 | 0.0816 | 2.2738E+05 | 0.0067 |
| AMEL | 1084.7391 | 90.71% | 606.0767 | 0.0408 | 3.6733E+05 | 0.0017 |
| D5S818 | 988.5956 | 90.71% | 588.5598 | 0.0580 | 3.4640E+05 | 0.0034 |
| FGA | 613.6335 | 91.46% | 361.2010 | 0.0443 | 1.3047E+05 | 0.0020 |
| Across Sample set | 768.2629 | 91.17% | 600.3572 | 0.0694 | 2.9946E+05 | 0.0048 |

**Table A1. Table A. Average Peak Height, Peak Height Ratio, Standard Deviation and Variance with Respect to Loci for Bloodstains.**

| Blood Samples | PH | PHR | Std. dev PH | Std. Dev. PHR | Var. PH | Var. PHR |
|---|---|---|---|---|---|---|
| D8S1179 | 1500.4194 | 90.55% | 683.4299 | 0.0490 | 4.6708E+05 | 0.0024 |
| D21S11 | 1340.2419 | 93.50% | 624.7027 | 0.0299 | 3.9025E+05 | 0.0009 |
| D7S820 | 1456.9942 | 93.03% | 850.8127 | 0.0358 | 7.2388E+05 | 0.0013 |
| CSF1PO | 1528.4740 | 93.31% | 1003.3207 | 0.0273 | 1.0067E+06 | 0.0007 |
| D3S1358 | 1050.8480 | 95.50% | 664.6696 | 0.0329 | 4.4179E+05 | 0.0011 |
| TH01 | 707.2824 | 96.13% | 415.9574 | 0.0302 | 1.7302E+05 | 0.0009 |
| D13S317 | 1000.7303 | 93.15% | 539.3789 | 0.8008 | 2.9093E+05 | 0.0011 |
| D16S539 | 1352.6272 | 92.86% | 779.6494 | 0.0303 | 6.0785E+05 | 0.0009 |
| D2S1338 | 1096.0000 | 90.35% | 644.6985 | 0.0646 | 4.1564E+05 | 0.0042 |
| D19S433 | 1051.8077 | 94.34% | 482.1367 | 0.0290 | 2.3246E+05 | 0.0008 |
| vWA | 1175.3086 | 92.36% | 640.4919 | 0.0463 | 4.1023E+05 | 0.0021 |
| TPOX | 1073.7468 | 93.74% | 620.7451 | 0.0466 | 3.8532E+05 | 0.0022 |
| D18S51 | 1094.8564 | 91.59% | 594.7609 | 0.0441 | 3.5374E+05 | 0.0019 |
| AMEL | 1231.5948 | 96.79% | 594.2771 | 0.0320 | 3.5317E+05 | 0.0010 |
| D5S818 | 1051.2337 | 93.30% | 473.2088 | 0.0430 | 2.2393E+05 | 0.0018 |
| FGA | 909.7487 | 93.15% | 430.0633 | 0.0349 | 1.8495E+05 | 0.0012 |
| Across sample set | 1162.1976 | 93.10% | 678.3612 | 0.0426 | 4.6017E+05 | 0.0018 |

**Table A2. Average Peak Area, Peak Area Ratio, Standard Deviation and Variance with Respect to Loci for Buccal Samples.**

| Buccal Samples | Avg. PA | Avg. PAR | Std. Dev. PA | Std. Dev. PAR | Var. PA | Var. PAR |
|---|---|---|---|---|---|---|
| D8S1179 | 1.41E+04 | 89.53% | 1.01E+04 | 0.0741 | 1.03E+08 | 0.0055 |
| D21S11 | 1.18E+04 | 93.81% | 7.61E+03 | 0.0412 | 5.79E+07 | 0.0017 |
| D7S820 | 1.42E+04 | 92.92% | 9.69E+03 | 0.0487 | 9.38E+07 | 0.0024 |
| CSF1PO | 1.02E+04 | 92.43% | 7.69E+03 | 0.0537 | 5.91E+07 | 0.0029 |
| D3S1358 | 1.03E+04 | 90.51% | 6.10E+03 | 0.0546 | 3.72E+07 | 0.0030 |
| TH01 | 4.91E+03 | 92.28% | 3.54E+03 | 0.0560 | 1.25E+07 | 0.0031 |
| D13S317 | 9.03E+03 | 93.74% | 5.64E+03 | 0.0457 | 3.18E+07 | 0.0021 |
| D16S539 | 8.94E+03 | 92.56% | 6.04E+03 | 0.0475 | 3.65E+07 | 0.0023 |
| D2S1338 | 7.56E+03 | 89.28% | 5.78E+03 | 0.0744 | 3.34E+07 | 0.0055 |
| D19S433 | 8.37E+03 | 92.80% | 5.18E+03 | 0.0454 | 2.69E+07 | 0.0021 |
| vWA | 1.00E+04 | 91.40% | 6.72E+03 | 0.1150 | 4.51E+07 | 0.0132 |
| TPOX | 7.74E+03 | 92.02% | 5.96E+03 | 0.0563 | 3.55E+07 | 0.0032 |
| D18S51 | 9.81E+03 | 90.63% | 7.69E+03 | 0.0750 | 5.92E+07 | 0.0056 |
| AMEL | 1.60E+04 | 91.18% | 9.97E+03 | 0.0426 | 9.94E+07 | 0.0018 |
| D5S818 | 1.44E+04 | 91.04% | 9.51E+03 | 0.0533 | 9.05E+07 | 0.0028 |
| FGA | 9.41E+03 | 92.20% | 5.97E+03 | 0.0444 | 3.57E+07 | 0.0020 |
| Across Sample Set | 1.03E+04 | 91.78% | 7.76E+03 | 0.0629 | 6.03E+07 | 0.0040 |

**Table A3. Average Area, Peak Area Ratio, Standard Deviation and Variance with Respect to Loci for Bloodstains.**

| Bloodstains | Avg. PA | Avg. PAR | Std. Dev. PA | Std. Dev. PAR | Var. PA | Var. PAR |
|---|---|---|---|---|---|---|
| D8S1179 | 1.774E+04 | 92.03% | 7.889E+03 | 0.0435 | 6.224E+07 | 1.89E-03 |
| D21S11 | 1.658E+04 | 95.11% | 7.390E+03 | 0.0278 | 5.461E+07 | 7.74E-04 |
| D7S820 | 2.004E+04 | 94.52% | 1.137E+04 | 0.0277 | 1.293E+08 | 7.69E-04 |
| CSF1PO | 2.318E+04 | 94.32% | 1.483E+04 | 0.0281 | 2.199E+08 | 7.92E-04 |
| D3S1358 | 1.290E+04 | 95.29% | 8.030E+03 | 0.0353 | 6.448E+07 | 1.24E-03 |
| TH01 | 8.160E+03 | 96.00% | 4.766E+03 | 0.0297 | 2.271E+07 | 8.81E-04 |
| D13S317 | 1.327E+04 | 94.24% | 6.934E+03 | 0.0300 | 4.808E+07 | 8.99E-04 |
| D16S539 | 1.951E+04 | 94.21% | 1.083E+04 | 0.0303 | 1.173E+08 | 9.16E-04 |
| D2S1338 | 1.703E+04 | 92.07% | 9.925E+03 | 0.0551 | 9.851E+07 | 3.03E-03 |
| D19S433 | 1.126E+04 | 94.34% | 5.007E+03 | 0.0781 | 2.507E+07 | 6.10E-03 |
| vWA | 1.413E+04 | 93.51% | 6.587E+03 | 0.0398 | 4.339E+07 | 1.58E-03 |
| TPOX | 1.500E+04 | 94.22% | 9.404E+03 | 0.0455 | 8.843E+07 | 2.07E-03 |
| D18S51 | 1.623E+04 | 93.07% | 8.308E+03 | 0.0354 | 6.901E+07 | 1.25E-03 |
| AMEL | 1.799E+04 | 97.93% | 8.614E+03 | 0.0179 | 7.419E+07 | 3.22E-04 |
| D5S818 | 1.529E+04 | 94.05% | 6.778E+03 | 0.0393 | 4.594E+07 | 1.54E-03 |
| FGA | 1.353E+04 | 94.30% | 5.781E+03 | 0.0327 | 3.342E+07 | 1.07E-03 |
| Across sample set | 1.568E+04 | 94.09% | 9.273E+03 | 0.0421 | 8.600E+07 | 1.77E-03 |

**Table B. Average Peak Height, Peak Height Ratio, Standard Deviation and Variance with Respect to Dye Color for Buccal Samples.**

| Buccal Samples | Avg. PH | Avg. PHR | Std. Dev. PH | Std. Dev. PHR | Var. PH | Var. PHR |
|---|---|---|---|---|---|---|
| Blue_6-FAM | 960.6076 | 91.23% | 148.2081 | 0.0328 | 2.998E+10 | 3.888E-05 |
| Green_VIC | 605.3778 | 91.03% | 63.8550 | 0.3277 | 2.344E+09 | 2.095E-05 |
| Yellow_NED | 699.1365 | 91.54% | 53.5693 | 0.0171 | 2.466E+09 | 4.809E-06 |
| Red_PET | 895.6561 | 90.96% | 136.6034 | 0.0091 | 1.720E+10 | 8.208E-07 |

**Table B1. Average Peak Height, Peak Height Ratio, Standard Deviation and Variance with Respect to Dye Color for Bloodstains.**

| Bloodstains | Avg. PH | Avg. PHR | Std. Dev.(PH) | Std. Dev. PHR | Var. PH | Var. PHR |
|---|---|---|---|---|---|---|
| Blue_6-FAM | 1456.5324 | 92.60% | 171.1559 | 0.0097 | 3.053E+10 | 6.108E-07 |
| Green_VIC | 1041.4976 | 93.60% | 137.4838 | 0.3408 | 2.690E+10 | 2.037E-06 |
| Yellow_NED | 1098.9299 | 93.01% | 70.7869 | 0.0084 | 6.208E+09 | 4.001E-07 |
| Red_PET | 1064.1924 | 94.41% | 85.1325 | 0.0057 | 7.753E+09 | 1.854E-07 |

**Table B2. Average Peak Area, Peak Area Ratio, Standard Deviation and Variance with Respect to Dye Color.**

| Buccal Samples | Avg. PA | Avg. PAR | Std. Dev. PA | Std. Dev. PAR | Var. PA | Var. PAR |
|---|---|---|---|---|---|---|
| Blue_6-FAM | 1.26E+04 | 92.17% | 8.78E+03 | 0.0544 | 7.84E+07 | 0.0031 |
| Green_VIC | 8.15E+03 | 91.67% | 5.42E+03 | 0.0557 | 3.03E+07 | 0.0032 |
| Yellow_NED | 8.98E+03 | 91.71% | 6.39E+03 | 0.0729 | 4.17E+07 | 0.0060 |
| Red_PET | 1.33E+04 | 91.47% | 8.49E+03 | 0.0468 | 7.52E+07 | 0.0022 |

**Table B3. Average Peak Area, Peak Area Ratio, Standard Deviation and Variance with Respect to Dye Color for Bloodstains.**

| Bloodstains | Avg. PA | Avg. PAR | Std. Dev. PA | Std. Dev. PAR | Var. PA | Var. PAR |
|---|---|---|---|---|---|---|
| Blue_6-FAM | 1.938E+04 | 93.99% | 1.037E+04 | 0.0318 | 1.165E+08 | 1.057E-03 |
| Green_VIC | 1.417E+04 | 94.36% | 8.097E+03 | 0.0360 | 7.021E+07 | 1.394E-03 |
| Yellow_NED | 1.415E+04 | 93.78% | 7.327E+03 | 0.0497 | 5.648E+07 | 2.750E-03 |
| Red_PET | 1.560E+04 | 95.43% | 7.058E+03 | 0.0300 | 5.119E+07 | 9.792E-04 |

**Table C. ANOVA for Peak Height Data with Respect to Locus.**

ANOVA: Two-Factor Without Replication (α = 0.05)

| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *Std. Dev.* |
|---|---|---|---|---|---|
| D8S1179 | 2 | 2705.2043 | 1352.6022 | 43699.8518 | 209.0451 |
| D21S11 | 2 | 2295.3230 | 1147.6615 | 74174.4419 | 272.3499 |
| D7S820 | 2 | 2482.7310 | 1241.3655 | 92991.4484 | 304.9450 |
| CSF1PO | 2 | 2185.3016 | 1092.6508 | 379883.7253 | 616.3471 |
| D3S1358 | 2 | 1892.5848 | 946.2924 | 21863.7284 | 147.8639 |
| TH01 | 2 | 1129.5941 | 564.7971 | 40604.1181 | 201.5046 |
| D13S317 | 2 | 1662.6364 | 831.3182 | 57400.9395 | 239.5849 |
| D16S539 | 2 | 1971.8891 | 985.9446 | 268912.3420 | 518.5676 |
| D2S1338 | 2 | 1577.6722 | 788.8361 | 188699.3093 | 434.3953 |
| D19S433 | 2 | 1811.8681 | 905.9341 | 42558.2297 | 206.2965 |
| vWA | 2 | 2001.0131 | 1000.5066 | 61111.4875 | 247.2074 |
| TPOX | 2 | 1634.3430 | 817.1715 | 131661.8110 | 362.8523 |
| D18S51 | 2 | 1745.0411 | 872.5206 | 98866.4030 | 314.4303 |
| AMEL | 2 | 2316.3340 | 1158.1670 | 10783.2979 | 103.8427 |
| D5S818 | 2 | 2039.8293 | 1019.9147 | 1961.7637 | 44.2918 |
| FGA | 2 | 1523.3822 | 761.6911 | 43842.1009 | 209.3851 |
| Buccal | 16 | 12352.8335 | 772.0521 | 51847.3376 | 227.7001 |
| Blood | 16 | 18621.9140 | 1163.8696 | 50583.4614 | 224.9077 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Loci | 1.2056E+06 | 15 | 8.0374E+04 | 3.6440 | 0.0085 | 2.4034 |
| Sample Set | 1.2282E+06 | 1 | 1.2282E+06 | 55.6829 | 2.00738E-06 | 4.5431 |
| Error | 3.3085E+05 | 15 | 2.2056E+04 | | | |
| Total | 2.7646E+06 | 31 | | | | |

**Table C1. Paired t-test for Peak Height Data with Respect to Locus.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 772.0521 | 1163.8696 |
| Variance | 51847.3376 | 50583.4614 |
| Observations | 16 | 16 |
| Pearson Correlation | 0.5694 | |
| Hypothesized Mean Difference | 0 | |
| df | 15 | |
| t Stat | -7.4621 | |
| P(T<=t) one-tail | 1.0037E-06 | |
| t Critical one-tail | 1.7531 | |
| P(T<=t) two-tail | 2.0074E-06 | |
| t Critical two-tail | 2.1314 | |

**Table D. ANOVA for Peak Height Data with Respect to Dye Color.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *Std. Dev.* | |
| Blue_6-FAM | 2 | 2417.1400 | 1208.5700 | 122970.6800 | 350.6718 | |
| Green_VIC | 2 | 1646.8753 | 823.4377 | 95100.2444 | 308.3833 | |
| Yellow_NED | 2 | 1798.0663 | 899.0332 | 79917.3745 | 282.6966 | |
| Red_PET | 2 | 1959.8485 | 979.9242 | 14202.2449 | 119.1732 | |
| Buccal | 4 | 3160.7779 | 790.1945 | 27537.1885 | 165.9433 | |
| Blood | 4 | 4661.1522 | 1165.2881 | 38257.0242 | 195.5940 | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Dye | 1.6658E+05 | 3 | 5.5527E+04 | 5.4085 | 0.0996 | 9.2766 |
| Sample Set | 2.8139E+05 | 1 | 2.8139E+05 | 27.4080 | 0.0136 | 10.1280 |
| Error | 3.0800E+04 | 3 | 1.0267E+04 | | | |
| Total | 478773.0036 | 7 | | | | |

**Table D1. Paired t-test for Peak Height Data with Respect to Dye Color.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 790.1945 | 1165.2881 |
| Variance | 2.7537E+04 | 3.8257E+04 |
| Observations | 4 | 4 |
| Pearson Correlation | 0.6972 | |
| Hypothesized Mean Difference | 0 | |
| df | 3 | |
| t Stat | -5.2353 | |
| P(T<=t) one-tail | 0.0068 | |
| t Critical one-tail | 2.3534 | |
| P(T<=t) two-tail | 0.0136 | |
| t Critical two-tail | 3.1824 | |

**Table E. ANOVA for Peak Height Data Ratio with Respect to Locus.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *Std. Dev.* | |
| D8S1179 | 2 | 1.8064 | 0.9032 | 0.0000 | 0.0033 | |
| D21S11 | 2 | 1.8624 | 0.9312 | 0.0000 | 0.0054 | |
| D7S820 | 2 | 1.8345 | 0.9173 | 0.0003 | 0.0184 | |
| CSF1PO | 2 | 1.8499 | 0.9250 | 0.0001 | 0.0115 | |
| D3S1358 | 2 | 1.8648 | 0.9324 | 0.0010 | 0.0319 | |
| TH01 | 2 | 1.8895 | 0.9447 | 0.0006 | 0.0235 | |
| D13S317 | 2 | 1.8500 | 0.9250 | 0.0001 | 0.0092 | |
| D16S539 | 2 | 1.8468 | 0.9234 | 0.0001 | 0.0073 | |
| D2S1338 | 2 | 1.7804 | 0.8902 | 0.0004 | 0.0188 | |
| D19S433 | 2 | 1.8673 | 0.9337 | 0.0002 | 0.0138 | |
| vWA | 2 | 1.8420 | 0.9210 | 0.0000 | 0.0037 | |
| TPOX | 2 | 1.8658 | 0.9329 | 0.0000 | 0.0064 | |
| D18S51 | 2 | 1.8069 | 0.9034 | 0.0003 | 0.0176 | |
| AMEL | 2 | 1.8750 | 0.9375 | 0.0019 | 0.0430 | |
| D5S818 | 2 | 1.8400 | 0.9200 | 0.0003 | 0.0183 | |
| FGA | 2 | 1.8461 | 0.9231 | 0.0001 | 0.0119 | |
| | | | | | 0.0000 | |
| Buccal | 16 | 14.5913 | 0.9120 | 0.0002 | 0.0141 | |
| Blood | 16 | 14.9365 | 0.9335 | 0.0003 | 0.0177 | |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Loci | 0.0059 | 15 | 0.0004 | 3.4094 | 0.0116 | 2.4034 |
| Sample Set | 0.0037 | 1 | 0.0037 | 32.2301 | 4.3897E-05 | 4.5431 |
| Error | 0.0017 | 15 | 0.0001 | | | |
| Total | 0.0114 | 31 | | | | |

**Table E1. Paired t-test for Peak Height Ratio Data with Respect to Locus.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 0.9120 | 0.9335 |
| Variance | 0.0002 | 0.0003 |
| Observations | 16 | 16 |
| Pearson Correlation | 0.5606 | |
| Hypothesized Mean Difference | 0 | |
| df | 15 | |
| t Stat | -5.6772 | |
| P(T<=t) one-tail | 2.1949E-05 | |
| t Critical one-tail | 1.7531 | |
| P(T<=t) two-tail | 4.3897E-05 | |
| t Critical two-tail | 2.1314 | |

**Table F. ANOVA for Peak Height Ratio Data with Respect to Dye Color.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *Std. Dev.* | |
| Blue_6-FAM | 2 | 1.8383 | 0.9192 | 9.3194E-05 | 0.0097 | |
| Green_VIC | 2 | 1.8463 | 0.9231 | 0.0003 | 0.0181 | |
| Yellow_NED | 2 | 1.8455 | 0.9227 | 0.0001 | 0.0104 | |
| Red_PET | 2 | 1.8537 | 0.9269 | 0.0006 | 0.0244 | |
| Buccal | 4 | 3.6477 | 0.9119 | 6.7514E-06 | 0.0026 | |
| Blood | 4 | 3.7362 | 0.9340 | 6.2143E-05 | 0.0079 | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Dye | 5.968E-05 | 3 | 1.989E-05 | 0.4060 | 0.7607 | 9.2766 |
| Sample Set | 0.0010 | 1 | 0.0010 | 19.9905 | 0.0208 | 10.1280 |
| Error | 0.0001 | 3 | 4.900E-05 | | | |
| Total | 0.0012 | 7 | | | | |

**Table F1. Paired t-test for Peak Height Ratio Data with Respect to Dye Color.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 0.9119 | 9.3404E-01 |
| Variance | 0.0000 | 6.2143E-05 |
| Observations | 4 | 4 |
| Pearson Correlation | -0.7105 | |
| Hypothesized Mean Difference | 0 | |
| df | 3 | |
| t Stat | -4.4711 | |
| P(T<=t) one-tail | 0.0104 | |
| t Critical one-tail | 2.3534 | |
| P(T<=t) two-tail | 0.0208 | |
| t Critical two-tail | 3.1824 | |

**Table G. ANOVA for Peak Area Data with Respect to Locus.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *Std. Dev* | |
| D8S1179 | 2 | 3.188E+04 | 1.594E+04 | 6.466E+06 | 2542.9224 | |
| D21S11 | 2 | 2.835E+04 | 1.417E+04 | 1.159E+07 | 3404.3468 | |
| D7S820 | 2 | 3.424E+04 | 1.712E+04 | 1.708E+07 | 4133.0432 | |
| CSF1PO | 2 | 3.337E+04 | 1.668E+04 | 8.435E+07 | 9184.3689 | |
| D3S1358 | 2 | 2.320E+04 | 1.160E+04 | 3.375E+06 | 1837.2197 | |
| TH01 | 2 | 1.307E+04 | 6.534E+03 | 5.289E+06 | 2299.7250 | |
| D13S317 | 2 | 2.230E+04 | 1.115E+04 | 8.970E+06 | 2994.9547 | |
| D16S539 | 2 | 2.844E+04 | 1.422E+04 | 5.583E+07 | 7471.7186 | |
| D2S1338 | 2 | 2.459E+04 | 1.229E+04 | 4.490E+07 | 6700.6483 | |
| D19S433 | 2 | 1.963E+04 | 9.814E+03 | 4.161E+06 | 2039.7797 | |
| vWA | 2 | 2.413E+04 | 1.206E+04 | 8.540E+06 | 2922.2979 | |
| TPOX | 2 | 2.274E+04 | 1.137E+04 | 2.634E+07 | 5132.5724 | |
| D18S51 | 2 | 2.603E+04 | 1.302E+04 | 2.061E+07 | 4540.0323 | |
| AMEL | 2 | 3.400E+04 | 1.700E+04 | 1.944E+06 | 1394.4207 | |
| D5S818 | 2 | 2.970E+04 | 1.485E+04 | 3.986E+05 | 631.3661 | |
| FGA | 2 | 2.294E+04 | 1.147E+04 | 8.504E+06 | 2916.2183 | |
| Buccal | 16 | 1.668E+05 | 1.042E+04 | 8.855E+06 | 2975.7820 | |
| Blood | 16 | 2.518E+05 | 1.574E+04 | 1.331E+07 | 3648.2371 | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Loci | 2.502E+08 | 15 | 1.668E+07 | 3.0418 | 0.0193 | 2.4034 |
| Sample Set | 2.261E+08 | 1 | 2.261E+08 | 41.2282 | 1.1513E-05 | 4.5431 |
| Error | 8.226E+07 | 15 | 5.484E+06 | | | |
| Total | 5.586E+08 | 31 | | | | |

**Table G1. Paired t-test for Peak Height Data with Respect to Locus.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 1.0423E+04 | 1.5739E+04 |
| Variance | 8.8553E+06 | 1.3310E+07 |
| Observations | 16 | 16 |
| Pearson Correlation | 0.5157 | |
| Hypothesized Mean Difference | 0 | |
| df | 15 | |
| t Stat | -6.4209 | |
| P(T<=t) one-tail | 5.7563E-06 | |
| t Critical one-tail | 1.7531 | |
| P(T<=t) two-tail | 1.1513E-05 | |
| t Critical two-tail | 2.1314 | |

**Table H. ANOVA for Peak Area Data with Respect to Dye Color.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *Std. Dev.* | |
| Blue_6-FAM | 2 | 3.196E+04 | 1.598E+04 | 2.320E+07 | 4.816E+03 | |
| Green_VIC | 2 | 2.232E+04 | 1.116E+04 | 1.815E+07 | 4.261E+03 | |
| Yellow_NED | 2 | 2.313E+04 | 1.157E+04 | 1.339E+07 | 3.659E+03 | |
| Red_PET | 2 | 2.888E+04 | 1.444E+04 | 2.714E+06 | 1.647E+03 | |
| | | | | | | |
| Buccal | 4 | 4.297E+04 | 1.074E+04 | 6.538E+06 | 2.557E+03 | |
| Blood | 4 | 6.331E+04 | 1.583E+04 | 6.083E+06 | 2.466E+03 | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Dye | 3.213E+07 | 3 | 1.071E+07 | 5.6050 | 0.0953 | 9.2766 |
| Sample Set | 5.172E+07 | 1 | 5.172E+07 | 27.0677 | 0.0138 | 10.1280 |
| Error | 5.732E+06 | 3 | 1.911E+06 | | | |
| | | | | | | |
| Total | 8.958E+07 | 7 | | | | |

**Table H1. Paired t-test for Peak Area Data with Respect to Dye Color.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 1.074E+04 | 1.583E+04 |
| Variance | 6.538E+06 | 6.083E+06 |
| Observations | 4 | 4 |
| Pearson Correlation | 0.6977 | |
| Hypothesized Mean Difference | 0 | |
| df | 3 | |
| t Stat | -5.2027 | |
| P(T<=t) one-tail | 6.900E-03 | |
| t Critical one-tail | 2.3534 | |
| P(T<=t) two-tail | 1.380E-02 | |
| t Critical two-tail | 3.1824 | |

**Table I. ANOVA for Peak Area Data Ratio with Respect to Locus.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *Std. Dev.* | |
| D8S1179 | 2 | 1.8156 | 0.9078 | 3.1261E-04 | 0.0177 | |
| D21S11 | 2 | 1.8892 | 0.9446 | 8.4212E-05 | 0.0092 | |
| D7S820 | 2 | 1.8744 | 0.9372 | 1.2734E-04 | 0.0113 | |
| CSF1PO | 2 | 1.8675 | 0.9338 | 1.7765E-04 | 0.0133 | |
| D3S1358 | 2 | 1.8580 | 0.9290 | 1.1458E-03 | 0.0338 | |
| TH01 | 2 | 1.8828 | 0.9414 | 6.9026E-04 | 0.0263 | |
| D13S317 | 2 | 1.8798 | 0.9399 | 1.2337E-05 | 0.0035 | |
| D16S539 | 2 | 1.8677 | 0.9338 | 1.3572E-04 | 0.0117 | |
| D2S1338 | 2 | 1.8136 | 0.9068 | 3.8944E-04 | 0.0197 | |
| D19S433 | 2 | 1.8713 | 0.9357 | 1.1846E-04 | 0.0109 | |
| vWA | 2 | 1.8491 | 0.9246 | 2.2285E-04 | 0.0149 | |
| TPOX | 2 | 1.8624 | 0.9312 | 2.4247E-04 | 0.0156 | |
| D18S51 | 2 | 1.8370 | 0.9185 | 2.9915E-04 | 0.0173 | |
| AMEL | 2 | 1.8912 | 0.9456 | 2.2765E-03 | 0.0477 | |
| D5S818 | 2 | 1.8509 | 0.9254 | 4.5338E-04 | 0.0213 | |
| FGA | 2 | 1.8649 | 0.9325 | 2.2073E-04 | 0.0149 | |
| Buccal | 16 | 14.6832 | 0.9177 | 1.8090E-04 | 0.0134 | |
| Blood | 16 | 15.0920 | 0.9432 | 2.0051E-04 | 0.0142 | |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Loci | 0.0040 | 15 | 2.6890E-04 | 2.3899 | 0.0511 | 2.4034 |
| Sample Set | 0.0052 | 1 | 5.2212E-03 | 46.4053 | 5.8706E-06 | 4.5431 |
| Error | 0.0017 | 15 | 1.1251E-04 | | | |
| Total | 0.0109 | 31 | | | | |

**Table I1. Paired t-test for Peak Area Ratio Data with Respect to Dye Color.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 0.9177 | 0.9432 |
| Variance | 1.8090E-04 | 2.0051E-04 |
| Observations | 16 | 16 |
| Pearson Correlation | 0.4106 | |
| Hypothesized Mean Difference | 0 | |
| df | 15 | |
| t Stat | -6.8121 | |
| P(T<=t) one-tail | 2.9353E-06 | |
| t Critical one-tail | 1.7531 | |
| P(T<=t) two-tail | 5.8706E-06 | |
| t Critical two-tail | 2.1314 | |

**Table J. ANOVA for Peak Area Ratio Data with Respect to Dye Color.**

| ANOVA: Two-Factor Without Replication (α = 0.05) | | | | | | |
|---|---|---|---|---|---|---|
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | *Std. Dev.* | |
| Blue_6-FAM | 2 | 1.8617 | 0.9308 | 1.6558E-04 | 0.0129 | |
| Green_VIC | 2 | 1.8604 | 0.9302 | 3.6115E-04 | 0.0190 | |
| Yellow_NED | 2 | 1.8549 | 0.9275 | 2.1521E-04 | 0.0147 | |
| Red_PET | 2 | 1.8690 | 0.9345 | 7.8143E-04 | 0.0280 | |
| Buccal | 4 | 3.6703 | 0.9176 | 8.7644E-06 | 0.0030 | |
| Blood | 4 | 3.7757 | 0.9439 | 5.3282E-05 | 0.0073 | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Rows | 5.01752E-05 | 3 | 1.6725E-05 | 0.3690 | 0.7827 | 9.2766 |
| Columns | 1.3874E-03 | 1 | 1.3874E-03 | 30.6124 | 0.0116 | 10.1280 |
| Error | 1.3596E-04 | 3 | 4.5322E-05 | | | |
| Total | 1.5735E-03 | 7 | | | | |

**Table J1. Paired t-test for Peak Area Ratio Data with Respect to Dye Color.**

| t-Test: Paired Two Sample for Means (α = 0.05) | | |
|---|---|---|
| | *Buccal* | *Blood* |
| Mean | 0.9176 | 0.9439 |
| Variance | 8.7644E-06 | 5.3282E-05 |
| Observations | 4 | 4 |
| Pearson Correlation | -0.6617 | |
| Hypothesized Mean Difference | 0 | |
| df | 3 | |
| t Stat | -5.5328 | |
| P(T<=t) one-tail | 5.8176E-03 | |
| t Critical one-tail | 2.3534 | |
| P(T<=t) two-tail | 0.0116 | |
| t Critical two-tail | 3.1824 | |

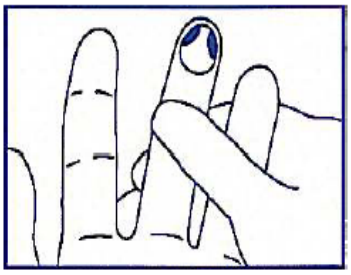
**Table K. Summary Table for All Data with Recovered Profiles.**

| | p-value (ANOVA) | p-value (paired t-test) | Chi-Squared Value |
|---|---|---|---|
| PH-Locus | 2.0074E-06 | 2.0074E-06 | 1.0000000 |
| PH-Dye | 1.3563E-02 | 1.3563E-02 | 0.9994033 |
| PHR-Locus | 4.3897E-05 | 4.3897E-05 | 1.0000000 |
| PHR-Dye | 2.0848E-02 | 2.0848E-02 | 0.9999970 |
| PA-Locus | 1.1513E-05 | 1.1513E-05 | 1.0000000 |
| PA-Dye | 1.3799E-02 | 1.3799E-02 | 0.9992310 |
| PAR-Locus | 5.8706E-06 | 5.8706E-06 | 1.0000000 |
| PAR-Dye | 1.1635E-02 | 1.1635E-02 | 0.9999994 |

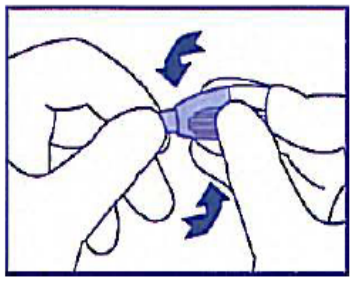
(α = 0.05)

# BD Microtainer©
## Contact-Activated Lancet

**QUICK REFERENCE CARD**

1 

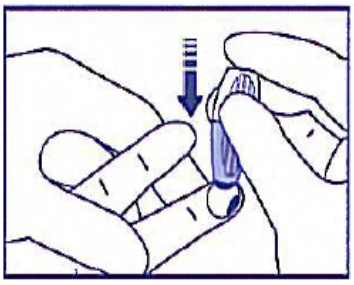
**Identify** the desired puncture site, (shaded area) and make sure it is properly cleansed according to your facility's established procedures.

2 

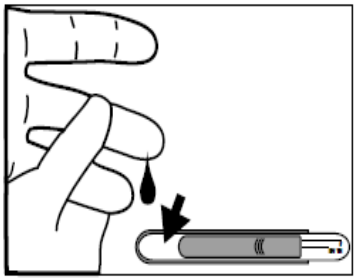
**Twist** off tab to break the seal and discard.

3 

**Position** safety lancet firmly against puncture site as illustrated. Hold safety lancet between fingers.

4 

To activate, **press** safety lancet firmly against the puncture site. Do not remove the device from the site until an audible click is heard.

5 

**Place** at least 3-5 drops of blood onto collector.

6 

**Discard** safety lancet into an approved sharps container.

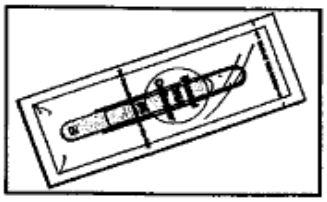
# How to Use the Buccal DNA Collector

**BUCCAL DNA COLLECTOR COMPONENTS**

A. Handle Base
B. Collection Paper
C. Slider Cover

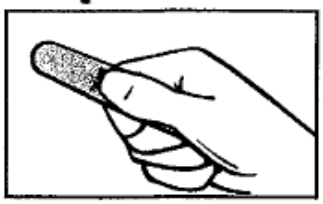

NOTE: Please read and follow instructions.
- If you are assisting in collection of sample, please read and follow steps 1 thru 6.
- If you are self-collecting, you are the subject in steps 2 thru 5.

## Step 1



Remove the Buccal DNA Collector from the pouch, holding the handle at the base. Move Slider Cover back if necessary to fully expose the Collection Paper.

## Step 2



Instruct the subject to hold the Buccal DNA Collector and put thumb on the area marked "Thumb" on the back of the Collector.

The subject does not need gloves to collect their own sample.

## Step 3

Instruct the subject to open mouth and place the white Collection Paper side flat against inside of cheek.

## Step 4

With Collection Paper pressed against inside of cheek, ask subject to drag it firmly toward lips and out of mouth.

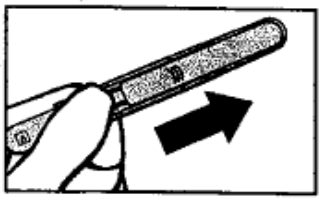
(This motion is similar to the "popping" of the cheek with a finger that children do.)

## Step 5


Repeat this action 7 more times. DO NOT rub Collector back and forth against cheek. It is not a toothbrush.

NOTE: It is important for collection personnel to observe subject pressing Collection Paper flat against cheek and dragging it across and bulging the cheek out during collection.

## Step 6



Push the Slider Cover forward towards the tip of the Collector, covering the Collection Paper.

## Step 7

Place one of the enclosed barcode labels onto area A (Handle Base) of the Buccal DNA Collector

APPENDIX E

The procedure that is contained in this Appendix was created for the lab that this project was performed in. The only modification that would be needed per lab would be how to access any high throughput worksheets or excel files.

## Manual AmpFLSTR® Identifiler® Direct PCR Amplification Kit Setup Using Buccal DNA Collector Samples

**Purpose:** To manually prepare amplifications of DNA samples collected by Buccal DNA Collector by using AmpFLSTR® Identifiler® Direct PCR Amplification Kit.

### Equipment and Supplies

- Centrifuge, vortex
- Heat block
- Pipettors and pipette tips (aerosol barrier)
- 96-well plates and base supports
- Buccal DNA Collector samples
- Blank paper (negative control)
- 1.5mL or 2mL microcentrifuge tubes
- GeneAmp® PCR System 9700
- Harris Punch (1.0mm or 1.2mm)
- Cutting mat
- Strip Caps

### Safety

Gloves, lab coats and eye protection must be worn during this procedure.

### Reagents

- Ethanol (70%)
- Bode PunchPrep™ Solution
- Control DNA 9947A (2.0ng/µL)
- $ddH_2O$

- AmpFLSTR® Identifiler® Direct Master Mix
- AmpFLSTR® Identifiler® Direct Primer Set

## Procedure

### A. DNA Extraction

**Note:** The Buccal DNA Collector will be used for both buccal and blood samples. Processing of these sample types needs to be performed on separate plates since amplification parameters are different.

1. Clean 1.2mm Harris Punch and cutting mat with 70% ethanol.

   **Note:** The protocol has been validated for both 1.0mm and 1.2mm punches for buccal samples.

   **Note:** Use one disposable Harris Punch per plate; one Harris Punch can punch approximately 90 samples.

2. Add 2µL of Bode PunchPrep™ Solution to each sample well for a maximum of 4 columns.

   **Note:** The effects of evaporation have been observed when processing a full plate.

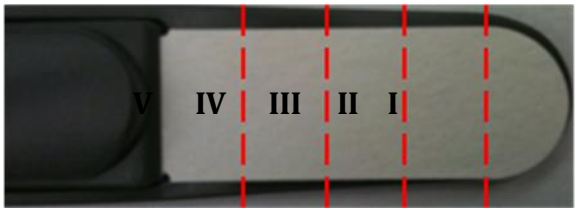
   **Note:** The positive and negative control wells are empty at this step.

3. Punch a disc from each Buccal DNA Collector sample and dispense into individual wells of 96-well plate.

   **Note:** The reagent blank well should contain a disc from an unused Buccal DNA Collector and 2µL of Bode PunchPrep™ Solution.

   **Note:** If possible, punch disc from Regions I or II on the Buccal DNA Collector (Figure 1). Optimal performance has been obtained from these regions.

**Figure 1:Regions of Buccal DNA Collector**




4. Visually confirm the presence of the disc in the Bode PunchPrep™ Solution.

   **Note:** Use a clean pipette tip to submerge disc in Bode PunchPrep™ Solution, if necessary.

5. Seal columns with strip caps.
6. Repeat steps 2-5 until plate is complete.
7. Incubate in heat block at 70ºC for 20 minutes.
8. Centrifuge for 20 seconds at approximately 3100 rpm.

## B. Positive Control and Negative Control Preparation

1. For a 1.2mm punch, add 2µL of 9947A to the positive control well(s).

   **Note:** If using a 1.0mm punch, add 1.5µL of 9947A and 0.5µL of $ddH_2O$ to the positive control well(s).

2. Add 2µL of $ddH_2O$ to the negative control well(s).

## C. Master Mix Calculations and Preparation

1. Launch the Y:\ network drive and open the folder "R&D Worksheets."

2. Open the Excel file "Manual Identifiler Direct Amplification Setup."

   Enter the number of samples to be setup for amplification in the specified cell. The spreadsheet will automatically populate all "Sample #" cells and calculate the volume needed for each component.

| Total volume of reagent | × | Volume of specified reagent needed per sample | Number of samples (*N*) | *Pipetting overage factor |
|---|---|---|---|---|

**Master Mix for Identifiler Direct**
(Example, *N* = 10)

| ***Reagent*** | ***Vol. per Sample (µL)*** | ***Total Volume (µL)*** |
|---|---|---|
| AmpFLSTR® Identifiler® Direct Master Mix | **12.5** | **137.5** |
| AmpFLSTR® Identifiler® Direct Primer Set | **12.5** | **137.5** |

***Pipetting overage factor = 1.1***

3. Allow reagents to equilibrate to room temperature.
4. Vortex for 3 seconds and centrifuge briefly.
5. Dispense 25µL of the reaction mix into each well.
6. Seal plate with strip caps.
7. Centrifuge at 3000 rpm for 20 seconds.

8. Amplify the samples inGeneAmp® PCR System 9700

## D. Thermal Cycling Parameters

1. Buccal

   a. Select program for 26 cycles.

   **Note:** Select program for 27 cycles for a 1.0mm punch.

   **Note:** Verify parameters.

| ***Identifiler Direct*** | | |
|---|---|---|
| **Number of cycles** | **Temperature** | **Time (min:sec)** |
| HOLD | 95ºC | 11:00 |
| 1.2mm: 26 CYCLES 1.0mm: 27 CYCLES | 94ºC | 0:20 |
| | 59ºC | 2:00 |
| | 72ºC | 1:00 |
| HOLD | 60ºC | 25:00 |
| HOLD | 4ºC | ∞ |

   b. Enter 27µL for the reaction volume.

   c. Select START.

   d. After the run is complete, samples can be electrophoresed or stored 14 days at 4°C.

2. Blood

   a. Select program for 25 cycles.

   **Note:** Verify parameters.

| ***Identifiler Direct*** | | |
|---|---|---|
| **Number of cycles** | **Temperature** | **Time (min:sec)** |
| HOLD | 95ºC | 11:00 |
| 1.2mm: 25 CYCLES | 94ºC | 0:20 |
| | 59ºC | 2:00 |
| | 72ºC | 1:00 |
| HOLD | 60ºC | 25:00 |
| HOLD | 4ºC | ∞ |

b. Enter 27µL for the reaction volume.

c. Select START.

d. After the run is complete, samples can be electrophoresed or stored 14 days at 4°C.

## References


1. Bode PunchPrep™ Instructions, Instructions for Use with the AmpFLSTR® Identifiler® Direct Amplification Kit.
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