

THE PENNSYLVANIA STATE UNIVERSITY  
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FORENSIC SCIENCE PROGRAM

VALIDATION OF PRESERVEI™ SWABSQUEEZER™ INDICATING MICROCARD FOR  
RELIABLE, RAPID GENOTYPING OF SALIVA REFERENCE SAMPLES

EMILY ROSE PARCHUKE  
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Reviewed and approved\* by the following:

Reena Roy, Ph.D.  
Associate Professor of Biochemistry and Molecular Biology (Forensic Science)  
Thesis Supervisor and Honors Advisor

Ralph R. Ristenbatt III, M.S., D-ABC, SCSA-IAI  
Senior Research Assistant and Instructor  
Faculty Reader

\* Signatures are on file in the Schreyer Honors College.

## ABSTRACT

The purpose of this experiment was to validate the PRESERVE<sub>i</sub><sup>TM</sup> SwabSqueezer<sup>TM</sup> Indicating MicroCard\* as a new, reliable preservation method for buccal swab collection. This device changes color upon deposition of the saliva on the PRESERVE<sub>i</sub><sup>TM</sup> paper. Saliva was collected from nine (9) donors, and each foam swab was pressed firmly on the indicating card. Following saliva transfer from the swab to the indicating card and after drying for at least 24 hours, areas were classified as white or pink based on their appearance on the storage card. Using a 1.2 mm Whatman<sup>TM</sup> Harris Micro Punch, various areas of the storage cards were punched for analysis. The areas for punching were selected based upon the color of the PRESERVE<sub>i</sub><sup>TM</sup> paper and location on the card. Short tandem repeat (STR) DNA profiles were obtained by direct amplification of the punched saliva stains using a direct autosomal amplification kit, capillary electrophoresis, and software analysis. The goal was to determine if there were significant differences in the quality of the profiles generated.

This research indicated that there was no statistically significant difference in the rate of success in obtaining complete profiles generated from white, pink, or center punches of the PRESERVE<sub>i</sub><sup>TM</sup> SwabSqueezer<sup>TM</sup> cards. When detectable quantities of DNA were obtained from the punches analyzed, the extracted DNA yielded complete profiles. It was concluded that in most instances it was possible to obtain complete autosomal profiles from the saliva stains whether the 1.2 mm punches were obtained from the pink or the white areas or from any location of the card.

\*Patent Pending

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## **Introduction**

### **Background and Justification**

According to the Uniform Crime Report released by the Federal Bureau of Investigation, 1,165,383 violent crimes occurred in the United States during the 2014 calendar year<sup>1</sup>. Of these crimes, only 47.4% were cleared; this shows that the majority of violent crimes in the United States go unsolved. As demonstrated by Locard's exchange principle, whenever a perpetrator commits a crime, there is a transfer of evidence between the culprit and the scene; often, the evidence is genetic in nature. With such a high rate of unsolved crimes versus the amount of evidence left at crime scenes, there must be a way to link criminals to their acts based on their genetics. Luckily there is, as DNA evidence has come to the forefront of forensic investigation.

At a crime scene, evidence is collected by either law enforcement officials or evidence collection units and is sent to a crime laboratory for processing. Many types of evidence that can be found at a crime scene may contain body fluids such as blood, saliva, or semen from which it is possible to extract DNA. Extracted DNA is then amplified with one of the several commercially available amplification kits. Following capillary electrophoresis and analysis with software, an autosomal STR DNA profile is generated from the evidence. This profile is then compared to a reference profile obtained from the saliva of a person of interest. Since the DNA profile is unique to each individual except for monozygotic (identical) twins, if the evidence profile appears to be the same as the reference profile, then it can be concluded that the evidence sample came from the person of interest. This process can be challenging, both in regards to obtaining a usable DNA sample as well as being able to generate an accurate profile within a short period of time.

Alternative methods of DNA collection and STR profile generation have the potential to aid in the solving of the large number of violent crimes committed each year.

The advent of DNA technology, such as DNA fingerprinting, has allowed for great advances in the field of forensic science. Obtaining DNA profiles from a source at a crime scene, or from a potential suspect, allows forensic scientists to draw conclusions about the relationship between a suspect and the DNA sample obtained from the evidence with absolute certainty. However, the suspect in a crime is not always the perpetrator, and detaining a suspect for extended period of time while DNA analysis is being performed is an all too common and unfortunate occurrence. The need for fast and reliable DNA analysis is evident, and the implementation of such technology would quickly confirm the identity of perpetrators as well as exonerate innocent individuals in a minimal amount of time.

In an effort to determine the amount of DNA on various areas of the cards, additional punches from white, pink, and center areas of each card were obtained. Instead of direct amplifications, these minute punches were robotically extracted, and the extracted DNA was quantified, amplified with the same amplification kit and DNA fragments were analyzed upon capillary electrophoresis and genetic analysis software. The goal was to determine if there was a significant difference in the quantity and quality of DNA present on the various areas of the card. The objective was to identify color or area of the card made any difference in the STR DNA profiles obtained in this study.

The authentication of this method has the potential to profoundly affect the law enforcement field by easing the collection of saliva from suspects and providing a complete and definitive DNA profile with increased ease and in significantly less time than before. The generation of STR profiles will ensure that the truly responsible party is held accountable in



criminal cases where DNA evidence is available, and that innocent parties walk free. The validation of the PRESERVE<sup>i</sup>™ SwabSqueezer™ method has the ability to assist personnel in generating genetic profiles faster and easier than ever before, and would be a valuable tool in the fields of law enforcement and forensic science.

### **Experimental Outline and Purpose**

The PRESERVE<sup>i</sup>™ SwabSqueezer™ by FITZCO® Fast Forward Forensics is a newly developed, portable saliva collection swab that has the potential to reduce the time it takes to generate a full DNA profile by its ability to be processed using direct amplification technology. This product is ideally utilized to collect a reference sample of DNA by the collection of a buccal swab of an individual. The product includes a lollipop foam swab and a PRESERVE<sup>i</sup>™ paper sample deposit area, where the DNA would adhere and have the ability to be collected and preserved. The purpose of this research is validate the PRESERVE<sup>i</sup>™ SwabSqueezer™ card so that it may be added to the arsenal of resources that forensic scientists and law enforcement may draw upon in order to solve crimes.

The product in question is a newly developed version of an FTA card, which has been validated in the past as a useful and reliable tool for the storage of DNA, leading to the eventual generation of DNA profiles<sup>2</sup>. The PRESERVE<sup>i</sup>™ card is known as a single indicating microcard. As the name suggests, there is only one application area for the buccal swab and that a color change indicates the application of a clear sample. The pink dye present on the PRESERVE<sup>i</sup>™ card changes to a white color with the application of a saliva sample from the buccal swab. It has been observed, however, that even with complete and thorough application of a saliva sample, not all areas of the card that have had saliva deposited on them turn from pink to white. Stemming from

this fact, a facet of this research will attempt to determine if a DNA analyst is more likely to obtain a DNA profile from either the white or the pink areas of the PRESERVE<sub>i</sub><sup>TM</sup> card. This has important implications for the future use of this product, as the results of this project will influence the recommended guidelines for appropriate use.

Based upon the results of this research, it may be recommended that punches be taken from one of the two colors for analysis of saliva sample, or that the color of the area for collection simply does not play a role in the likelihood of obtaining a DNA profile. If the latter is determined to be true, the analyst can select any area of the card he or she so chooses, so long as saliva has surely been deposited in that region. Furthermore, the intended amplification for this product is direct amplification, in contrast to the “traditional” sequence of extraction and quantitation followed by amplification. The STR DNA profile generation in this experiment is to be completed by use of direct amplification only, to keep in line with the intended use of the product to produce fast and reliable results. Direct amplification has been validated as a fast and reliable method of DNA profile generation in previous studies<sup>3,4</sup>.

Quantitation will be performed, however, in an attempt to further support or refute the assumption that white areas of the indicating card would be the preferable sampling area. DNA will be extracted from both white and pink areas of the card for every donor and quantified in order to determine if there is DNA present in appreciable amounts in both the white and pink areas of the card. Results of this analysis may corroborate findings that one area of the indicating card should be sampled in favor over the other, or may provide insight that a certain threshold must be met in order for direct amplification to be a viable option for rapid genotyping.

## **Materials and Methods**

### **Saliva Collection**

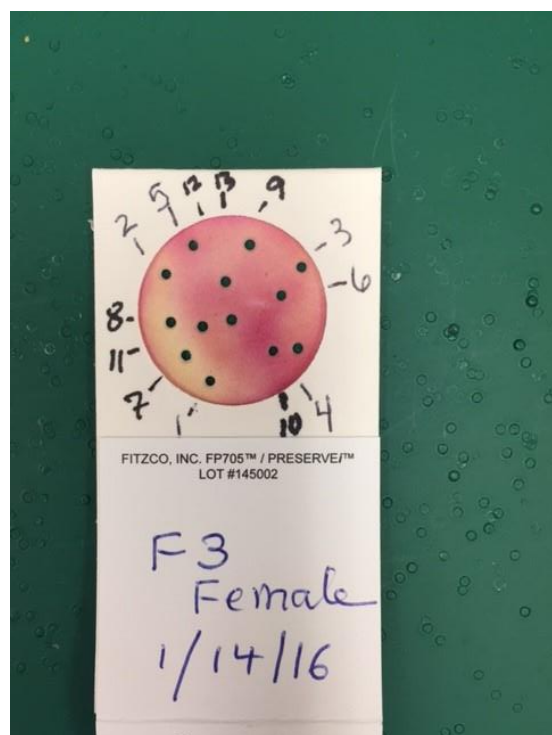
Nine (9) total saliva samples were obtained from Penn State faculty and staff associated with the Department of Biochemistry and Molecular Biology, and Chemistry. Each package containing the individual indicator card was opened and handled using proper sterile technique. The foam tipped applicator associated with the indicator card was placed under the subject's tongue and swept along the gum line and under the tongue in order to ensure absorption of a sufficient amount of saliva on the applicator. The applicator tip was then swabbed along the inside of the cheek a minimum of ten (10) times. The foam tip was then rotated one hundred and eighty (180) degrees and swabbed along the inside of the opposite cheek a minimum of ten (10) times to ensure adequate collection of simple squamous epithelial cells. The applicator was removed from the mouth, and placed in the center of the pink PRESERVE<sup>i</sup>™ paper on the card. The cover of the card was pressed firmly on top of the applicator for ten (10) seconds in order to effectively transfer the DNA-containing cells to the card. Foam applicators were discarded after saliva transfer to the storage card. Storage cards were allowed to dry for a minimum of twenty-four (24) hours post-saliva deposition. After drying, cards were stored in separate, labeled envelopes for later analysis.

### **Labeling Classifications**

After drying, each card was individually labeled according to their anonymous donor assignment, and the date on which the saliva was collected. Male donor samples were designated

by the letter ‘M,’ followed by a number correlated to the order in which they were collected in reference to the other male subjects. For example, the third male saliva sample collected was designated “M3.” Female saliva donor samples were labeled according to the same method, designated by the letter ‘F’ followed by the number corresponding to the order in which the sample was collected.

Every punch from a subject’s card was labeled according to the order in which it was removed from the card, beginning with ‘1’ and ending with the last punch that was removed. An example of a card with the labeling classification clearly shown to the right (Figure 1). The F3 donor card had 13 punches removed from it, with the punch labeled ‘1’ being the first removed,



and the punch labeled ‘13’ being the last removed.

Thus, the first punch for this donor would be designated as ‘F3.1,’ referring to both the donor and the order in which the punch was removed from the card. This naming system was applied to all punches of the card and each name (F3.1, F3.2, and so on) remained tied to that punch throughout the study.

This classification system was used for all of the saliva samples, which included both male and female donors.

**Figure 1. Labeling system example**

## **Sample Collection**

Each punch was removed from the storage cards using a 1.2 mm Whatman™ Harris Micro Punch tool. Before the collection of each sample from a donor card, the punch tool was deconstructed and cleaned with a bleach and alcohol wash to eliminate cross-contamination. For donors F1, F2, F3, M1, and M2, punches 1-3 were removed from the white areas of the card and punches 4-6 were removed from the pink areas of the card. This pattern was repeated with punches 7-9 and 10-12, respectively. Punch 13 was removed from the center of the indicating card, regardless of the color. Donors F4, F5, M3, M4, and M5 followed a slightly different pattern; punches 1-3 were removed from the white areas of the card, punches 4-6 were removed from the pink area of the card, and punch 7 was removed from the center of the card. The pattern was repeated if necessary for these donors, where punch 8-10 were from white areas, 11-13 were from pink areas, and punch 14 was from the center of the card. Additional punches were only generated in the case where there was an amplification or capillary failure.

## **Polymerase Chain Reaction**

Samples removed from the donor cards were placed in labeled Polymerase Chain Reaction (PCR) tubes. PCR reactions were prepared using Promega PowerPlex® Fusion 6C reagents. Reactions were prepared according to the Promega PowerPlex® Fusion 6C protocol for direct amplification of DNA from storage card punches.<sup>5</sup> Once it was determined that consistent results were observed with both the 25 µL reaction volume and the 12.5 µL reaction volume, all subsequent reactions were performed using the half-reaction volume (12.5 µL). The recommended thermal cycling protocol was followed for the samples using the Applied

Biosystems Veriti™ 96-well Thermal Cycler.<sup>6</sup> Twenty-five amplification cycles were performed on each set of samples as a baseline cycle number and cycle number was increased to twenty-six (26) cycles, if necessary, for samples that did not yield complete profiles when amplified for the first time.

### **Capillary Electrophoresis**

Samples were prepared for capillary electrophoresis according to the Penn State Forensic Science Program protocol for the Applied Biosystems 3130xl Genetic Analyzer.<sup>7</sup> The internal lane standard utilized for this experiment was WEN ILS 500. The recommended protocol for the Genetic Analyzer was followed for most samples. If a complete profile could not be obtained on the first run, then the injection time and injection voltage were increased from 7 kV, 10 seconds to 10 kV, 12 seconds. In samples, which showed over-amplification, amplified DNA fragments were diluted; The diluted amplified products were injected using the capillary electrophoretic system using the recommended voltage and injection time.

### **Genetic Software Analysis**

GeneMarker® HID 2.7.1 software was utilized to visualize the generated STR profiles. Software analysis was utilized to determine whether the samples yielded full STR profiles, partial STR profiles, or no profiles.

### **Extraction of Punches**

Six (6) punches were obtained from each saliva stained card for extraction. Three (3) punches were made from the white regions of the card and three (3) punches from pink regions of the card. DNA was extracted with stain extraction buffer which consisted of 76 mL 10 mM Tris-HCl; 2 mL 0.5 M EDTA; 2 mL 5 M NaCl; 20 mL 10% SDS. Samples were incubated at 56° Celsius for one (1) hour. Each digested punch containing saliva was then extracted using the Qiagen EZ1® DNA Investigator® using the Normalization protocol, Tris/EDTA (TE) extraction, and an elution volume of 50 µL.<sup>8</sup>

### **Quantification**

Extracted DNA was quantified using the Applied Biosystems 7500 Real-Time PCR System and the Quantifiler® Human Quantification Kit. The protocol for quantification was as recommended by the manufacturer of the kit<sup>9</sup>. A total of 54 extracted samples were quantified.

## Results and Discussion

### Characterization of Profiles Generated

The first run of the Genetic Analyzer 3130xl (capillary electrophoresis) produced no results for any samples including the positive and negative control. Due to complete capillary failure, the first run was disregarded as the first ‘true’ run that was made. Therefore, the designation that a profile was obtained on the “first attempt” was truly the second run of the capillary electrophoresis, but it was the first attempt that produced viable results. For this reason, the classification was shifted and should be noted when interpreting results.

The data results from the capillary electrophoresis for all samples were analyzed using GeneMarker<sup>®</sup> HID 2.7.1. The determination of a ‘full profile’ was based upon the presence of alleles at the 27 loci detected by the system. Alleles were either called by the software independent of human input or were determined to be the only alleles present when disregarding pull-up peaks present. A peak height threshold of 50 relative fluorescence units (RFU) was set in order to determine allelic drop out.

Table 1 displays the results of profile generation and timeliness of achieving each profile. The first two categories describe at what attempt the first full profile was obtained, and the number of full profiles obtained for a donor, regardless of if it took one (1), two (2), or three (3) attempts to generate a full profile for the donor for either color of punch. The third category describes how many total full profiles were generated for each donor from all of the punches removed from the card. Lastly, DNA profiles were attempted to be generated from the center punch of each of the donor cards, and the results are displayed in the last column.



**Table 1. Results of direct amplification with Fusion 6C on PRESERVEi™ SwabSqueezer™ Indicating Cards**

Donor	White Punches	Pink Punches	Center Punches
M1	C1, 1, P≥3	C1, 1, P≥3	NR
M2	C1, 1, P≥3	C2, 0, P≥3	T
M3	C1, 2, P≥3	C1, 2, P2	T
M4	C1, 1, P≥3	C1, 3, P≥3	T
M5	C1, 2, P≥3	C1, 3, P≥3	NR
F1	C1, 1, P≥3	C2, 0, P≥3	NR
F2	C1, 1, P≥3	C2, 0, P≥3	T
F3	C1, 1, P≥3	C1, 2, P≥3	T
F4	C1, 3, P≥3	C1, 2, P≥2	T
C1 = at least 1 complete profile from donor on first attempt at profile generation			
C2 = at least 1 complete profile from donor on second attempt at profile generation			
C≥3 = at least 1 complete profile from donor on third or greater attempt at profile generation			
0 = No complete profile generated on first attempt			
1 = 1 complete profile generated on first attempt			
2 = 2 complete profiles generated on first attempt			
3 = 3 or more complete profiles generated on first attempt			
P1 = one complete profile was generated out of the population of punches removed from a particular colored area			
P2 = two complete profiles were generated out of the population of punches removed from a particular colored area			
P≥3 = three complete profiles were generated out of the population of punches removed from a particular colored area			
T = complete profile from donor was obtained from the punch removed from the center of the card			
NR = No results			

### Successes and Failure Rates

A “success” for this portion of the results was defined as when at least one (1) complete STR DNA profile was generated from the first set of three (3) punches taken from either the white or pink area of the card. A failure is defined as not generating a complete profile from any of the punches in the white or pink areas on the first set of three (3) punches.

Punches from the white areas of the cards produced at least one (1) full profile during the first attempt at profile generation for all donors; thus, a 100% success rate was achieved in the sample set.

Of the pink punches removed from the storage cards, six (6) of the donors had full profiles generated from the first round of amplification and analysis, yielding a 67% success rate for the sample set.

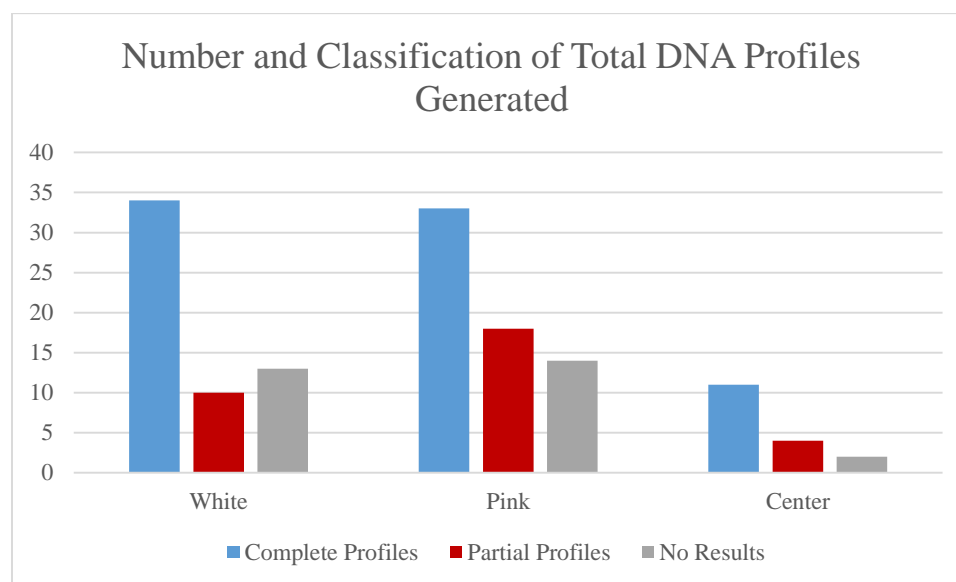
Analysis of the total profiles generated was used to determine success and failure rate. Generation of one hundred and thirty-nine (139) profiles was attempted. Table 2 highlights the number of complete and partial profiles obtained from each area of the card tested. Total numbers of complete profiles, partial profiles, and lack of profiles (no results) were tabulated, as shown below.

**Table 2. Results of direct amplification with Fusion 6C on PRESERVEi™ SwabSqueezer™ Indicating Cards**

	Complete Profiles	Partial Profiles	No Results	Total
White	34	10	13	57
Pink	33	18	14	65
Center	11	4	2	17
Total	78	32	29	139

Figure 2 provides a graphical representation of the data presented in Table 2. It is easy to discern that the number of complete profiles generated dominates the number of partial profiles and absence of profiles observed in this experiment. The results for punches from both the pink and center areas follow the same trend, where complete profiles were observed most often, followed by partial profiles and then absence of profiles. The trend does not hold for the punches taken from the white areas as there were more “no results” claimed for the punches than partial

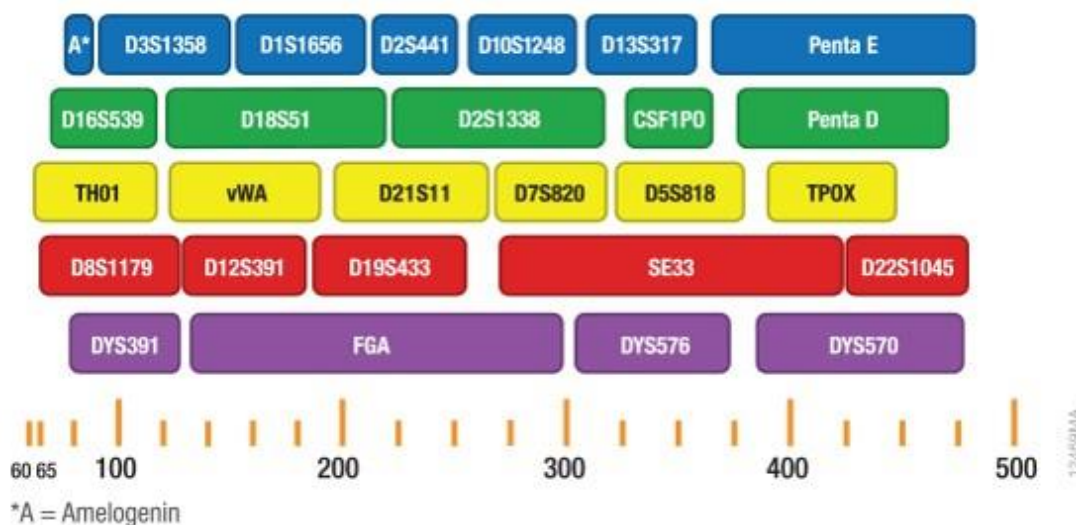
profiles. There are two likely causes of the uncharacteristically high number of “no results” for the white areas of the cards: incomplete amplification or inhibition in the punches that results in an inability to interpret a DNA profile for a donor. Without further experimentation, it is impossible to characterize, with accuracy, what factor produced the different trend observed for the white punches.



**Figure 2. Total number of complete and partial DNA profiles generated for white, pink, and center areas of SwabSqueezer® storage cards**

It should be noted that a larger number of complete profiles were generated when compared to the combined results of partial profiles and no profiles. Partial profiles were determined by analyzing the STR profiles generated from the storage cards; if one or more alleles failed to appear in the profile in comparison with a known standard from that same donor, the profile was designated as “partial.” The most common reason for the designation of a profile as “partial” was due to failure of detection of alleles in the Penta E locus of the profile. Figure 3 is a representation of the 27 alleles detected by the Promega PowerPlex® Fusion 6C system,

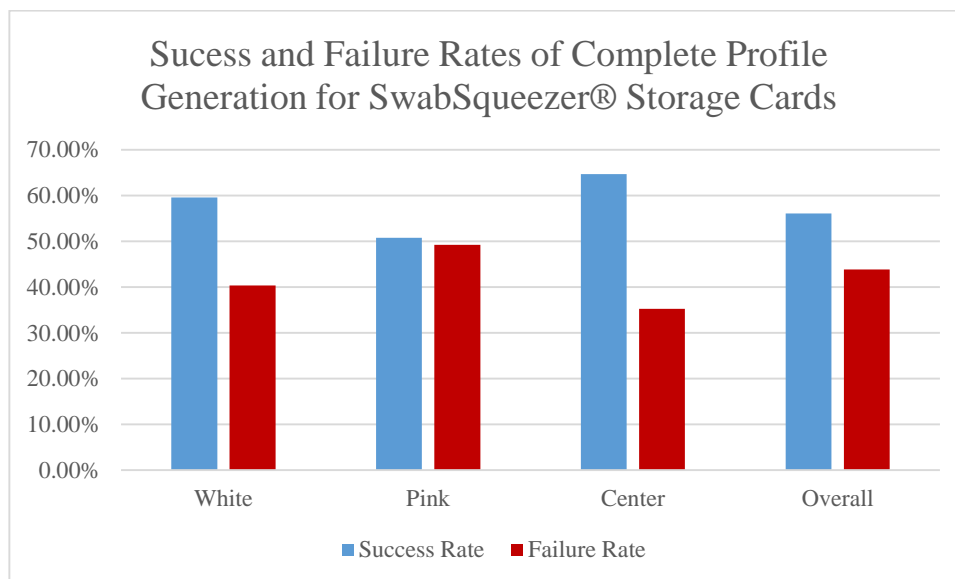
where the Penta E locus can be seen at top right (retrieved from [www.promega.com](http://www.promega.com)). Since the alleles in the Penta E locus are larger, the frequency of receiving partial profiles is explainable and not entirely unexpected.



**Figure 3. Loci detected by Promega PowerPlex® Fusion 6C system**

It was hypothesized that increased PCR amplification cycles and increased injection time and voltage when performing capillary electrophoresis would increase the likelihood of yielding a full profile from a particular punch, but this standard protocol did not always generate a full profile upon re-amplification and/or re-injection.

Figure 3 (below) shows success and failure rates for each area of the SwabSqueezer™ card, as well as the overall calculated success and failure rate for all trials performed in this study. Success rates (%) for the white, pink, and center areas of the card were calculated by multiplying the ratio of the number of complete profiles obtained from the area by the total number of profiles generated by 100. Failure rates were also calculated to provide an accurate comparison.



**Figure 4. Success and Failure Rates of different areas of SwabSqueezer® cards**

The center punches from the SwabSqueezer™ card had the highest rate of success. However, this should be taken in stride, as the sample size for the center punches was much less than the sample sizes for either the white or pink areas of the card (see Table 2). Nonetheless, it does appear that simply taking the center punch from the storage card might have the highest probability of returning a full profile. With proper application of the foam swab to the storage card, a high concentration of DNA from the buccal swab is likely to be deposited in that area.

The white areas of the storage card were observed to have the next highest rate of success; nearly 60%. It should also be noted from the data contained in Table 1 that the white areas of the card returned at least one (1) full profile from each donor on the first attempt at generating an STR profile. Though a 60% success rate may not appear so impressive, at least one complete profile was obtained on the first attempt with direct amplification. This finding is

important, as it solidifies that direct amplification is a valid and viable option for providing quick and reliable results with the SwabSqueezer™ Indicating Microcard.

The number of profiles generated from punches obtained from the pink areas of the card were such that the failure rate for this colored area was higher than the observed rate of success. As observed in Table 2, there were thirty-three (33) complete profiles generated from pink areas of the card, out of sixty-five (65) attempts. Though only one (1) more complete profile was generated for the white areas of the cards (34), less total attempts were made at deriving an STR profile on the white areas of the card, as results were produced more frequently on earlier attempts in comparison to other areas of the card (refer to Figure 1).

Taking into account all areas of the card, overall success and failure rates were computed for the SwabSqueezer™ card. The success rate was slightly greater than 56%, even when taking into account the high failure rate of the pink areas of the cards.

### **Statistical Analysis of Success**

In order to find statistical significance in the trends observed in this experiment, a statistical test was employed. The statistical analysis performed was based upon the observed success or failure of receiving a complete STR DNA profile from either the white, pink, or center area of the SwabSqueezer™ storage card by direct amplification and subsequent genetic analysis. A chi-squared statistical analysis was utilized to analyze the data.

The null hypothesis for this analysis is that the proportion of success in each category is equal to the population proportion of success, which is estimated by  $\hat{p}$ . The alternative

hypothesis is that the calculated rate of success in each category differs from the overall rate of success.

The observed and expected values for each category can be seen in Table 3:

**Table 3. Observed and expected values utilized for chi-squared test statistic**

	<b>Observed Value</b>	<b>Expected Value</b>
<b>White</b>	33	36.4
<b>Pink</b>	34	31.92
<b>Center</b>	11	9.52
<b>Total</b>	78	77.84

The expected values were calculated using the sample proportion of successes,  $\hat{p}$ , which was estimated to be 0.56. Therefore, it follows that this proportion of successes should be viewed in every category examined. The test was computed using the chi-squared test statistic:

$$\chi^2 = \sum_{i=1}^n \frac{(\text{Observed}_i - \text{Expected}_i)^2}{\text{Expected}_i}$$

The chi-squared value obtained for the data collected was 0.6832. The degrees of freedom used to analyze the test statistic was 1; degrees of freedom is calculated as:

$$df = n - 1 - \text{number of categories estimated}$$

As the category size 'n' was three (3), and one (1) parameter was estimated ( $\hat{p}$ ), one (1) degree of freedom should be utilized. Consulting a chi-squared table at  $\alpha = 0.05$ , the critical value is 3.841. The chi-squared value calculated is less than the critical value, therefore, the null hypothesis cannot be rejected. With no evidence to support the alternative hypothesis, the notion that sampling a particular area of the SwabSqueezer™ card has a higher success rate than the overall success rate cannot be concluded.

### **Quantification**

Six (6) 1.2 mm punches from every donor were robotically extracted and quantified. Three (3) punches were from the white areas of the card and three (3) punches were from the pink areas of the card. The range of DNA concentration observed (nanograms/microliter) was 0.0016 to 0.2143, providing evidence that detectable levels of DNA were present on all areas of the card that were subject to analysis. Ten (10) of the fifty-four (54) samples that were quantified were flagged by the instrument as "low quantity of DNA." No samples were noted as having significantly large quantities of DNA.

This analysis does not provide statistically significant information as to the quantity of DNA in the white versus pink areas of the storage card as observed DNA quantities for all punches were at low, but clearly detectable, levels.



## Conclusions and Future Research

### Recommendations for Use

It was originally hypothesized from early compilation of the data in the study that the white or center areas of the SwabSqueezer™ Indicating Microcard were the most promising areas to be sampled in order to obtain a full STR DNA profile from the sample. However,  $\chi^2$  analysis affirmed that there is no statistical difference in success rates between different colors/areas of the storage card.

Following this analysis, it can be summarized for the benefit of the users of this storage card that as long as saliva has been placed on the storage card through application with the foam swab, punches from any area should be sufficient in generating a complete DNA profile. Following high failure rates of the pink areas of the card, however, it may be stated that white and center areas of the card (regardless of apparent color) would be more desirable.

Due to a large number of complete profiles obtained for all areas of the card and an overall success rate of approximately sixty percent (60%), it can be concluded that direct amplification is a suitable method for the production of rapid and reliable STR profiles when saliva samples are collected on the the SwabSqueezer™ Indicating Microcard.

### Direction of Future Research

It is recommended that the SwabSqueezer™ Indicating Microcard be validated by one or more additional independent scientists in order to provide more weight regarding the storage abilities of the card, leading to the production of complete STR DNA profiles. It is also

recommended that the storage cards are subjected to various environmental conditions that have the potential to destroy DNA samples to determine if the cards would be sufficient for long-term storage of samples and/or have the integrity to remain a reliable source of reference DNA following potential damage.

Furthermore, color-changing indication systems may need to be reevaluated, as it was originally hypothesized that the areas in which color change was observed had a more significant deposition of sample on said area. Due to the lack of statistically significant difference in the rates of success of profile generation between the different colored areas of the card, the indicating system should be considered null in this case. Drawing on these conclusions, these indication systems warrant further research into their efficacy.

Due to the success observed with direct amplification in this instance, it would be beneficial for future research to be aimed at producing products conducive to use with direct amplification, or improving the technology of direct amplification itself. The ability to receive rapid and – most importantly – accurate STR DNA results has the ability to revolutionize the standard timeframe in which DNA results are produced and interpreted in the field. With large case backlogs, especially sexual assault cases, forensic science as a discipline would greatly benefit from new technological developments that would increase the speed and accuracy with which samples are processed and results are interpreted.

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## Academic Vita of Emily Rose Parchuke

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**Thesis Title:** Validation of PRESERVE<sup>i</sup><sup>TM</sup> SwabSqueezer<sup>TM</sup> Indicating Microcard for Reliable, Rapid Genotyping of Saliva Reference Samples

**Thesis Supervisor:** Reena Roy, Ph. D.

**Education:** The Pennsylvania State University

Schreyer Honors College

University Park, PA

Bachelor of Science in Biology, Honors in Forensic Science

**Activities:** Undergraduate Student Researcher

Primary Investigator: Reena Roy, Ph.D.

Student Research Assistant

Conservation/Sustainability of Select Tropical Ecosystems, Spring 2014

**Relevant Coursework:**

Criminalistics: Biological Evidence with Lab

Molecular Biology of the Gene

Essential Practices in Forensic Science

Genetic Analysis

Analysis of Biological Data

Scientific Approach to Crime Scene Investigation

Population Genetics

**Grants Received** Eberly College of Science Grant Recipient (2015-2016)

Schreyer Honors College Travel Grant Recipient (2013, 2014)

**International Education:** Eight (8) credits of Conservational Biology and Tropical Ecology

Field Practicum in Panama and Costa Rica (Summer 2013, 2014)