Predicting the Quality of DNA Profiles through the Evaluation of the ParaDNA® Screening Instrument

Miranda Aufiero, BS, *Marshall University*
Stacey K. Anderson, MS,*
Amy B. McGuckian, MS,*
Julie Conover Sikorsky, MS,*
**Palm Beach County Sheriff's Office*
and
Pamela J. Staton, PhD, *Marshall University*

Abstract

In order to determine if the Palm Beach County Sheriff's Office (PBSO) Forensic Biology Unit could limit the number of low quality samples processed in the laboratory, the LGC Forensics ParaDNA® Screening Instrument with the Screening Kit was evaluated. The ParaDNA® Instrument processes evidentiary samples in 75 minutes, reporting a percent score indicative of whether the sample will yield a positive result from STR analysis and whether the DNA originates from a male or female. In order to determine if the ParaDNA® Instrument could be implemented at PBSO, 82 samples were analyzed on the ParaDNA® Instrument and a portion of the same sample processed through current PBSO protocols. The ParaDNA® percent score of each sample was compared to the quantification value, the profile obtained, and the average RFU value for each dye channel. These comparisons were used to determine the correlation between the ParaDNA® percent score and the STR profile result. The results of the evaluation showed that for all samples that the ParaDNA® Instrument rated greater than 75%, the quantification value was greater than the target amplification value of 0.8 ng and the sample yielded a full or full mixture profile. Of the samples rated between 25% and 74%, only 33% were above the target amplification value, yet 91% of these samples produced full or full mixture profiles. Only 4% of samples rated between 1% and 24% were above the target amplification value, with 46% resulting in full profiles. None of the samples rated 0% were above the target amplification value, yet 36% were able to yield a full profile. The results of this evaluation indicated that the ParaDNA® Screening Instrument with the Screening Kit may serve as a useful tool to prioritize evidentiary samples to be processed for STR analysis by helping to determine which samples may yield the most interpretable DNA profiles.

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Introduction

Forensic DNA laboratories throughout the United States are experiencing a backlog of casework due to an influx of requests to process all cases that contain biological evidence. As awareness of the ability of DNA to assist in investigations increases, more samples are being submitted to forensic laboratories for testing than ever before[8]. Requests for DNA analysis can be submitted faster than cases can be analyzed and reports written. Additionally, cold cases that were not previously analyzed for DNA evidence are being reopened and advancements in DNA technology have made it possible to obtain a DNA profile from a number of sources, such as touch DNA evidence. In order to meet the growing demand for DNA analysis in criminal investigations and decrease case turnaround time, it is essential to find a solution that will assist in sample analysis.

One solution is to hire additional analysts, however this is usually not a feasible option due to budgetary constraints. Another solution is to implement technologies that are faster, more efficient, and aid in streamlining laboratory procedures. The National Institute of Justice (NIJ) is working with accredited public-sector forensic laboratories and providing grants to help reduce the DNA backlog by providing funding for the hiring of new employees, purchasing of high throughput instruments, and contracting with private vendors to not only assist in the validation and implementation of instrumentation, but also in outsourcing casework. The DNA Backlog Reduction Program has allowed for the testing of numerous cases that would have otherwise remained backlogged[8].

Another alternative is the addition of a sample screening or prioritization process to current analytical procedures. Many samples analyzed for DNA may not generate an interpretable DNA profile or even a quantifiable amount of DNA, which has the potential to

consume valuable time and resources. For this purpose, LGC Forensics has developed the ParaDNA® Screening instrument. The ParaDNA® Instrument is a benchtop instrument designed to screen a sample for the presence of DNA in 75 minutes using melt curve analysis[1]. There are four heads on the instrument that can each process one sample at a time, either simultaneously or independently from one another. By using the ParaDNA® Instrument, it may be possible to reduce the number of samples tested in the forensic laboratory as well as sort and prioritize samples based on the results of this screening test.

The ParaDNA® Instrument requires a sample collector and reaction cartridge unique to the instrument. The sample collector is a device with four nibs that are designed to fit directly into the four wells of the reaction cartridge. Each of the four wells of the cartridge contain all reagents necessary for analysis and each well within the cartridge amplifies different regions of DNA, as seen in Table 1. There are two cartridges that are designed for use with the ParaDNA® Instrument – the Screening Kit and the Intelligence Kit (LGC Forensics, Teddington, UK). The Screening Kit amplifies short tandem repeat (STR) loci D16S539 and TH01, as well as Amelogenin. The results of the test show a percent value, which is designed to indicate the presence and quality of DNA in the sample, as well as a gender call. The Intelligence Kit amplifies the STR loci D3S1358, D8S1179, and D18S51, in addition to those amplified by the Screening Kit, but the instrument reports the actual allele calls. With regard to the Screening Kit, the loci amplified in each well of the cartridge correspond to a specific allele range, as seen in Table 1. For wells A, B, and C, alleles that are larger than the detected allele range will be categorized as the largest allele present within that range (i.e. for Well A, all alleles above 12 repeats will be called as a 12). The cartridge sits inside one head of the instrument, which has four cameras that read the fluorescence in each well individually[1].

Table 1: *Loci Tested by the ParaDNA® Screening Kit*[7]

Well	Locus	Allelic Range Detected
A	D16S539	8-12+
B	D16S539	11-15+
C	TH01	5-9.3+
D	Amelogenin	X, Y

The ParaDNA® Instrument utilizes a HyBeacon™ fluorescence detection assay to detect DNA in a sample and was shown to be a reliable method to rapidly genotype alleles[1]. Though initially developed for use in the medical field to analyze single nucleotide polymorphisms (SNPs) as a potential diagnostic method, this assay has been adapted for use with short tandem repeats (STRs) and for use in forensic laboratories[2]. HyBeacon™ analysis utilizes an asymmetric PCR reaction, melt curve analysis, and highly specific probes to differentiate between alleles based on the behavior of the DNA and bound probes at certain temperatures. HyBeacon™ probes are single-stranded oligonucleotides designed to be complimentary to the target DNA strand, and are labeled with a fluorophore, which increases in fluorescence when the probe is annealed to single stranded DNA[5,6]. When the probe is bound to the template DNA, the conformation of the probe changes and shifts the fluorophore closer to the DNA strand, thus increasing the fluorescence[4,6]. Using multiple probes with different fluorescent dye labels allows for multiplexing of the HyBeacon™ system, allowing either several loci to be analyzed in one reaction or separate probes to be used for different alleles of the same locus[10]. Initial testing of the HyBeacon™ probes showed that larger repeat alleles have too small of a difference in melting temperature (ΔT_M) to be able to accurately determine the allele length[3]. To eliminate this problem, a blocking oligonucleotide was added to the HyBeacon™ assay. These oligonucleotides are designed to bind to a specific number of the STR repeats (number of repeats varies by locus), as well as a portion of the template DNA prior to the repeat called the anchor.

This anchor ensures the blocker has bound to the template correctly and prevents slippage along the STR template. Once this is bound, a smaller HyBeacon™ probe is able to bind to the reduced-length STR. The reduced size of the target makes it possible for differentiation between the allele melting temperatures (T_M)[3].

The ParaDNA® assay begins with a direct asymmetric PCR step. Asymmetric PCR utilizes two primers in unequal concentrations. The DNA strands amplified by the primer that is less concentrated will bind to the excess of strands created by the primer with higher concentration. This will result in leftover single-stranded copies of the DNA template, in addition to double stranded copies that are not analyzed[9]. This reduction in the number of copies analyzed assists in the specificity of the assay. The wells are then cooled to 20° C, during which time a blocking oligonucleotide and HyBeacon™ probe anneal to the single-stranded DNA. Depending on the length of the allele, the probe will either anneal partially or fully. Alleles that are as long as or longer than the target probe length will bind completely and have a higher affinity for the probe. Once the probes are bound, they begin to fluoresce (Figure 1).

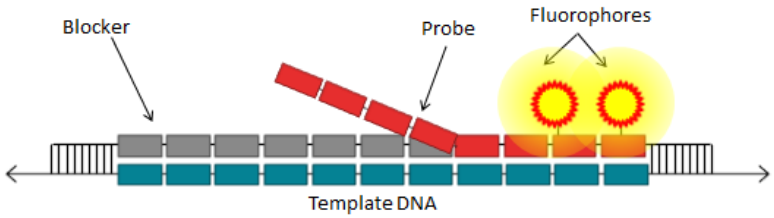

Figure 1: *HyBeacon™ probes and blocking oligonucleotide bound to template DNA, causing the bound fluorophores to fluoresce*[7]

The sample wells are then heated again and the probes separate from the DNA strand, at which point the probe will stop fluorescing. The point at which the probe separates from the DNA strand is the T_M for that specific allele. Shorter alleles have a lower T_M, while the T_M for longer alleles will be higher due to the increased affinity. The decrease in fluorescence is

measured by the change in relative fluorescence units (ΔRFU) of the sample and plotted to create a melt curve, showing the change in temperature versus the change in RFU (Figure 2). Based on the temperature at which the probe separated from the DNA strand, an allele call can be made for the sample[3,7].

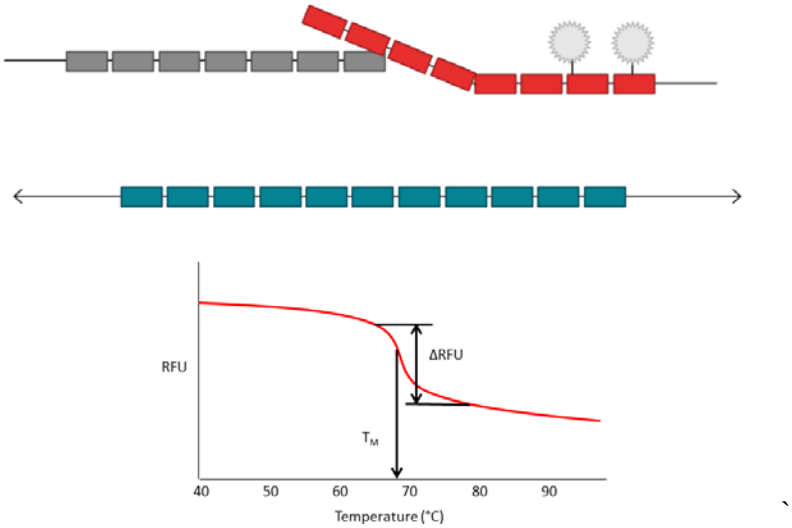

Figure 2: *Probe separated from template DNA (eliminating fluorescence of bound fluorophores) and the resulting melt curve*[7]

According to the ParaDNA® developmental validation, the probes used in the ParaDNA® Screening Kit are human and higher primate specific[1]. No cross reactivity was observed with any of the other species tested. Sensitivity tests demonstrated that the limit of detection of the Screening Kit was 62.5 pg. The developmental validation also showed that using the sample collector on a swab prior to STR analysis did not have a detrimental effect on the STR analysis, as the amount required for the test and removed by the sample collector was so low. Contamination studies showed that contamination was not occurring between samples or between the heads of the instruments, as each reaction is occurring in a sealed cartridge[1].

The Palm Beach County Sheriff's Office (PBSO) Forensic Biology Unit in West Palm Beach, FL, performed a pilot evaluation of the ParaDNA® Instrument and Screening Kit. Their goals in this evaluation included:

- Test the usability of the ParaDNA® Instrument and sample collector
- Test the efficiency of the sample collector to recover DNA from various types of evidence, including blood, saliva, mixed semen, and touch samples
- Determine the correlation of the results obtained with the ParaDNA® Instrument to the results obtained through traditional STR testing using current PBSO protocols
- Define the routes of implementation of the ParaDNA® Instrument in the PBSO laboratory
- Determine if there was any potential for cost savings within the PBSO Forensic Biology Unit with the addition of the ParaDNA® Instrument

In order to accomplish these goals, various sample types were tested using the ParaDNA® Instrument, and the results of a full STR analysis on these samples were compared to the percent score reported by the ParaDNA® Instrument.

Materials and Methods

Sample Collection and Preparation

Various sample types were tested on the ParaDNA® Instrument and through traditional STR analysis. Samples were identified as blood, mixed semen, saliva, or touch. A full list of samples evaluated can be viewed in Appendix A.

Whole blood samples were provided by the Palm Beach County Medical Examiner's Office. Both male and female blood was provided and genotyped. Samples were prepared by spotting the blood on various items to represent mock casework evidence. Neat blood, both male and female, was spotted onto a glass slide and denim and allowed to dry. The female blood was used for a 1:16 and a 1:200 dilution using pyrogenated water. Neat blood and the dilutions were spotted onto a cotton sock in triplicate. Two mixtures were prepared using the male and female blood samples – a 1:16 male/female mixture and a 16:1 male/female mixture. All blood samples were swabbed with sterile cotton swabs for analysis. A total of 15 blood samples were evaluated.

Mixed semen samples were prepared using known donors. A 1:1 saliva/semen biological fluid mixture was prepared, as well as a 1:16 saliva/semen mixture. Three swabs were created from each of the saliva/semen mixtures. Four vaginal swabs were collected and approximately 100 μL of neat semen was added to the swabs.

Saliva samples were collected either directly, with the ParaDNA® sample collector, or indirectly, with a cotton swab. Saliva was collected from a known donor and used for comparison of the two different collection methods using the ParaDNA® sample collector. The samples tested were neat, a 1:16 dilution and a 1:200 dilution using pyrogenated water. Other saliva samples were collected by taking a swab of the rims of various used soda bottles (6

samples), soda cans (3 samples), and chewed gum (2 samples). Cigarette butts (5 samples) were extracted from a cutting of the filter paper. A total of 34 saliva samples were evaluated.

Touch samples were collected by swabbing various items throughout the PBSO laboratory and other locations. Swabs were pre-wet with sterile water prior to sample collection, and two swabs were collected per item. A total of 23 touch samples were evaluated.

ParaDNA® Sample Collection

A total of 82 samples were evaluated, as well as 12 blank swabs and 6 blank ParaDNA® sample collectors. Two collection methods were used for ParaDNA® testing – direct and indirect. Direct collection refers to using the ParaDNA® sample collector to collect DNA directly from the source of the evidence, i.e. the stain or sample itself. This method was used in triplicate on the neat, 1:16, and 1:200 dilutions of saliva in order to determine if DNA removed from the sample by the ParaDNA sample collector would affect the results of downstream STR analysis. Six ParaDNA® sample collectors were used for each sample – three were run on the ParaDNA® Instrument and three were swabbed for STR analysis. The direct sampling method was also used on cigarette butts.

The remainder of the samples was tested using the indirect collection method. This method requires first taking a swab of the sample evidence item as would be used for traditional STR analysis and then using the ParaDNA® sample collector to sample those swabs. Swabs were taken from the prepared mock evidence items and allowed to dry, then rubbed with the ParaDNA sample collector for one minute (recommended by the ParaDNA® Training Manual). The original swab was then saved for further STR analysis.

ParaDNA® Testing

All 82 evaluation samples were tested using the ParaDNA® Instrument and the Screening Kit. Screening Kit cartridges were stored in a freezer at -20° C as per manufacturer directions. The cartridges for each run were thawed for at least 15 minutes prior to the run. The cartridges were labeled with the sample name and the foil seal was then removed. The nibs of the sample collectors were aligned with the wells of the cartridges and inserted. The cartridges were checked to ensure a proper seal between the sample collector head and the top of the cartridge. The sample collector handles were snapped off, and the cartridge with the sample collector head was placed into a head of the ParaDNA® Instrument. The corresponding sample information was entered into the ParaDNA® software linked to the instrument. The software requires two pieces of information to be entered for a run – Case Number and Item Number. The sample type (saliva, blood, touch, or mixed semen) was entered as the Case Number, and the name of the sample was entered as the Item Number. The instrument ran for 75 minutes, and the percent score and gender call assigned by the instrument and software was recorded for all samples.

STR Analysis

STR analysis was performed on all 82 samples evaluated, following current PBSO validated procedures. For all swabs, the entire swab was cut and extracted. All samples were extracted on an EZ1 Advanced XL using the DNA Investigator Kit (Qiagen, Gaithersburg, MD). Mixed semen samples were separated using the PBSO protocol for differential extractions, and resulted in two extracts per sample. An extraction positive control (blood sample that is well characterized and has been genotyped by PBSO) and negative control (reagent blank) were included with every extraction. Quantification was performed using Plexor® HY (Promega,

Madison, WI) and a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Samples were amplified using PowerPlex® 16 (Promega, Madison, WI) and a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA). Samples were normalized to target an input of 0.8 ng prior to amplification based on the quantification value. Capillary electrophoresis was performed on a 3130*xl* Genetic Analyzer (Applied Biosystems, Foster City, CA). Profiles were analyzed using the GeneMapper® ID-*X* Software version 1.3 (Applied Biosystems, Foster City, CA).

Comparisons

Comparisons were made between the results of the ParaDNA® testing and the results of the in-house STR analysis. Raw data from the ParaDNA® Instrument was sent to LGC, and the melt curves associated with each sample were analyzed and returned to PBSO. The ParaDNA® percent score for each sample was compared to the quantification value of that sample to determine if there was a correlation between the percent score and the quantification value. The percent score was also compared to the profile obtained (full, partial, mixture, or none) and the average relative fluorescence (RFU) across each dye channel.

Results

ParaDNA® Percent Scores and Gender Calls

The results of the ParaDNA® testing are listed in Appendix B. The percent scores reported by the ParaDNA® Instrument were categorized into four groups: 75-100%, 25-74%, 1-24%, and 0%. Figure 3 below shows the number of samples and the percentage of total scores that fell within each category. Fourteen samples had a ParaDNA® percent score in the 75 to 100% category, 30 samples had a ParaDNA® percent score between 25 and 74%, and 24 samples were between 1 and 24%. The remaining 14 samples were all assigned a ParaDNA® percent score of 0%.

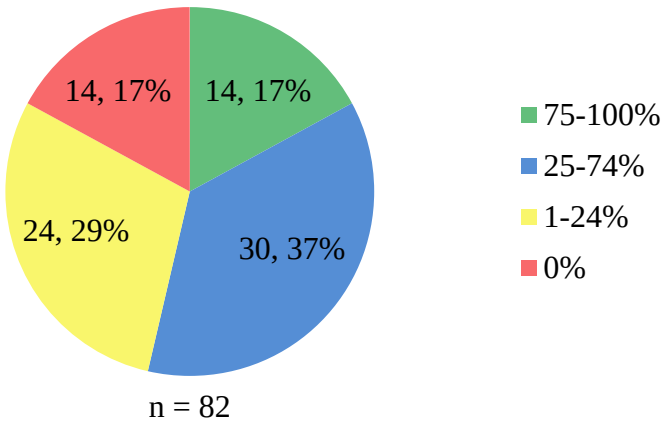

Figure 3: *Percent scores assigned by the ParaDNA® Instrument (segments are labeled with number of scores and percent of total scores within each range)*

Figure 4 shows the gender calls assigned to the evaluation samples. Sixty-two percent of samples were not assigned a gender call by the ParaDNA® Instrument.

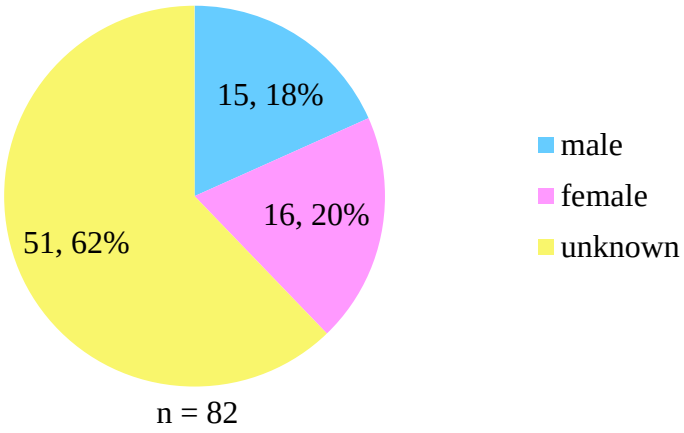

Figure 4: *Gender calls assigned by the ParaDNA® Instrument (segments are labeled with number of calls and percent of total calls for male, female, and unknown)*

Table 2 shows the percent scores assigned by the ParaDNA® Instrument by sample type. The blood samples were distributed among all percent score categories. All of the mixed semen samples were assigned a percent score higher than 25%, with 80% of the samples assigned a percent score between 75 and 100%. No saliva samples were assigned a percent score higher than 75%, however 53% were assigned a percent score between 25 and 74%, and 41% were assigned a percent score between 1 and 24%. Of the touch samples, 43% were assigned a percent score of 0%. No touch samples were assigned a percent score higher than 75%.

Table 2: *Percent scores assigned by ParaDNA® Instrument by sample category*

	75-100%	**25-74%**	**1-24%**	**0%**
Blood	6	4	3	2
Mixed Semen	8	2	0	0
Saliva	0	18	14	2
Touch	0	6	7	10

ParaDNA® Melt Curve Classifications

All raw data from samples evaluated on the ParaDNA® Instrument was sent to LGC for analysis. The melt curves from each sample were classified by LGC into one of five categories: good, leakage, mixture, dropout, or not enough DNA. Leakage occurs when the sample collector head is not properly sealed on the cartridge. Because of this, fluorescence can "leak" from one well and be detected by the camera of a different well, which can generate incorrect calls by the ParaDNA® Instrument. Leakage is due to an error in the technique of loading the sample collector into the cartridge. Leakage was present in several of the samples analyzed at the beginning of the study due to improper sealing of the cartridges and sample collector heads. According to LGC, this could result in inaccurate percent scores and gender determination as reported by the instrument. Samples that indicated that leakage was occurring were not included in the results for later studies. After the cartridge sealing procedures were corrected, LGC continued to provide feedback as to the amount of leakage seen in the analyzed samples. Figure 5 shows the breakdown of melt curve classifications, as provided by LGC, for all four cartridge wells for all samples evaluated (n = 4 wells x 82 samples).

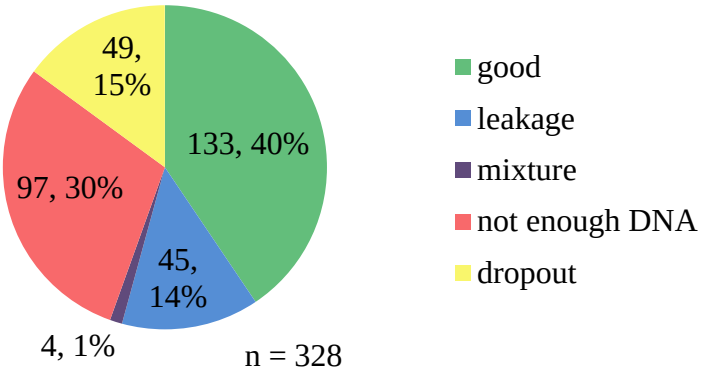

Figure 5: *ParaDNA® melt curve classifications (per LGC) in all four cartridge wells (segments are labeled with number of calls and percent of total calls for each classification)*

The melt curve classifications were broken down by well, as seen in Table 3. Wells A and B had more samples that were classified as good than wells C and D. Wells A and C exhibited a higher percentage of leakage, 18% and 26% respectively, than wells B and D. Mixtures were only seen in wells A and B; however the occurrence was very low. All wells showed between 22% and 34% of samples were classified as not enough DNA. The 14 samples that were assigned a percent score of 0% by the ParaDNA® Instrument are all included in this category for each well. Wells C and D exhibited a higher percentage of dropout, 20% and 29% respectively, than wells A and B.

Table 3: *ParaDNA® melt curve classifications (per LGC) by well*

	Well A: D16 (8-12+)	**Well B: D16 (12-15+)**	**Well C: TH01 (5-9.3+)**	**Well D: Amelogenin**
good	34	44	27	28
leakage	15	7	21	2
mixture	3	1	0	0
not enough DNA	23	28	18	28
dropout	7	2	16	24

ParaDNA® Percent Scores vs. Quantification Values

The quantification values for the evaluation samples can be seen in Appendix C. This also shows the normalized quantification value, which corresponds to the amount of DNA, in nanograms, that was added to the amplification reaction. Table 4 shows the average quantification value for each ParaDNA® percent score category. It also shows the number of samples that were above or below the PBSO target amplification value of 0.8 ng in each category. Finally, it shows the number of samples that were above target after normalization, or if >1 µL of extract were amplified. As the maximum sample volume allowed by PowerPlex® 16

is 19.2 µL, several samples were still below the 0.8 ng target with amplification of the full amount allowed.

Table 4: *Average quantification values, samples above and below PBSO target amplification value of 0.8 ng, and samples above target after sample normalization (amplification of >1 µL) for each ParaDNA® percent score category*

ParaDNA® Percent Score	Number of samples	Average Quant Value (ng/µL)	Samples Below Target	Samples Above Target	Percent Above Target	Normalized Samples Above Target	Normalized Percent Above Target
100-75%	22	22.12	0	22	100%	22	100%
74-25%	32	5.96	21	11	34%	20	63%
24-1%	24	0.08	23	1	4%	6	25%
0%	14	0.04	14	0	0%	3	21%

ParaDNA® Percent Scores vs. Profile Obtained

After STR analysis was complete, the profiles obtained were classified into one of five categories: full, partial, mixture – full, mixture – partial, or none. For partial or mixture – partial, the number of loci that had allele calls was recorded. The profiles obtained from each sample, as well as the number of loci that had an allele call, can be seen in Appendix D. Figure 6 shows the profiles obtained from all of the evaluation samples, where n = 92 due to the mixed semen samples resulting in two separate profiles each (10 differential samples = 20 profiles). Of the 92 samples, 73% were full profiles, with 46% of the total being single-source profiles and 27% of the total being full mixed profiles. Twenty-six percent of samples yielded a partial profile, with 18% of the total being single-source profiles and 8% of the total being partial mixed profiles. A total of 32 samples (35%) were determined to be mixtures. Only one sample (wooden handle of hammer) yielded no profile whatsoever. This sample was run through a Microcon® Centrifugal

Filter (Millipore, Billerica, MA) to determine if inhibition was occurring, but re-analysis still yielded no profile.

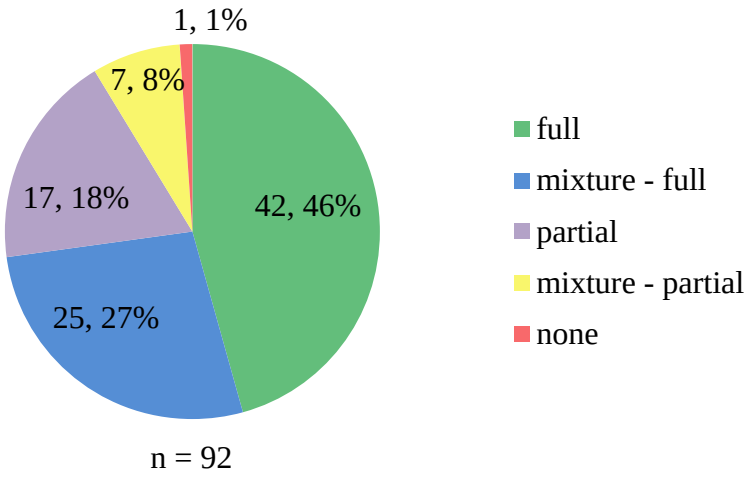

Figure 6: *STR profiles obtained from all evaluation samples (segments are labeled with number of profiles and percent of total profiles for each classification)*

Table 5 shows the profiles obtained for each sample type. The majority of the blood samples, 87%, gave full profiles. Two of the diluted blood samples yielded partial profiles. Ninety percent of the differential samples gave a full mixed profile; however, all of these had a clear major and minor contributor. One sample gave a full single-source profile, and one gave a partial single-source profile. The partial profile, however, had allele calls at 15 of 16 loci. Of the saliva samples, 74% yielded a full single-source profile and 3% yielded a full mixed profile. The remaining 23% gave a partial single-source profile. The 8 saliva samples that gave partial profiles were the three 1:200 dilution samples and the five cigarette butt samples. The touch samples yielded a range of profiles, with 23% giving a full single-source profile, 17% giving a full mixed profile, 26% giving a partial single-source profile, 30% giving a partial mixed profile, and 4% giving no profile.

Table 5: *Profiles obtained for each sample type*

	full	mixture - full	partial	mixture - partial	none
Blood	11	2	2	0	0
Mixed Semen	1	18	1	0	0
Saliva	25	1	8	0	0
Touch	5	4	6	7	1

In order to determine the correlation between the ParaDNA® percent scores and the profiles obtained, the results were further broken down by the profiles obtained in each percent score category. Figure 7 shows the breakdown of the profiles obtained for each ParaDNA® percent score category. Of the samples that were assigned a ParaDNA® percent score between 75 and 100%, all of the profiles obtained were full, whether single-source or mixed profiles. Though 14 samples were categorized by the ParaDNA® Instrument as between 75 and 100%, 22 profiles total were obtained due to 8 of these samples being differential samples (2 profiles each). Of the samples that were assigned a ParaDNA® percent score between 25 and 74%, 91% were full profiles, with 66% of the total being single-source and 25% of the total being mixed profiles. Two of the samples that fell into this category were differential samples, so these produced a total of 4 profiles. The remaining 9% of profiles were partial. In the 1-24% range, 46% of samples yielded full profiles, all of which were single-source. Fifty percent of profiles in this range were partial, with 29% of the total being single-source and 21% of the total being mixed profiles. The one sample that yielded no profile was in this range. Of the samples assigned a ParaDNA® percent score of 0%, 36% yielded full profiles, with 29% of the total giving a single-source profile and 7% of the total giving a mixed profile. Sixty-four percent of the samples in

this category gave a partial profile, with 50% of the total being single-source and 14% of the total being mixed profiles. None of the samples in this category yielded a negative profile.

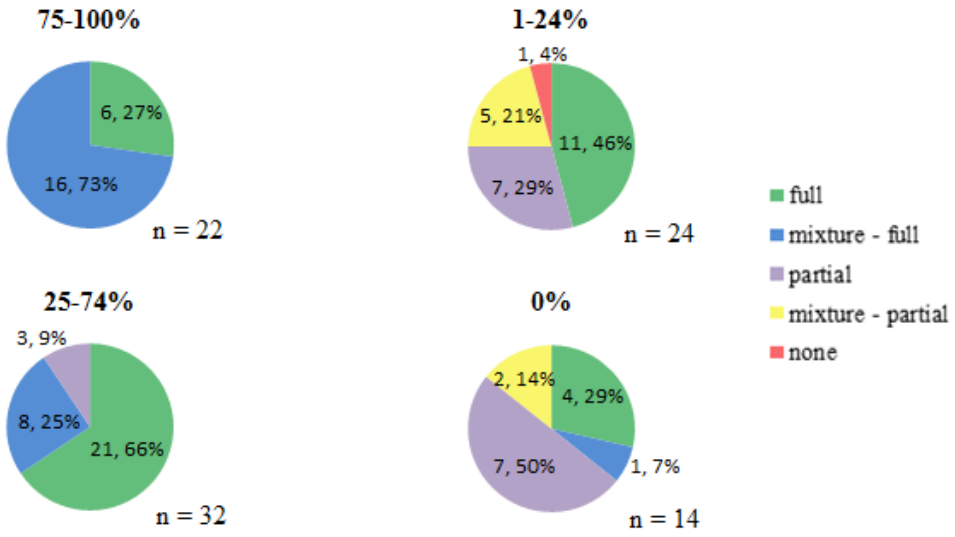

Figure 7: *Profiles obtained for each ParaDNA® percent score category (segments are labeled with number of profiles and percent of total profiles for each classification)*

For the 24 samples that gave partial profiles (single-source and mixed), as well as the one sample that gave no profile, the number of loci that had allele calls were counted. Figure 8 shows the number of profiles that had allele calls at less than the full 16 loci. Of these profiles, 72% had allele calls for at least half of the loci (8 loci). The remaining 28% had 7 or less loci called.

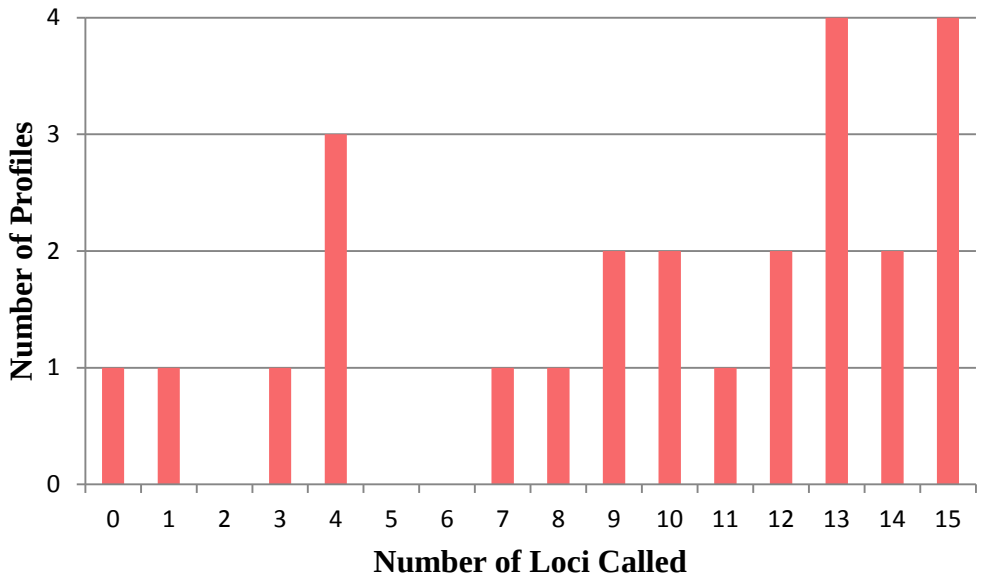

Figure 8: *Number of loci called for all profiles categorized as partial, mixture - partial, or none*

ParaDNA® Percent Scores vs. Average Profile RFU

The average RFU was calculated, by dye channel, for each sample in order to determine how many samples were above the PBSO stochastic threshold of 208 RFU in each ParaDNA® percent score range. This was used to determine the correlation between the ParaDNA® percent score and the quality of the profile obtained. The average RFU, by dye channel, for each sample can be seen in Appendix D. Figure 9 shows the average RFU for all dye channels, split by profiles within each ParaDNA® percent score range, that are above stochastic threshold and those that are below stochastic threshold. The average RFU was plotted across all dye channels rather than each individually because the results were mostly the same for all dye channels, with the exception of one additional sample above stochastic threshold in the green channel (JOE). However, the overall average for this sample was below stochastic threshold. All samples that were assigned a ParaDNA® percent score above 75% gave a profile that was above stochastic

threshold. Additionally, all of these were full profiles (see Figure 7). Of the samples assigned ParaDNA® percent scores between 25 and 74%, 94% were above stochastic threshold. The 6% (2 samples) that were below stochastic threshold were also both partial single-source profiles. For the samples assigned a ParaDNA® percent score between 1 and 24%, 58% were above stochastic threshold. For the samples assigned a ParaDNA® percent score of 0%, 36% were above stochastic threshold.

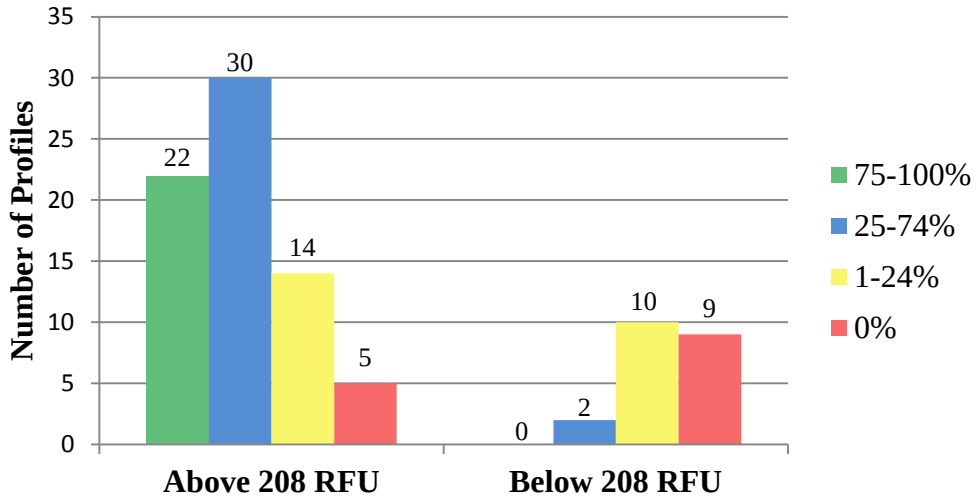

Figure 9: *Average RFU for profiles obtained, where 208 RFU is the PBSO stochastic threshold*

Discussion

ParaDNA® Percent Scores and Gender Calls

The percent scores reported by the ParaDNA® Instrument were, in general, consistent with what would be expected from the sample types. The neat blood samples were all assigned high percent scores, while the dilutions (1:16 and 1:200) were assigned lower scores. The mixed semen samples were all assigned high percent scores, with 80% of samples assigned a percent score above 75%. However, the prepared saliva samples (neat and dilutions) did exhibit more variability than expected which may have been the result of the improper sealing of the sample cartridge. The additional saliva samples, taken from chewed gum, soda bottles and cans, and cigarette butts had ParaDNA® results more consistent with what would be expected from these items of evidence. These samples were all below 75% but greater than 0%, indicating that DNA was present, but not in significant amounts. The touch samples had ParaDNA® percent scores below 75%, with 43% assigned a percent score of 0%. The highest percent score assigned to a touch sample was 54%. These low results were expected of the touch samples, as they typically contain less DNA than other sample types.

Sixty-two percent of the samples were not assigned a gender call by the ParaDNA® Instrument. Of the 51 samples without a gender call, 14 were samples that were assigned a percent score of 0%. Of the remaining 37 samples, 16 were due to low level DNA concentrations in the sample well, 20 were due to possible dropout, and one was classified as leakage. For the samples classified as dropout, it was apparent that DNA was present in the sample; however the decrease in fluorescence was not enough of a change for the ParaDNA® Instrument to make the allele call. Though the total number of samples that actually had a gender call was low (38%), 97% of gender calls were consistent with the known STR profile data.

ParaDNA® Melt Curve Analysis

The first 18 samples run on the ParaDNA® Instrument were the saliva samples prepared from a known source and generated results that were lower than expected. The raw data from these samples was analyzed by LGC, and the data indicated that leakage occurred in 15 of these samples. These 15 samples accounted for 64% of the total leakage results seen (see Figure 5). With the help of LGC, the problem was remedied for further testing. The rest of the cartridges in the evaluation were checked for proper seals prior to analysis. The cause of leakage in the remaining 36% of samples is unknown. Melt curve analysis by LGC, however, was only used during this evaluation and was important for troubleshooting errant results. In casework scenarios, the melt curves would not be provided to the lab, so it is essential that users are properly trained and have knowledge of how to seal the cartridges in order to ensure that accurate results are obtained.

ParaDNA® Percent Scores vs. Quantification Values

When compared to the quantification values, it can be seen that there is a correlation between the ParaDNA® percent scores and the quantity of DNA present in the sample, although the ParaDNA® Instrument does not directly quantify DNA during its analysis. All samples assigned a percent score above 75% had a quantification value that was greater than the PBSO target amplification value of 0.8 ng. The percentage of samples above this target decreases with each decreasing percent score range (see Table 4). However, with amplification of up to 19.2 μL (the maximum amount of sample volume allowed by PowerPlex® 16) it is possible to achieve the target amplification value in 63% of samples with a percent score between 25 and 74%, 25% of

samples with a percent score between 1 and 24%, and 21% of samples with a percent score of 0%. This downward trend is consistent with the decreasing percent scores.

From the quantification results, it can be inferred that samples with a percent score between 75 and 100% are likely to contain a quantity of DNA higher than the target amplification value, samples with a percent score between 25 and 74% may be below target quantity but can likely be normalized to amplify the target value, and samples below 25% (including 0%) may be normalized to amplify the target value, but are more likely to be below the target amplification value even after normalization.

ParaDNA® Percent Scores vs. Profile Obtained and Average RFU

A correlation could be made between the quality of the STR profiles obtained from the samples and the ParaDNA® percent scores. Seventy-three percent of the profiles obtained were full profiles, whether single-source or mixed. Of the partial profiles, 72% had allele calls at greater than half of the loci.

The STR profiles obtained for each sample category were, for the most part, as expected. The neat blood samples all generated complete profiles, as did 67% of the diluted samples. Two of the 1:200 dilution samples resulted in partial profiles, but this result was expected due to the nature of the samples. The two blood mixtures (1:16 and 16:1 male to female) resulted in full mixed profiles, with a clear major and minor contributor, as expected. The mixed semen samples resulted in full mixed profiles 90% of the time, however a major female contributor could be seen in the non-sperm fractions, and a major male contributor could be seen in the sperm fractions. These mixtures were due to incomplete separation. One mixed semen sample resulted in a partial profile, however only one locus was not called. The saliva samples prepared from a

known source also yielded profiles as expected. All prepared saliva samples collected indirectly (by teasing a swab and performing STR analysis on this swab) resulted in a full profile. Of the samples collected directly (STR analysis performed on a swab of a ParaDNA® sample collector), the neat and 1:16 dilution samples gave full profiles, while all three 1:200 dilutions resulted in partial profiles that were below stochastic threshold. This was expected, as the amount of DNA collected by the ParaDNA® sample collector is minimal. Twenty-two out of the 23 touch samples did provide some profile, even if only at a few loci.

All of the samples that were assigned a ParaDNA® percent score between 75 and 100% gave a full single-source or mixed profile. Additionally, all of these profiles were above the PBSO stochastic threshold of 208 RFU. These profiles are consistent with the high ParaDNA® percent scores.

The samples assigned a ParaDNA® percent score between 25 and 74% resulted in mostly full single-source or mixed profiles, with a small percentage (9%) of partial single-source profiles. Of the three samples that did not yield full profiles, all had allele calls at greater than 11 loci. Two of the partial profiles were below stochastic threshold. The rest of the samples in this category were above stochastic, including the one remaining partial profile. From these results, it can be inferred that samples with a ParaDNA® percent score between 25 and 74% are likely to result in a full profile, but this may not always be the case.

For the samples assigned a ParaDNA® percent score between 1 and 24%, a full profile was obtained from less than half (46%) of the samples. The remainder of the samples yielded either a single-source or mixed partial profile, with one sample yielding no profile. This sample was processed through a Microcon-100® to remove any inhibitors and re-analyzed. After re-analyzing the sample, Amelogenin was called and signals were visible at 9 loci, however these

were below the analytical threshold of 50 RFU so no calls were made. Of the profiles that were obtained, 58% were above stochastic threshold, while the other 42% were below stochastic threshold. Though the results show that it is possible to obtain an interpretable profile from the samples assigned a ParaDNA® percent score between 1 and 24%, there is also a high chance that the profile obtained will not be of good quality.

All of the samples assigned a ParaDNA® percent score of 0% yielded some sort of profile, although 64% were partial single-source or mixed profiles. Five profiles (36%) were full profiles and were above stochastic threshold. One of the samples with a ParaDNA® percent score of 0% that yielded a full profile, a direct sampling of neat saliva, was in the first round of testing when leakage issues were still occurring. The 0% score can be attributed to the leakage issues for this sample. For the remaining samples, it is unknown why the ParaDNA® Instrument did not detect the presence of DNA. It is possible that these samples may have had uneven sampling by the ParaDNA® sample collector, resulting in little to no DNA detected by the ParaDNA® Instrument. Though the results seem to imply that even a sample with a ParaDNA® percent score of 0% will result in a profile, this may be due to the nature of the samples evaluated. Samples that were chosen for this study were expected to give at least a partial profile. Approximately 12% of all samples tested at the PBSO lab do not result in a DNA profile. Had samples such as those been evaluated, it is likely that they would have received a percent score of 0% by the ParaDNA® Instrument. Additionally, 64% of the samples with a ParaDNA® percent score of 0% evaluated were below stochastic threshold and dropout was known to be occurring. Though results were obtained through STR analysis, they are not always good quality profiles, and in a casework scenario, may not have needed to be tested.

Overall, there is a correlation between the ParaDNA® percent score and the STR profile obtained. Higher percent scores are more likely to result in a quality profile. Lower percent scores have the possibility of yielding a profile, but this profile may be partial or below stochastic threshold.

ParaDNA® Cost Analysis

A cost analysis was attempted to determine if the ParaDNA® Instrument would be a cost efficient method of screening evidentiary samples in the PBSO laboratory. This analysis was based on the costs of all reagents and consumables used in traditional STR analysis, but do not account for analysts' salaries, benefits or facility costs. A full list of the costs of all reagents and consumables can be seen in Appendix E. Table 6 shows the estimated total cost of analysis for January through June of 2013, based on the number of samples processed in the PBSO laboratory during this time. The cost per case includes all standards and controls necessary for STR analysis, however the cost per sample only includes the reagents and consumables necessary for a single sample, with no controls included. The total cost of analysis was calculated based on the cost per case. The success rate of the PBSO laboratory was calculated using data from January through June of 2013. Any sample that resulted in a profile with at least one allele call was considered a successful sample. This resulted in an overall success rate of 88% in the PBSO laboratory during the six-month period analyzed. During this time period, 85 samples (12%) yielded either no quantification results or no STR profile.

Table 6: *Total cost of STR analysis per 6 months, based on PBSO data from Jan-Jun 2013*

Cases per 6 Months	125
Samples per 6 Months	698
Average Samples per Case	5.6
Cost per Sample	$78.59
Cost per Case	$593.25
Success Rate	88%
Total Cost per 6 Months	**$73,944.38**

From these results, the total cost of ParaDNA® screening and STR analysis was calculated as if the 85 negative samples had been eliminated prior to STR analysis. Table 7 shows the total cost both with and without ParaDNA® screening, based on the 88% success rate and the number of samples analyzed in January through June of 2013. The cost of analyzing one sample on the ParaDNA® Instrument is $50. Had the 85 negative samples been screened prior to STR analysis, it would have cost the PBSO laboratory $4,250. However, the cost of STR analysis on the remaining 613 positive samples would have been only $64,940, as opposed to the cost of $73,944 for analyzing all 698 samples. The total cost of ParaDNA® screening and STR analysis in this scenario would be $69,190. This amounts to a total savings of $4,755. This data is based on the assumption that only the negative samples would have been screened with the ParaDNA® Instrument, which is unlikely.

Table 7: *Cost of in-house STR analysis with and without ParaDNA® screening, based on PBSO data from Jan-Jun 2013 and an 88% success rate*

	Without Screening	**With Screening**
Samples Screened	N/A	85
Positive Samples	N/A	0
Samples Sent to Lab	698	613
Useable Profiles Generated	613	613
Total Screening Costs	**$0**	**$4,250**
Total Lab Costs	**$73,944**	**$64,940**

Additionally, a cost analysis was also examined based on the percentage of PBSO samples that resulted in a profile with allele calls at less than six loci. A total of 67 profiles had at least one but less than six loci called, resulting in a total of 152 profiles when combined with the 85 negative profiles. This would make the overall success rate 78%, as opposed to the previous 88%. Table 8 shows the total cost of analysis both with and without ParaDNA® screening, based on this 78% success rate and the number of samples analyzed in January through June of 2013. Had the 152 samples been screened prior to STR analysis, it would have cost $7,600. However STR analysis would have only been performed on 546 samples, at a cost of $57,842. The total cost of ParaDNA® screening and STR analysis in this scenario would be $65,442. This amounts to a total savings of $8,503. Again, this data is based on the assumption that only these 152 samples were screened with the ParaDNA® Instrument, which is again unlikely. With the available data, it cannot accurately be determined at this time what, if any, cost benefit utilizing the ParaDNA Screening Instrument with Screening Kit would have on the PBSO Laboratory.

Table 8: *Cost of in-house STR analysis with and without ParaDNA® screening, based on PBSO data from Jan-Jun 2013 and a 78% success rate*

	Without Screening	**With Screening**
Samples Screened	N/A	152
Positive Samples	N/A	0
Samples Sent to Lab	698	546
Useable Profiles Generated	546	546
Total Screening Costs	**$0**	**$7,600**
Total Lab Costs	**$73,944**	**$57,842**

Conclusions and Recommendations

From the results of the ParaDNA® Instrument with the Screening Kit when compared to traditional STR analysis, the expected quality of the STR profile can be inferred as the ParaDNA® percent score seems to correlate with the quality of the profiles and the quantity of DNA in the sample. It can be expected that a sample assigned a ParaDNA® percent score between 75 and 100% will yield a full profile that is above stochastic threshold and the heterozygote peaks can be expected to be balanced. In general, samples in this category can be expected to yield a good quality profile. Samples assigned a ParaDNA® percent score between 25 and 74% will mostly yield a full profile that is above stochastic threshold and has balanced heterozygote peaks. Some of these samples may yield partial profiles or profiles below the stochastic threshold. Samples in this category also typically result in good quality profiles, but there is a possibility the profile obtained may only be of average quality. Samples assigned a ParaDNA® percent score between 1 and 24% may often result in partial profiles or profiles below stochastic threshold. Many of these profiles have imbalanced heterozygote peaks, indicating that dropout may be occurring. Though some full profiles were obtained in this sample category, most samples resulted in poor quality profiles. Finally, samples that were assigned a ParaDNA® percent score of 0% often result in a partial profile or a profile that is below stochastic threshold and has imbalanced heterozygote peaks. Some samples did yield a full profile; however, these may have been outliers due to sampling technique or leakage as defined by LGC. In a casework scenario, these samples may not have been tested initially based on the ParaDNA® percent score, but they may be analyzed at a later date, depending on the case.

In casework, many samples are received that have no detectable DNA. Based on the PBSO data from January through June of 2013, there were 85 such samples. Had these samples

been screened, and assuming they were truly negative, they should have been assigned a ParaDNA® percent score of 0%. This would enhance the correlation between the ParaDNA® percent score of 0% and the profile, or lack thereof, that will be obtained. Though several negative sample collectors and swabs were tested on the ParaDNA® Instrument, these were not carried through to STR analysis and therefore cannot be included in the comparison data. Of these, one sample collector and one swab gave a percent score above 0%, but the LGC melt curve analysis showed that leakage was occurring and the allele calls were spurious. Additionally, 67 PBSO casework samples resulted in a profile with between one and six loci with allele calls. It is likely that these samples would have received a ParaDNA® percent score between 1 and 24% had they been screened. However, without actual evaluation of these samples on the ParaDNA® Instrument, it is impossible to know for sure.

Based on the correlation found between the ParaDNA® percent scores and the STR profiles obtained, a prioritization system can be created. Screening with the ParaDNA® Instrument is not necessary for every sample, as many samples tested are reference standards or samples that are expected to contain a high quantity of DNA. For these samples, screening would likely yield a high percent score, and would only increase the cost and time of analysis. However, many samples received by the PBSO laboratory are touch samples, or are samples that may contain a very low quantity of DNA, if any at all. Often, these samples result in a profile that cannot be interpreted. It is these samples that would benefit from screening with the ParaDNA® Instrument. Analyst discretion must be used when selecting which samples to screen with the ParaDNA® Instrument.

Table 9 shows the prioritization of samples based on the ParaDNA® percent scores. Samples assigned a ParaDNA® percent score between 75 and 100% should be analyzed first as

they are most likely to give the best quality profile, followed by samples assigned a ParaDNA® percent score between 25 and 74% . If more samples are needed, those with a ParaDNA® percent score between 1 and 24% could then be analyzed. Samples with a ParaDNA® percent score of 0% should be saved in the instance that more testing is required for the case, but do not need to be analyzed immediately as they are unlikely to yield an interpretable profile. The decision whether to screen samples with the ParaDNA® Instrument may rely on analyst discretion given the sample types and the case scenario.

Table 9: *Prioritization of samples based on ParaDNA® percent scores*

ParaDNA® Percent Score	**Priority**	**Comments**
75-100%	High	test first; likely to obtain interpretable results
25-74%	High/Medium	test if needed; likely to obtain interpretable results
1-24%	Low	test if needed; may or may not obtain interpretable results
0%	Low	save for later testing; not likely to obtain interpretable results

The ParaDNA® Instrument with the Screening Kit may serve as a useful tool for the PBSO laboratory in allowing for the prioritization of evidentiary samples prior to STR analysis. The PBSO hopes to continue their evaluation of the ParaDNA® Instrument by next evaluating the Intelligence Kit, which would report allele calls for six forensic loci.

Screening samples with the ParaDNA® Instrument prior to STR analysis has the potential to increase laboratory efficiency. By prioritizing samples and focusing on the highest quality samples, the laboratory would analyze the most interpretable samples first, potentially eliminating low quantity or quality DNA samples from the analysis process. This may have a direct effect on backlog reduction and a related decrease in analyst time and other laboratory resources. This study has shown the ParaDNA® Instrument to be a reliable method to prioritize evidentiary samples.

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Appendix A: List of Evaluation Samples

Sample	Type	Collection Method
Male blood on denim, neat	Blood	Indirect
Female blood on denim, neat	Blood	Indirect
Male blood on glass slide, neat	Blood	Indirect
Female blood on glass slide, neat	Blood	Indirect
Female blood on sock, neat (1)	Blood	Indirect
Female blood on sock, neat (2)	Blood	Indirect
Female blood on sock, neat (3)	Blood	Indirect
Female blood on sock, 1:16 dilution (1)	Blood	Indirect
Female blood on sock, 1:16 dilution (2)	Blood	Indirect
Female blood on sock, 1:16 dilution (3)	Blood	Indirect
Female blood on sock, 1:200 dilution (1)	Blood	Indirect
Female blood on sock, 1:200 dilution (2)	Blood	Indirect
Female blood on sock, 1:200 dilution (3)	Blood	Indirect
1:16 male/female blood mixture	Blood	Indirect
16:1 male/female blood mixture	Blood	Indirect
Vaginal swab/semen mixture (1)	Differential	Indirect
Vaginal swab/semen mixture (2)	Differential	Indirect
Vaginal swab/semen mixture (3)	Differential	Indirect
Vaginal swab/semen mixture (4)	Differential	Indirect
1:1 saliva:semen mixture (1)	Differential	Indirect
1:1 saliva:semen mixture (2)	Differential	Indirect
1:1 saliva:semen mixture (3)	Differential	Indirect
1:16 saliva:semen mixture (1)	Differential	Indirect
1:16 saliva:semen mixture (2)	Differential	Indirect
1:16 saliva:semen mixture (3)	Differential	Indirect
Saliva, neat (1)	Saliva	Direct
Saliva, neat (2)	Saliva	Direct
Saliva, neat (3)	Saliva	Direct
Saliva, 1:16 dilution (1)	Saliva	Direct
Saliva, 1:16 dilution (2)	Saliva	Direct
Saliva, 1:16 dilution (3)	Saliva	Direct
Saliva, 1:200 dilution (1)	Saliva	Direct
Saliva, 1:200 dilution (2)	Saliva	Direct
Saliva, 1:200 dilution (3)	Saliva	Direct
Saliva, neat (1)	Saliva	Indirect
Saliva, neat (2)	Saliva	Indirect
Saliva, neat (3)	Saliva	Indirect
Saliva, 1:16 dilution (1)	Saliva	Indirect
Saliva, 1:16 dilution (2)	Saliva	Indirect

Saliva, 1:16 dilution (3)	Saliva	Indirect
Saliva, 1:200 dilution (1)	Saliva	Indirect
Saliva, 1:200 dilution (2)	Saliva	Indirect
Saliva, 1:200 dilution (3)	Saliva	Indirect
Chewed gum (1)	Saliva	Indirect
Chewed gum (2)	Saliva	Indirect
Cigarette butt (1)	Saliva	Direct
Cigarette butt (2)	Saliva	Direct
Cigarette butt (3)	Saliva	Direct
Cigarette butt (4)	Saliva	Direct
Cigarette butt (5)	Saliva	Direct
Rim of soda bottle (1)	Saliva	Indirect
Rim of soda bottle (2)	Saliva	Indirect
Rim of soda bottle (3)	Saliva	Indirect
Rim of soda bottle (4)	Saliva	Indirect
Rim of soda bottle (5)	Saliva	Indirect
Rim of soda bottle (6)	Saliva	Indirect
Rim of soda can (1)	Saliva	Indirect
Rim of soda can (2)	Saliva	Indirect
Rim of soda can (3)	Saliva	Indirect
Baseball cap (1)	Touch	Indirect
Baseball cap (2)	Touch	Indirect
Baseball cap (3)	Touch	Indirect
Car steering wheel	Touch	Indirect
Clothes hanger	Touch	Indirect
Coffee mug	Touch	Indirect
Computer keyboard	Touch	Indirect
Computer mouse	Touch	Indirect
Desk drawer handles	Touch	Indirect
Door handle (inside building)	Touch	Indirect
Door knob and lock	Touch	Indirect
iPod screen and buttons	Touch	Indirect
Light switch (1)	Touch	Indirect
Light switch (2)	Touch	Indirect
Microwave buttons	Touch	Indirect
Office copier buttons	Touch	Indirect
Office desk phone	Touch	Indirect
Pen (1)	Touch	Indirect
Pen (2)	Touch	Indirect
Plier handles	Touch	Indirect
Refrigerator door handle	Touch	Indirect
Smartphone screen and buttons	Touch	Indirect

Wooden handle of hammer	Touch	Indirect
Blank Swab (1)	Negative	Indirect
Blank Swab (2)	Negative	Indirect
Blank Swab (3)	Negative	Indirect
Blank Swab (4)	Negative	Indirect
Blank Swab (5)	Negative	Indirect
Blank Swab (6)	Negative	Indirect
Blank Swab (7)	Negative	Indirect
Blank Swab (8)	Negative	Indirect
Blank Swab (9)	Negative	Indirect
Blank Swab (10)	Negative	Indirect
Blank Swab (11)	Negative	Indirect
Blank Swab (12)	Negative	Indirect
Blank Sample Collector (1)	Negative	N/A
Blank Sample Collector (2)	Negative	N/A
Blank Sample Collector (3)	Negative	N/A
Blank Sample Collector (4)	Negative	N/A
Blank Sample Collector (5)	Negative	N/A
Blank Sample Collector (6)	Negative	N/A

Appendix B: ParaDNA® Percent Scores and Gender Calls

Note: For the ParaDNA® Percent Score column, cell color matches the color assigned by the instrument. Percent Scores above 2.5% are green, and below 2.5% are red.

Sample	ParaDNA® Percent Score	ParaDNA® Gender Call
Male blood on denim, neat	76	male
Female blood on denim, neat	70	female
Male blood on glass slide, neat	76	male
Female blood on glass slide, neat	56	male
Female blood on sock, neat (1)	83	female
Female blood on sock, neat (2)	91	female
Female blood on sock, neat (3)	86	female
Female blood on sock, 1:16 dilution (1)	33	unknown
Female blood on sock, 1:16 dilution (2)	0	unknown
Female blood on sock, 1:16 dilution (3)	12	unknown
Female blood on sock, 1:200 dilution (1)	17	unknown
Female blood on sock, 1:200 dilution (2)	0	unknown
Female blood on sock, 1:200 dilution (3)	7	unknown
1:16 male:female blood mixture	80	female
16:1 male:female blood mixture	73	male
Vaginal swab/semen mixture (1)	83	female
Vaginal swab/semen mixture (2)	89	female
Vaginal swab/semen mixture (3)	70	female
Vaginal swab/semen mixture (4)	82	unknown
1:1 saliva:semen mixture (1)	100	male
1:1 saliva:semen mixture (2)	95	male
1:1 saliva:semen mixture (3)	82	male
1:16 saliva:semen mixture (1)	89	male
1:16 saliva:semen mixture (2)	82	male
1:16 saliva:semen mixture (3)	57	male
Saliva, neat (1) – direct	0	unknown
Saliva, neat (2) – direct	73	female
Saliva, neat (3) – direct	46	female
Saliva, 1:16 dilution (1) – direct	6	unknown
Saliva, 1:16 dilution (2) – direct	71	female
Saliva, 1:16 dilution (3) – direct	42	unknown
Saliva, 1:200 dilution (1) – direct	0	unknown

Saliva, 1:200 dilution (2) – direct	21	unknown
Saliva, 1:200 dilution (3) – direct	14	unknown
Saliva, neat (1) – indirect	28	unknown
Saliva, neat (2) – indirect	33	female
Saliva, neat (3) – indirect	64	female
Saliva, 1:16 dilution (1) – indirect	23	unknown
Saliva, 1:16 dilution (2) – indirect	21	unknown
Saliva, 1:16 dilution (3) – indirect	30	female
Saliva, 1:200 dilution (1) – indirect	5	unknown
Saliva, 1:200 dilution (2) – indirect	6	unknown
Saliva, 1:200 dilution (3) – indirect	5	unknown
Chewed gum (1)	20	male
Chewed gum (2)	27	unknown
Cigarette butt (1)	22	unknown
Cigarette butt (2)	21	unknown
Cigarette butt (3)	19	unknown
Cigarette butt (4)	25	male
Cigarette butt (5)	62	male
Soda bottle (1)	46	unknown
Soda bottle (2)	30	unknown
Soda bottle (3)	4	unknown
Soda bottle (4)	43	unknown
Soda bottle (5)	5	unknown
Soda bottle (6)	32	unknown
Soda can (1)	71	unknown
Soda can (2)	42	unknown
Soda can (3)	52	unknown
Baseball cap (1)	48	male
Baseball cap (2)	7	unknown
Baseball cap (3)	29	male
Car steering wheel	35	unknown
Clothes hanger	0	unknown
Computer keyboard	54	female
Computer mouse	18	unknown
Desk drawer handles	0	unknown
Door handle (inside building)	0	unknown
Door knob and lock	0	unknown
Handles of pliers	0	unknown
iPod screen and buttons	0	unknown
Light switch (1)	8	unknown

Light switch (2)	0	unknown
Microwave buttons	9	unknown
Office copier buttons	21	unknown
Office desk phone	0	unknown
Pen (1)	21	unknown
Pen (2)	50	unknown
Refrigerator door handle	0	unknown
Rim of coffee mug	0	unknown
Smartphone screen and buttons	40	unknown
Wooden handle of hammer	24	female
Blank Swab (1)	0	unknown
Blank Swab (2)	0	unknown
Blank Swab (3)	3	unknown
Blank Swab (4)	0	unknown
Blank Swab (5)	0	unknown
Blank Swab (6)	0	unknown
Blank Swab (7)	0	unknown
Blank Swab (8)	0	unknown
Blank Swab (9)	0	unknown
Blank Swab (10)	0	unknown
Blank Swab (11)	0	unknown
Blank Swab (12)	0	unknown
Blank Sample Collector (1)	12	unknown
Blank Sample Collector (2)	0	unknown
Blank Sample Collector (3)	0	unknown
Blank Sample Collector (4)	0	unknown
Blank Sample Collector (5)	0	unknown
Blank Sample Collector (6)	0	unknown

Appendix C: Quantification Values and Normalized Quantification Values

Note: Normalized quant values are after dilution or with amplification of >1 µL to achieve the PBSO target of 0.8 ng.

Sample	Quant Value (ng/µL)	Normalized Quant Value (ng amplified)
Male blood on denim, neat	8.79	0.80
Female blood on denim, neat	3.27	0.82
Male blood on glass slide, neat	3.47	0.87
Female blood on glass slide, neat	4.85	0.81
Female blood on sock, neat (1)	6.15	0.82
Female blood on sock, neat (2)	8.54	0.78
Female blood on sock, neat (3)	4.50	0.82
Female blood on sock, 1:16 dilution (1)	0.05	0.79
Female blood on sock, 1:16 dilution (2)	0.02	0.38
Female blood on sock, 1:16 dilution (3)	0.02	0.30
Female blood on sock, 1:200 dilution (1)	0.01	0.18
Female blood on sock, 1:200 dilution (2)	0.00	0.04
Female blood on sock, 1:200 dilution (3)	0.01	0.10
1:16 male:female blood mixture	4.42	0.80
16:1 male:female blood mixture	5.77	0.82
Vaginal swab/semen mixture (1) - non-sperm fraction	77.33	0.80
Vaginal swab/semen mixture (1) - sperm fraction	28.68	0.80
Vaginal swab/semen mixture (2) - non-sperm fraction	58.86	0.80
Vaginal swab/semen mixture (2) - sperm fraction	34.19	0.80
Vaginal swab/semen mixture (3) - non-sperm fraction	74.76	0.80
Vaginal swab/semen mixture (3) - sperm fraction	24.77	0.80
Vaginal swab/semen mixture (4) - non-sperm fraction	75.89	0.80
Vaginal swab/semen mixture (4) - sperm fraction	12.08	0.81
1:1 saliva:semen mixture (1) - non-sperm fraction	8.17	0.82
1:1 saliva:semen mixture (1) - sperm fraction	16.24	0.81
1:1 saliva:semen mixture (2) - non-sperm fraction	11.16	0.80
1:1 saliva:semen mixture (2) - sperm fraction	19.93	0.80
1:1 saliva:semen mixture (3) - non-sperm fraction	7.22	0.80
1:1 saliva:semen mixture (3) - sperm fraction	19.90	0.80
1:16 saliva:semen mixture (1) - non-sperm fraction	11.93	0.80
1:16 saliva:semen mixture (1) - sperm fraction	35.15	0.80
1:16 saliva:semen mixture (2) - non-sperm fraction	8.68	0.79

1:16 saliva:semen mixture (2) - sperm fraction	25.39	0.79
1:16 saliva:semen mixture (3) - non-sperm fraction	12.25	0.82
1:16 saliva:semen mixture (3) - sperm fraction	28.72	0.80
Saliva, neat (1) – direct	0.27	0.80
Saliva, neat (2) – direct	0.02	0.31
Saliva, neat (3) – direct	0.03	0.64
Saliva, 1:16 dilution (1) – direct	0.01	0.09
Saliva, 1:16 dilution (2) – direct	0.01	0.15
Saliva, 1:16 dilution (3) – direct	0.01	0.20
Saliva, 1:200 dilution (1) – direct	0.01	0.11
Saliva, 1:200 dilution (2) – direct	0.00	0.01
Saliva, 1:200 dilution (3) – direct	0.00	0.05
Saliva, neat (1) – indirect	11.87	0.79
Saliva, neat (2) – indirect	11.44	0.76
Saliva, neat (3) – indirect	10.12	0.78
Saliva, 1:16 dilution (1) – indirect	0.26	0.78
Saliva, 1:16 dilution (2) – indirect	1.03	1.03
Saliva, 1:16 dilution (3) – indirect	1.56	0.78
Saliva, 1:200 dilution (1) – indirect	0.08	0.77
Saliva, 1:200 dilution (2) – indirect	0.07	0.84
Saliva, 1:200 dilution (3) – indirect	0.10	0.82
Chewed gum (1)	0.03	0.66
Chewed gum (2)	0.03	0.51
Cigarette butt (1)	0.00	0.08
Cigarette butt (2)	0.00	0.03
Cigarette butt (3)	0.00	0.03
Cigarette butt (4)	0.01	0.19
Cigarette butt (5)	0.01	0.17
Soda bottle (1)	0.01	0.16
Soda bottle (2)	0.01	0.13
Soda bottle (3)	0.01	0.10
Soda bottle (4)	0.11	0.88
Soda bottle (5)	0.02	0.46
Soda bottle (6)	0.02	0.34
Soda can (1)	0.12	0.80
Soda can (2)	0.09	0.80
Soda can (3)	0.05	0.00
Baseball cap (1)	0.06	0.79
Baseball cap (2)	0.03	0.58
Baseball cap (3)	0.02	0.30

Car steering wheel	0.00	0.00
Clothes hanger	0.00	0.05
Computer keyboard	0.23	0.81
Computer mouse	0.02	0.45
Desk drawer handles	0.04	0.76
Door handle (inside building)	0.01	0.16
Door knob and lock	0.01	0.14
Handles of pliers	0.00	0.07
iPod screen and buttons	0.07	0.81
Light switch (1)	0.00	0.08
Light switch (2)	0.00	0.08
Microwave buttons	0.03	0.55
Office copier buttons	0.04	0.69
Office desk phone	0.07	0.82
Pen (1)	0.03	0.49
Pen (2)	0.28	0.85
Refrigerator door handle	0.03	0.53
Rim of coffee mug	0.00	0.01
Smartphone screen and buttons	0.14	0.79
Wooden handle of hammer	0.07	0.80

Appendix D: Profiles Obtained and Average RFU (By Dye)

Note: For average RFU columns, green cells indicate that the average RFU is above the PBSO stochastic threshold (208 RFU). Red cells indicate that the average RFU is below the PBSO stochastic threshold.

Sample	Profile Obtained	Number of Loci	Average RFU – Blue (FL)	Average RFU – Green (JOE)	Average RFU – Yellow (TMR)
Male blood on denim, neat	full	16	940	1122	1004
Female blood on denim, neat	full	16	1189	1384	1345
Male blood on glass slide, neat	full	16	986	1343	875
Female blood on glass slide, neat	full	16	1000	1180	1032
Female blood on sock, neat (1)	full	16	1539	1846	1654
Female blood on sock, neat (2)	full	16	1405	1633	1322
Female blood on sock, neat (3)	full	16	1315	1346	1265
Female blood on sock, 1:16 dilution (1)	full	16	966	1008	790
Female blood on sock, 1:16 dilution (2)	full	16	489	584	397
Female blood on sock, 1:16 dilution (3)	full	16	348	452	332
Female blood on sock, 1:200 dilution (1)	partial	15	201	197	156
Female blood on sock, 1:200 dilution (2)	partial	4	64	72	73
Female blood on sock, 1:200 dilution (3)	full	16	173	189	139
1:16 male:female blood mixture	mixture - full	16	613	540	590
16:1 male:female blood mixture	mixture - full	16	576	670	602
Vaginal swab/semen mixture (1) - non-sperm	mixture - full	16	660	722	668
Vaginal swab/semen mixture (1) - sperm	mixture - full	16	971	967	966
Vaginal swab/semen mixture (2) - non-sperm	mixture - full	16	808	1103	777
Vaginal swab/semen mixture (2) - sperm	mixture - full	16	1649	1859	1475
Vaginal swab/semen mixture (3) - non-sperm	mixture - full	16	638	854	541
Vaginal swab/semen mixture (3) - sperm	mixture - full	16	1239	1266	1347
Vaginal swab/semen mixture (4) - non-sperm	mixture - full	16	633	729	559
Vaginal swab/semen mixture (4) - sperm	mixture - full	16	947	1113	1262
1:1 saliva:semen mixture (1) - non-sperm	mixture - full	16	352	425	428
1:1 saliva:semen mixture (1) - sperm	mixture - full	16	1824	2370	1687
1:1 saliva:semen mixture (2) - non-sperm	mixture - full	16	597	737	641
1:1 saliva:semen mixture (2) - sperm	mixture - full	16	1532	1832	1501
1:1 saliva:semen mixture (3) - non-sperm	mixture - full	16	525	438	518
1:1 saliva:semen mixture (3) - sperm	full	16	1590	1601	1152
1:16 saliva:semen mixture (1) - non-sperm	mixture - full	16	1406	1436	1176
1:16 saliva:semen mixture (1) - sperm	mixture - full	16	1663	1871	1475

1:16 saliva:semen mixture (2) - non-sperm	mixture - full	16	1293	1568	1365
1:16 saliva:semen mixture (2) - sperm	mixture - full	16	1660	1839	1777
1:16 saliva:semen mixture (3) - non-sperm	mixture - full	16	973	1004	807
1:16 saliva:semen mixture (3) - sperm	partial	15	890	342	724
Saliva, neat (1) – direct	full	16	1108	1449	1173
Saliva, neat (2) – direct	full	16	590	813	524
Saliva, neat (3) – direct	full	16	1119	1358	992
Saliva, 1:16 dilution (1) – direct	full	16	378	408	283
Saliva, 1:16 dilution (2) – direct	full	16	425	576	308
Saliva, 1:16 dilution (3) – direct	full	16	546	701	415
Saliva, 1:200 dilution (1) – direct	partial	15	146	227	184
Saliva, 1:200 dilution (2) – direct	partial	4	50	89	64
Saliva, 1:200 dilution (3) – direct	partial	13	101	117	101
Saliva, neat (1) – indirect	full	16	584	707	601
Saliva, neat (2) – indirect	full	16	873	1047	934
Saliva, neat (3) – indirect	full	16	1117	1410	1137
Saliva, 1:16 dilution (1) – indirect	full	16	1238	1457	1199
Saliva, 1:16 dilution (2) – indirect	full	16	1279	1527	1279
Saliva, 1:16 dilution (3) – indirect	full	16	1086	1416	1007
Saliva, 1:200 dilution (1) – indirect	full	16	1285	1574	1026
Saliva, 1:200 dilution (2) – indirect	full	16	1787	1910	1621
Saliva, 1:200 dilution (3) – indirect	full	16	1604	1970	1529
Chewed gum (1)	full	16	924	1102	910
Chewed gum (2)	full	16	1112	1305	1107
Cigarette butt (1)	partial	9	110	87	76
Cigarette butt (2)	partial	7	78	58	103
Cigarette butt (3)	partial	1	0	50	0
Cigarette butt (4)	partial	11	155	90	111
Cigarette butt (5)	partial	14	170	147	126
Soda bottle (1)	mixture - full	16	1143	1346	930
Soda bottle (2)	full	16	305	296	294
Soda bottle (3)	full	16	943	999	837
Soda bottle (4)	full	16	481	462	565
Soda bottle (5)	full	16	1339	1227	1165
Soda bottle (6)	full	16	1257	1164	744
Soda can (1)	full	16	742	760	677
Soda can (2)	full	16	723	668	671
Soda can (3)	full	16	998	1150	985
Baseball cap (1)	mixture - full	16	330	423	277
Baseball cap (2)	mixture - partial	12	431	412	255

Baseball cap (3)	mixture - full	16	285	232	241
Car steering wheel	mixture - full	16	663	939	717
Clothes hanger	partial	14	71	95	147
Computer keyboard	full	16	624	834	606
Computer mouse	mixture - partial	13	281	283	214
Desk drawer handles	mixture - partial	12	180	162	177
Door handle (inside building)	partial	8	87	71	59
Door knob and lock	mixture - partial	15	105	96	102
Handles of pliers	partial	9	70	86	77
iPod screen and buttons	full	16	738	764	640
Light switch (1)	partial	10	99	84	97
Light switch (2)	partial	3	50	85	56
Microwave buttons	mixture - partial	13	347	322	217
Office copier buttons	mixture - partial	10	182	140	105
Office desk phone	mixture - full	16	325	314	363
Pen (1)	mixture - partial	13	272	330	280
Pen (2)	full	16	1121	1261	1156
Refrigerator door handle	full	16	511	734	338
Rim of coffee mug	partial	4	59	56	0
Smartphone screen and buttons	full	16	974	1130	911
Wooden handle of hammer	none	0	0	0	0

Appendix E: Costs of Reagents and Consumables for STR Analysis

Item	Step	Cost	Samples	Cost per sample	Cost per case
EZ1 Kit	Extraction	$402.00	48	$8.38	$63.65
SpinEze® Spin Baskets	Extraction	$35.00	100	$0.35	$2.66
Capless Tubes	Extraction	$92.60	100	$0.93	$7.04
Plexor® HY Kit	Quantification	$2,985.00	800	$3.73	$91.79
MicroAmp® Plate	Quantification	$59.22	10	$5.92	$5.92
MicroAmp® Optical Adhesive	Quantification	$185.40	100	$1.85	$1.85
TE	Quantification	$34.00	178	$0.19	$0.19
PowerPlex® 16 Kit	Amplification	$6,746.00	400	$16.87	$246.23
AmpliTaq® Gold	Amplification	$1,612.80	750	$2.15	$31.40
MicroAmp® Plate	Amplification	$59.22	10	$5.92	$5.92
Strip Caps	Amplification	$77.40	1000	$0.08	$0.15
Formamide	Capillary Electrophoresis	$34.34	25	$1.37	$25.55
MicroAmp® Plate	Capillary Electrophoresis	$59.22	10	$5.92	$5.92
Plate Septa	Capillary Electrophoresis	$341.31	20	$17.07	$17.07
Reservoir Septa	Capillary Electrophoresis	$187.68	20	$1.68	$1.68
1X Running Buffer	Capillary Electrophoresis	$108.30	250	$0.43	$8.06
POP-4® Polymer	Capillary Electrophoresis	$346.00	60.2	$5.75	$78.17
Total				**$78.59**	**$593.25**