FINAL TECHNICAL REPORT

Automated Processing of Sexual Assault Cases
Using Selective Degradation

Award No: 2009-DN-BX-K039

Authors: Dr. Christian Carson (PI), Dr. Alex Garvin (co-PI), Kim Gorman

Abstract

This research addressed the problem of obtaining a sperm DNA profile from sexual assault evidence samples that also contain a large amount of epithelial cell DNA, and the ability to automate the differential extraction process. The current standard differential extraction method leaves some amount of epithelial cell DNA in the sperm fraction of the sample, which will create a mixture of epithelial and sperm cell DNA and may partially or completely mask the sperm DNA profile. This may prevent conviction or even detection of the perpetrator.

This research used a nuclease to digest the remaining epithelial DNA in the sperm fraction of mock sexual assault samples. The research demonstrated the ability to obtain clean single-source male DNA profiles from mixed stains, under a variety of sample conditions. In addition, the research optimized this procedure, including reagent concentrations and volumes, and then validated the procedure for sensitivity, reproducibility and precision. Following that, the results of this selective degradation approach were compared to the results obtained with the current standard method of differential extraction. The selective degradation methodology was demonstrated to be compatible with the DNA purification methods currently used in public crime laboratories.

This methodology was designed to be compatible with a single tube method and with a 96 well plate automated method of differential extraction. It was specifically designed to be compatible with multiple liquid handling platforms in order to utilize existing equipment in public laboratories.

This research resulted in an optimized and validated process for obtaining single source male DNA profiles from mixed stains, greatly improving the ability of the law enforcement community to process and prosecute sexual assault cases. This process eliminates sperm fraction DNA profile mixtures from almost all sexual assault evidence containing sperm cells. It often permits crime laboratories to obtain single source male DNA profiles even in situations where the standard method of differential extraction does not produce a sperm fraction male profile.

As a result, in some cases where law enforcement was unable to identify any suspect for a sexual assault, they will now be able to obtain a full DNA profile of the perpetrator. This will allow certain current and cold cases to be solved and closed. This procedure saves time for DNA criminalists in performing differential extractions. It also eliminates the time drain and uncertainty caused by mixed DNA profiles. And, it allows the differential extraction process to be easily automated, which will help reduce the backlog of sexual assault evidence.

Table of Contents

Executive Summary

Synopsis of Problem

This research was designed to address the problem experienced in crime laboratories of obtaining a single source sperm DNA profile from sexual assault evidence containing both sperm cell and epithelial cell DNA. The removal of epithelial cell DNA from the sperm fraction of the sample has been a time consuming step that has met with limited success. Another aspect of the problem is that traditionally this process was very difficult or impossible to successfully automate.

The method used in a crime laboratory to obtain the sperm cell and epithelial cell DNA profiles from the mixed stain is referred to as differential extraction. The method of differential extraction in use for over two decades has relied on preferential lysis of the epithelial cells using a solution that will lyse epithelial cells but not sperm cells. This allows the scientist to separate the two types of cells.

The problem arises because there is often an extreme excess of epithelial cells as compared to the number of sperm cells recovered from the sexual assault evidence. Traditionally, a mild lysis solution is introduced to the tube containing the evidentiary sample. This lysis solution will lyse the epithelial cells, but is not stringent enough to lyse the sperm cells. After epithelial cell lysis is complete the tube is centrifuged and the sperm cells create a pellet in the bottom of the tube.

As much of the lysis solution as possible, without disturbing the sperm pellet, is removed from the tube. The sperm pellet is then diluted with a buffer, mixed, and again centrifuged to create a pellet. This dilution or washing step is generally repeated 3 to 5 times. The dilutions are time consuming and tedious and ultimately it is impossible to dilute away all of the epithelial DNA. When there is an extreme excess of epithelial DNA as compared to sperm DNA it is nearly impossible to dilute the epithelial DNA to a degree that there is more DNA from the sperm cells than from the epithelial cells. When significant amounts of epithelial DNA as compared to sperm DNA remain, a DNA mixture will be obtained. In cases where there is an excess of epithelial DNA it may be impossible to deconvolute the sperm profile or no sperm profile may be obtained.

Obtaining a mixture profile causes extra work involving the statistical analysis of the profile. It also provides defense counsel with an opportunity to raise doubts in court about whether the client's profile is even represented in the mixture, and may lead to acquittal.

If no sperm profile is obtained because of an excess of epithelial DNA, then the DNA evidence is not useful in identifying the suspect even though sperm were identified by the laboratory.

excess of 5 minutes generally gave balanced X and Y Amelogenin amplification. However, some postcoital swabs did not give balanced Y and X Amelogenin peaks until 15 minutes of nuclease incubation time. Earlier experiments indicated that at 0 minutes of nuclease incubation time there was a significant amount of epithelial DNA remaining in the sperm fraction.

This experiment indicates that a 15 minute incubation time is optimal for elimination of the epithelial cell DNA from the sperm fraction.

Table 1, Summary of Incubation Time Data

Swab	PC16		PC22		PC49		PC75		PC77		PC80		PC67	
Hours postcoital	0-6		0-6		25-30		31-36		31-36		37-42		43-48	
Incubation Time (minutes)	5	15	5	15	5	15	5	15	5	15	5	15	5	15
Percent Y Amelogenin	49%	50%	45%	49%	49%	50%	28%	50%	32%	50%	41%	47%	34%	49%
Improvement	1%		4%		1%		22%		21%		6%		15%	

Summary of data comparing nuclease incubation times of 5 minutes and 15 minutes. Percent Y Amelogenin values were determined by dividing the Y Amelogenin peak value by the sum of X and Y Amelogenin peak values. Thus, 50% Y Amelogenin values indicate equal X and Y peaks and that therefore the sperm cell DNA fraction consisted of only male DNA. The data indicate that while the epithelial cell DNA was eliminated from some samples in 5 minutes, others required a 15 minute incubation before Y Amelogenin peak values were equal to the X Amelogenin peak values.

(iv)(k) Additional Nuclease Variables Considered

Spiking with Extra Nuclease

During the developmental optimization stages, there were occurrences of apparent incomplete digestion of epithelial cells. Experiments were designed to determine if nuclease added sequentially with additional incubation time periods would eliminate excess epithelial DNA. Spiking with extra nuclease for longer incubation times did not help to remove excess epithelial cell DNA (data not shown). It is not know if the remaining epithelial DNA is due to incomplete epithelial cell lysis or the inability of the nuclease to degrade all of the DNA.

Semen Extender to Protect Sperm from Nuclease

As noted previously, sperm cells that had been hydrated from dried samples were intact morphologically. However, there were indications that the nuclease was digesting some of the sperm DNA. This experiment was designed to determine if protecting the sperm cells using semen extender would shield the sperm DNA from the nucleae. Semen extender has been used to protect living sperm cells from freezing and thawing damage and subsequent chromatin damage (4). For the purpose of protecting forensic sperm cells from damage during the selective degradation extraction process, semen extender was prepared in Solution #1 of the Er*ase* extraction kit using two of the main ingredients found in human semen extenders. Er*ase* extractions were run in triplicate comparing Solution #1 with and

without extender reagents. While some experiments that used only sperm cells indicated that the semen extender helped to increase sperm fraction DNA yield (data not shown), subsequent experiments using postcoital samples indicated otherwise. Figure 21 (in Section III-1-A-(v), Sperm Cell Lysis) reveals no significant differences between Er*ase* reactions with or without Solution #1 semen extender. Extender was also added to the Er*ase* extraction buffer, but did not increase sperm DNA yield (data not shown).

Performance of Differential Extraction Methods with Menstrual Blood.

Postcoital swabs were collected from a donor during menses 12 hours postcoitus. The swabs were obviously stained with blood. The swabs were processed using both the selective degradation method and the standard differential extraction method. The samples were purified using the BioRobot EZ1 Workstation. The samples were eluted in 50ul of water and concentrated to 10ul with a speed vacuum. The samples were quantified using QuantIt and 2ng DNA was amplified using PP16. Supplemental Figure 18 shows the epithelial cell (♀) and sperm cell (♂) fraction profiles from these postcoital swabs.

Both differential extraction methods produced single source epithelial cell fractions. The selective degradation process produced a single source male profile in the sperm cell fraction. The standard differential extraction produced mixed profiles in the sperm fraction.

See Figure 18 (in Supplement), Comparison of Methods on Vaginal Swab with Menstrual Blood.

(v) Sperm Cell Lysis --- Solution #3

This step in the procedure was designed to stop nuclease activity and to lyse the sperm cells. This step in the originally published selective degradation procedure (1) consisted of the addition of a solution of EDTA and DTT, and incubation for 5 minutes at 56°C. EDTA inactivates the nuclease remaining in the sample and the DTT lyses the sperm cells. Initial experiments testing this step in the procedure determined that a 15 minute incubation period at room temperature was just as effective as 5 minutes at 56°C (data not shown). Microscopic observations of samples after this step were made and sperm lysis always appeared complete. Fifteen minutes at 56°C was chosen for the final protocol because it allowed for variables in heating the test tube. Even if the sample is not exposed to the proper heat the sperm cells will be fully lysed in 15 minutes.

EGTA Treatments During Sperm Cell Lysis

Experiments were designed in order to determine if the nuclease remained active after introduction of solution #3. If the nuclease remained active after sperm lysis the sperm cell DNA would be digested as it was released from the lysed sperm cells, effectively reducing sperm cell DNA yield. The selective degradation

procedure used EDTA to chelate Mg^{2+} in the sample to prevent its availability for activating the nuclease. Er*ase* already included an excess amount of EDTA so that there was a sufficient amount to bind all of the Mg^{2+}.

But Ca^{2+} was also included in Solution #1 and was needed for nuclease activity. EDTA does not bind Ca^{2+} as well as Mg^{2+}. EGTA binds Ca^{2+} ions better than EDTA. EGTA was tested in the sperm cell lysis step of Er*ase* to determine if there was residual Ca^{2+}-dependent nuclease activity. Equivalent numbers of sperm cells were extracted with Er*ase* and comparisons were made with and without EGTA added before or included with Solution #3.

Figure 19 shows the results of these experiments. No significant differences in Er*ase*-extracted sperm cell DNA yield were found between the treatments as indicated on the chart. No Ca^{2+}-dependent nuclease activity was detected and the amount of EDTA used in the Er*ase* extraction was found to be sufficient for inactivating nuclease.

Figure 19

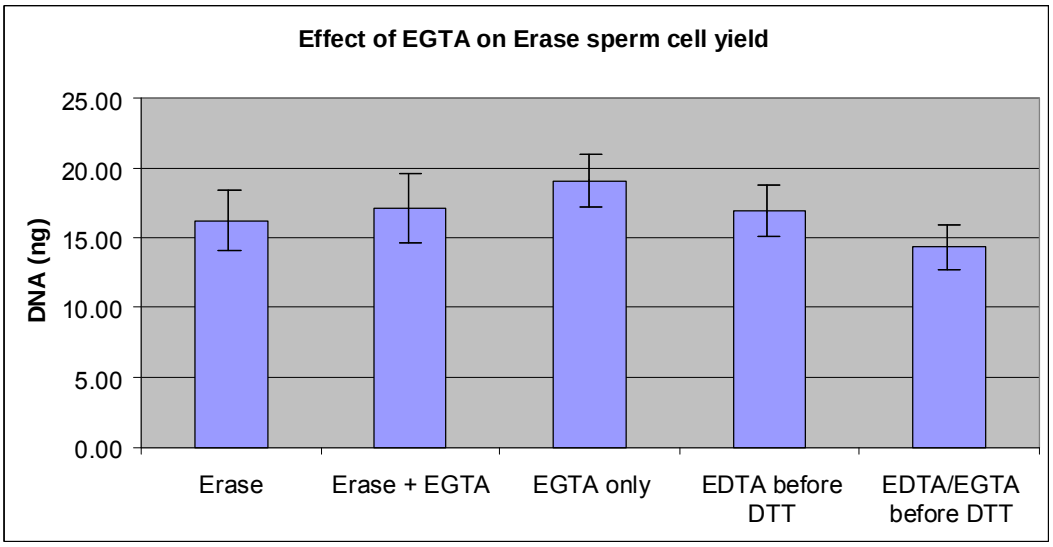


Figure 19 shows the total DNA recovered from Er*ase* reactions on 50,000 sperm cells from semen only samples with and without EGTA added before or during incubation with Solution #3. The chart shows the averages and standard deviations of three replications of each treatment.

Heat Treatments during Sperm Cell Lysis

Ultimately, heat inactivation has been shown to physically destroy nuclease activity [16]. Heat treatments of 65°C, 85°C and 100°C were applied before adding Solution #3 (see Figure 20). Because the yield of sperm cell DNA did not increase with heat treatment, it can be assumed that there must be no residual nuclease activity during the sperm lysis step in the selective degradation extraction.

Figure 20

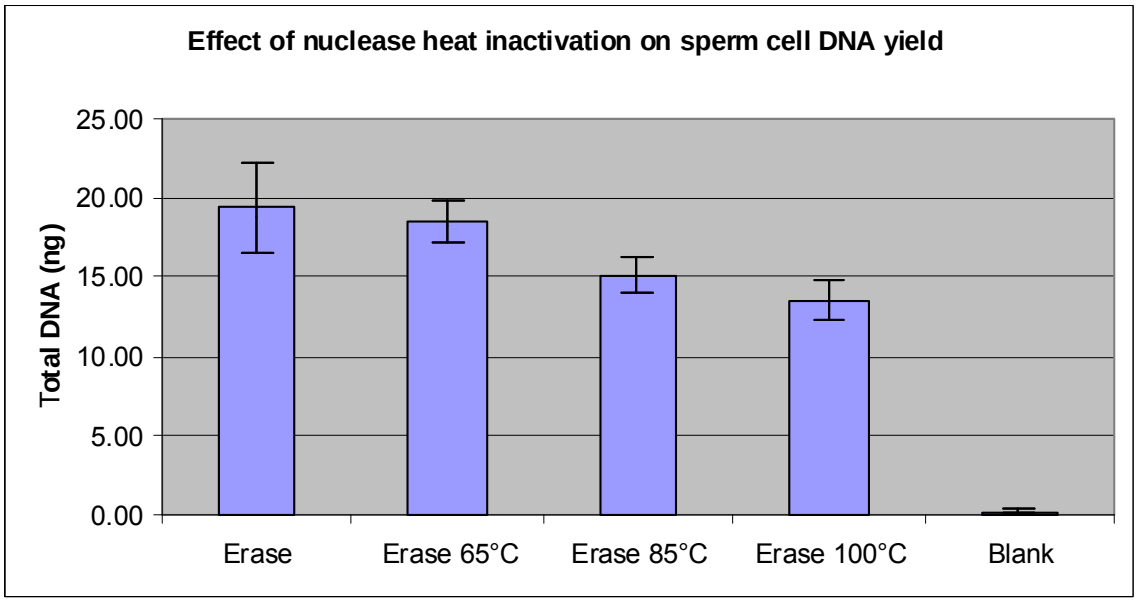


Figure 20 presents the results from E*rase* extraction experiments using 50,000 sperm cells from semen only samples where the samples were treated with 65°C, 85°C or 100°C heat after nuclease incubation and before sperm cell fraction lysis. The average of three replications with the standard deviations are shown.

Including SDS during Sperm Cell Lysis

The standard differential extraction used SDS in the sperm lysis step and E*rase* did not. Detergent from the initial extraction buffer remained in the E*rase* extraction during the sperm lysis step, but the concentration was lower than in the initial epithelial cell lysis and the detergent may be less effective than SDS. Because SDS is known to be an efficient detergent for sperm cell lysis, SDS was added to Solution #3 to determine if any additional sperm cell DNA could be recovered. Figure 21 shows the results from experiments comparing E*rase* extractions with and without 2% SDS (final concentration). There was no increase in sperm cell DNA yield when SDS was added to Solution #3. This experiment supported previous microscopic data that indicated sperm cell lysis was complete after addition of solution #3 and incubation.

Figure 21

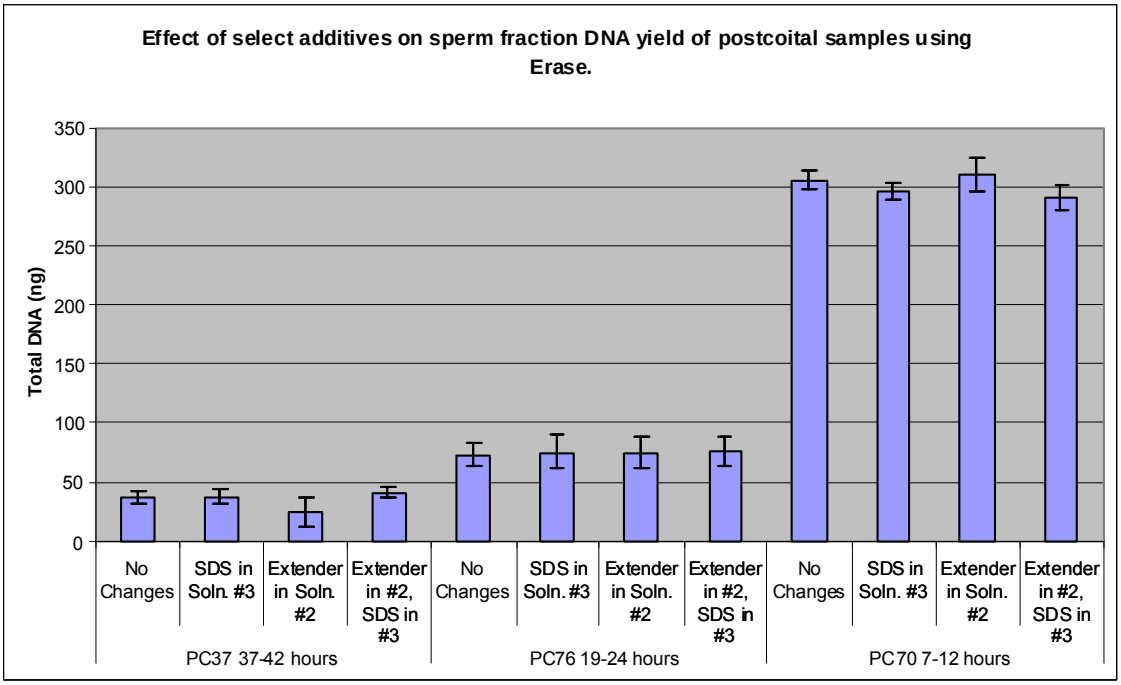


Figure 21 represents the averaged results for total DNA yield from the male fraction of E*rase* extractions of postcoital samples using various additives. Each postcoital swab sample was treated with Erase *proteinase* K extraction buffer and then divided equally for the step shown. The basic E*rase* procedure was compared with and without the addition of SDS, with egg yolk extender added to solution #2 and with both extender and SDS added. The results represent the average total DNA yield and their standard deviations for three replications.

(vi) Retaining Substrate Material in Extraction throughout Selective Degradation Procedure

The Erase procedure calls for removing the substrate after the sperm pelleting centrifugation step. The centrifugation step pelleted most sperm cells, but it is assumed that some of the sperm cells remained in the swab after centrifugation. It is common knowledge in the forensic DNA community that if a sexual assault substrate is re-extracted after the initial extraction process it is often possible to obtain sperm cells that remained on the substrate after the initial extraction process. Experiments were designed in order to determine whether introducing the substrate back into the sperm fraction tube after the sperm pelletting centrifugation step would increase the sperm cell DNA yield. The experimental data varied in significance, but the overall trend was that the addition of the substrate tended to lower the sperm fraction DNA yield. It is surmised that some of the sperm cell DNA may be trapped in the substrate after final centrifugation.

Figure 22

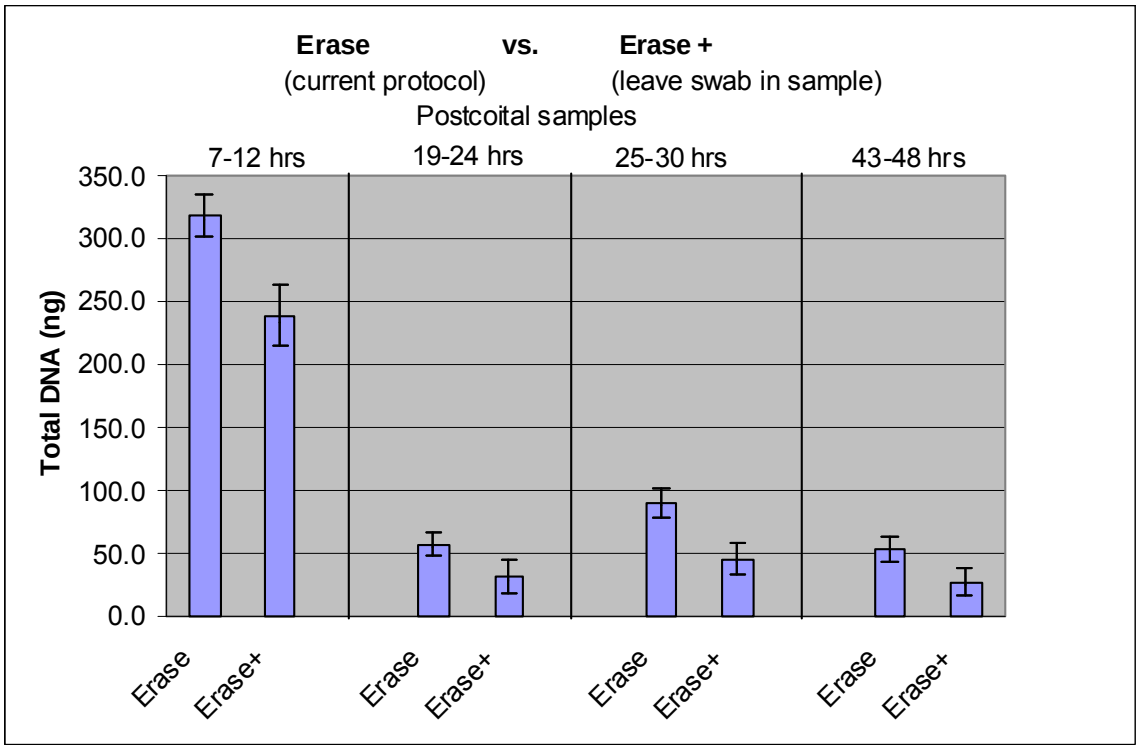


Figure 22 presents the results from Er*ase* extractions where the substrate material (swab) was either removed from the extraction (Er*ase*) or was added back after centrifugation (Er*ase+*). The chart shows the average total DNA yields and standard deviations for three replications each using four different postcoital swabs. Leaving the substrate in the extraction tended to decrease the yield of sperm DNA.

B. Validation

PTC also performed experiments to establish the developmental validation of the Erase kit. The developmental validation consists of experiments designed to determine the efficacy and reliability of this method for forensic casework analysis, including the determination of the conditions and limitations of operation of the new method.

The developmental validation studies include sensitivity, substrate storage stability, reproducibility, comparison to current methods with case type samples, mixtures, and precision and accuracy.

The Developmental Validation Study is contained in a separate attachment. We apologize for any redundancy that may have been caused by the fact that the Developmental Validation Study was drafted as a separate, stand-alone document, with its own recitation of methods, etc.

Please note also that Figures and Tables are numbered in their own, self-contained sequence in the Validation paper, without reference to the figure and table numbering in this Technical Report.

The significance of the developmental validation studies are discussed in this Technical Report, both in Section III-1-A regarding the comparison of performance of selective degradation to other methods, and in the three subsections of the Conclusion, Section IV-1, 2, and 3.

C. Beta Test Site Results

(i) Validation by Crime Laboratories

Erase has been validated by or will undergo validation in many crime laboratories around the country. Laboratories in Missouri, Illinois, Arizona, California, Louisiana, Maryland, Michigan, Texas, Florida, Virginia, Idaho, New York, Pennsylvania, Wisconsin and the FBI are currently validating the Erase method of differential extraction, along with several laboratories in Europe.

Several laboratories have either completed validation and are online with the method or are awaiting publication of the developmental validation of this process to go online.

With only one exception, every laboratory has reported positive findings with the Erase validation kit. In one instance an intern was allowed to proceed with testing the Erase kit. After speaking with her she stated that she had made several mistakes in the procedure during the testing. In addition, she did not have a clear understanding of how to make dilutions and of how many sperm were necessary to obtain a profile. Some of her work apparently involved dilutions to only10 sperm on a vaginal swab. It would not be expected that a male profile would be obtained under those conditions.

(ii) Difficult Case, St. Louis County Police Crime Laboratory

The St. Louis County Police Crime Laboratory (SLCPCL) worked a case in which they were unable to obtain a sperm DNA profile using traditional differential extraction methods, even though sperm were present on the slide for the sample. After two attempts using the standard differential extraction method, only a female profile with a trace of male DNA had been obtained by the crime laboratory from the sperm fraction.

At the time this case was in progress, E*rase* kits were not commercially available. The crime laboratory delivered a portion of the sample to PTC. PTC performed the differential extraction process on one half of a vaginal swab, using the Er*ase* method. PTC then returned purified DNA from the sperm and epithelial fractions to the St. Louis County Crime Laboratory.

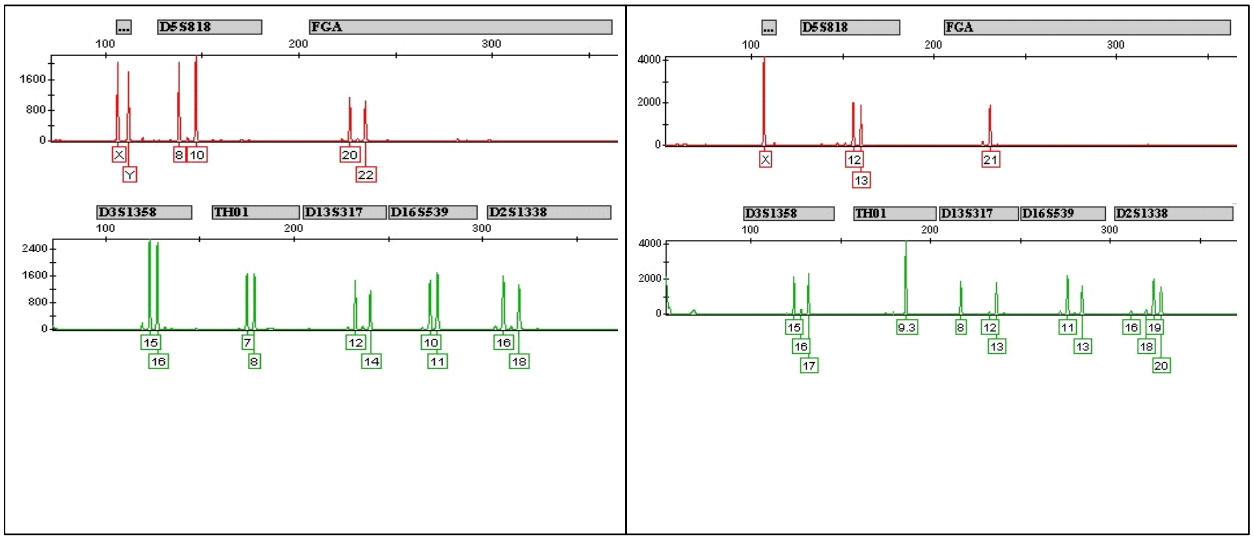
Using the purified DNA from the E*rase* extraction, the crime laboratory successfully produced a full 16 loci sperm DNA profile.

The electropherograms for some of the tested loci from the sperm fraction, representing both the traditional and selective degradation methods, are included in Figure 24. The full profile is not made available for privacy reasons.

Figure 24: E*rase* vs. Standard Method on a Difficult Sample

The sperm fraction of a sexual assault sample was processed by a crime laboratory using a standard differential extraction method. The profile obtained by the crime laboratory was female with a trace of male contributor. Subsequent attempts by the laboratory produced the same result. The sample was sent to PTC for processing with E*rase* and then the extract was returned to the crime laboratory. A full, mixture free male profile was obtained by the crime laboratory using the E*rase* extract.

E*rase* Profile **Standard Method Profile**



The profile produced by Erase had no above threshold alleles from the victim and only a trace of the victim below threshold. The sperm fraction profile was a full male profile and the statistics were straight forward.

The St. Louis County analyst was Ms. Margaret Walsh. Ms. Walsh is available to discuss the details of this case as they pertain to the use of E*rase*. In addition, the St. Louis County Police Crime Laboratory is now using Erase routinely, and both Ms. Walsh and Kyra Lienhop, DNA Technical Leader, would be happy to discuss the laboratory's experience with Erase generally.

In this instance, Erase made the difference in a DNA identification of the suspect as opposed to a trace profile where he could not be excluded.

(iii) Substrates other than Swabs

Several of the crime laboratories introduced various substrates for internal validation of E*rase*. The E*rase* method worked well with every substrate that the

crime laboratories tested. A partial list of substrates tested include those substrates most likely to be encountered in a forensic case, as follows: condom, tampon, sanitary napkin, denim, black cotton, nylons, and mesh underwear.

The Missouri State Highway Patrol Crime Laboratory (MSHP) tested the Erase kit and a number of substrates. Table 2, below, shows the substrates tested, and the results.

Table 2, Substrates Tested by Missouri State Highway Patrol Crime Laboratory

Differential Description	Sample type	Quantity (ng/uL)	Profile Description	Gender Characteristics
Post-coital	Swab of condom (inside) (NS)	0.0317	No profile	N/A
Post-coital	Swab of condom (inside) (S)	1.41	Single source	Male
Post-coital	Swab of condom (outside) (NS)	9.52	Single source	Female
Post-coital	Swab of condom (outside) (S)	0.0053	Partial, single source	Male
Post-coital (7 hrs.)	Tampon (NS)	0.0672	Partial, single source (one additional allele)	Female
Post-coital (7hrs.)	Tampon (S)	10.55	Single Source	Male
Postcoital	Sanitary napkin (NS)	19.16	Mixture	Male/Female
Postcoital	Sanitary napkin (S)	0.566	Single Source	Male
Neat semen	Denim (NS)	0.235	Single Source (one additional allele)	Male
Neat semen	Denim (S)	0.00933	Single Source	Male
1:1 female blood:semen	Black cotton (NS)	0.366	Partial, mixture	Male/Female
1:1 female blood:semen	Black cotton (S)	3.61	Single Source	Male
1:1 female blood:semen	blue mesh underwear (NS)	0.223	Mixture	Male/Female
1:1 female blood:semen	blue mesh underwear (S)	2.43	Single Source	Male
1:1 female blood:semen	white nylons (NS)	0.809	Mixture	Male/Female
1:1 female blood:semen	white nylons (S)	1.2	Single Source	Male
Reagent Blank	BLANK (NS)	0	No profile	N/A
Reagent Blank	BLANK (S)	0	No profile	N/A

S – Sperm Fraction NS – Non-Sperm Fraction

The Missouri State Highway Patrol Crime Laboratory tested Er*ase* with various substrates that are typical of those from sexual assault cases. Table 2 is a compilation of the results of those tests. The expected results were obtained on all substrates.

(iv) Differex Comparison

Differex is a commercially available product sold by Promega Corporation, and it is used by some crime laboratories as an alternative to the standard method of differential extraction. Selective degradation differential extraction results were compared to Differex results by the crime laboratory in Lausanne, Switzerland. It is an attachment to this report entitled Differex Comparison Data. The data from this comparison is available in an attachment labeled Differex Comparison Data.

PTC was not familiar with the Differex product and it was necessary to verify that the differences in results were not due to PTC's inexperience with Differex. The advantage of this crime laboratory study was that both Differex and Er*a*se could be tested by a laboratory with extensive experience using Differex.

The results of the Differex study are discussed below in Section E., Performance of Selective Degradation Compared to Other Methods.

D. Automation of Selective Degradation

The standard method of differential extraction that has been used in crime labs for many years is not amenable to automation. Using standard methods, a substrate is introduced to a mild lysis buffer that is stringent enough to lyse epithelial cells, but will not lyse sperm cells. After the extraction incubation period, the substrate is placed in a basket and the tube is centrifuged in order to remove the liquid from the substrate and to pellet the sperm cells. Then the analyst attempts to pipette as much of the supernatant (epithelial cell fraction) as possible from the tube with the sperm pellet without disturbing the sperm pellet.

The sperm fraction is diluted/washed several times with buffer in order to dilute the remaining epithelial cell DNA. Using this method, it can be very difficult to remove enough of the epithelial cell DNA so that the resulting DNA profile will be entirely from the sperm cell DNA. When the amount of epithelial cell DNA is very large and there are relatively few sperm cells it is often impossible to remove enough of the epithelial DNA that a sperm DNA profile is obtained. A mixed DNA profile often results.

The dilution/washing steps along with the need for very carefully avoiding the sperm pellet while removing as much of the supernatant as possible make the standard differential extraction process very difficult or impossible to automate.

The protocol for the selective degradation method as compared with other methods is extraordinarily simple to automate. There are no dilution and precise

pipetting steps. There is no need to attempt to wash away the epithelial cell DNA remaining in the sperm fraction. There is no need for additional centrifugation steps. After the initial epithelial lysis buffer is introduced, there is one centrifugation step that will allow for the removal of liquid from the substrate and will simultaneously pellet the sperm cells. After that, the process of elimination of epithelial DNA from the sperm fraction can be completely hands off. The robot performs a transfer of a portion of the epithelial fraction to another plate. Basic liquid handlers can be programmed to remove liquid to a designated height leaving the sperm pellet undisturbed. The robot introduces solutions #1 and #2 into the sperm fraction, mixes and incubates the samples, then transfers the samples to a new plate. The robot then adds solution #3 buffer to the sperm fraction and incubates the sample. At that point, both the epithelial and sperm DNA fractions are ready for DNA purification.

A wide variety of automated DNA purification method can be used from that point forward.

During this research, DNA extracted using the selective degradation process was successfully purified with the following purification systems:

InVitrogen Charge/Switch
Promega DNA IQ
Qiagen Mini-Amp
Qiagen EZ-1 Automated System
Maxwell 16 LEV System
Phenol/chloroform, ethanol precipitation
Vivacon100

The E*rase* Sperm Isolation Kit was tested using a simulated automation experiment using an 8-channel pipette and whole postcoital swabs to determine the suitability of selective degradation for automation. Similar experiments were also performed using a Tecan MiniPrep 75 liquid handler.

The JOE labeled electropherograms of the AmpFl STR® SGM Plus® amplifications from one column of a 96 well plate extracted using simulated automation and the E*rase* Sperm Isolation Kit are found in Supplemental Figure 23. Postcoital swabs, collected at various intervals following coitus, were placed into wells 1A, 1C, 1E, 1G, and 1H. DNA-free swabs were placed into wells 1D and 1F. A noncoital vaginal swab was placed into well 1B.

See Figure 23 (in Supplement), Profiles from Selective Degradation Extractions Performed in Column 1 of a 96 Well Plate

Expected results were obtained from every tested sample and all profiles were consistent with previous manual testing of similar samples.

The resulting sperm and epithelial cell fractions are single source male and female profiles, respectively, for all postcoital sample intervals tested (ranging from 0 - 36 hours). In addition, the DNA-free amplifications are not contaminated by the

neighboring wells. The noncoital sample in 1B demonstrates the efficiency E*rase*'s removal of the epithelial DNA from the sperm cell DNA fraction even when no sperm are present.

Forensic laboratories have numerous liquid handling devices available to choose for automation of case work. Scripts will need to be written and validations performed on each model of robot, but the Erase validation for automation has been developed to support a general application that can be adapted to any liquid handling device.

In house, automation was performed using a very basic Tecan model. External laboratories have been recruited for validation on other instruments, but those experiments are not complete. The key steps to optimize the procedure are 1) removal of the supernatant to leave 50ul over the pelleted sperm cells, 2) addition of 10ul each of Solution 1 and Solution 2, thoroughly mixing, then transferring to a new 96 well plate, and 3) the addition of 10ul of Solution 3, and then mixing. There are incubation steps along the way that may or may not be handled by the robot, depending on the robot model available to the laboratory. The procedure is straightforward.

Different liquid handling devices use different programs for operation. To set up the Erase method for automating differential extractions, three robotics companies have been contacted and asked to prepare scripts for their devices that can be used for Erase automation. Those companies are Hamilton, Tecan, and Beckman-Coulter.

For further information, including the Erase Sperm Isolation Kit protocol and references to Solutions 1, 2, and 3, see the Methods section of this Final Technical Report.

E. Performance of Selective Degradation Compared to Other Methods

In demonstrating the effectiveness (or ineffectiveness) of the selective degradation technology, one important aspect of this research was a head to head comparison with existing technologies.

The research performed at PTC included such comparisons, as did testing performed by other laboratories.

The comparisons performed in various laboratories repeatedly show the same results obtained by PTC. Because of the ability to remove more epithelial DNA from the male fraction, the selective degradation method of differential extraction provides substantially better results. On samples with larger amounts of sperm, it provides clean single source male profiles rather than mixed sperm and epithelial profiles. On samples containing fewer sperm, selective degradation allows the recovery of a male profile even when other current methods of differential extraction provide only a partial male profile or no male profile at all.

(i) Research Performed at PTC

There were three different sets of experiments at PTC that directly compare the performance of selective degradation to the performance of the traditional method of differential extraction.

(a) Comparison with Samples with Five Hundred Sperm -- After completing the optimization experiments for selective degradation, PTC performed a test on noncoital vaginal swabs, with 500 sperm cells added to each, as discussed above in Section III-1-A-(iv)(d). The experiment used the selective degradation method and the traditional method for differential extraction.

As shown in Figure 6, the traditional method primarily produced an epithelial cell DNA profile in the sperm fraction, although traces of the male profile could be seen at some loci. But no sperm DNA alleles could be called In this case, the traditional method of differential extraction was not capable of removing enough epithelial DNA to isolate the sperm cells from the epithelial DNA, and as a result no sperm profile was identified.

In this same experiment, the selective degradation method produced a clean male profile. Sufficient epithelial DNA was removed from the sperm fraction, by degradation, to allow the male profile to be easily identified.

(b) Comparison with Samples containing Menstrual Blood -- In another experiment, described in Section III-1-A-(iv)(k), a comparison between the selective degradation and traditional methods was conducted on samples that contained menstrual blood. The samples were vaginal swabs, collected at 12 hours postcoitus.

As can be seen in Figure 18, the standard differential extraction method produced sperm cell fraction samples that were mixed with DNA from both the epithelial cell donor and the semen donor. The sperm fraction under the selective degradation method provided a clean male DNA profile, with no need for mixture interpretation.

(c) Sensitivity Study – In the Developmental Validation Study, PTC performed a sensitivity study, comparing the traditional method of differential extraction with selective degradation. These experiments were devised to determine the minimum number of sperm cells necessary to obtain a single source sperm DNA profile using selective degradation. These experiments are more fully described in the Validation Study.

Duplicate mock forensic samples were prepared using noncoital vaginal swabs spiked with varying amounts of sperm. Samples were prepared using 2660, 1330, 665, 333, and 166 sperm cells. Traditional and selective degradation differential extractions were performed. The DNA was amplified using the PowerPlex16 system and separated using polyacrylamide gel electrophoresis. The results at the D21S11 locus are displayed in Figure 1 of the Validation Study, and Table 1 of that study summarizes the data.

Using the traditional method, the sperm fraction showed a mixed profile for all samples. The 2,660 sperm sample was a mixture with the sperm donor as the major contributor. At both 1,330 and 665 sperm, the contributions from the sperm and epithelial donors were approximately equal. At 333 and 166 sperm, the standard method produced on the epithelial donor's DNA profile. No sperm DNA profile could be identified.

The selective degradation method gave a clean male profile, with no significant epithelial cell DNA, at 2,660, 1,330, 665, and 333 sperm. For the sample with 166 sperm, the selective degradation method produced a mixed DNA profile, with approximately equal contributions from the sperm cell and epithelial cell donors.

(ii) St. Louis County Police Crime Laboratory

The St. Louis County Police Crime Laboratory has had experience with the selective degradation method of differential extraction both in actual case work, and in performing some of the experiments for the developmental validation of E*rase*.

(a) Difficult Case -- The St. Louis County Police Crime Laboratory (SLCPCL) worked a case in which they were unable to obtain a sperm DNA profile using traditional differential extraction methods, even though sperm were present on the slide for the sample. After two attempts using the standard differential extraction method, only a female profile with a trace of male DNA had been obtained by the crime laboratory from the sperm fraction.

Because E*rase* kits were not yet available, SLCPCL delivered a portion of the sample to PTC. PTC performed the E*rase* differential extraction process on one half of a vaginal swab. PTC then returned purified DNA from the sperm and epithelial fractions to the St. Louis County Crime Laboratory.

Using the purified DNA from the E*rase* extraction, the crime laboratory successfully produced a full 16 loci sperm DNA profile. That profile had no above threshold alleles from the victim and only a trace of the victim below threshold. The statistics were straightforward, and the case went to court.

Electropherograms for a portion of the sperm fraction profile under both methods are shown in Figure 24, above.

As this actual case indicates, E*rase* has the potential to obtain male profiles for many cases that could not previously be solved using autosomal DNA from a sexual assault.

Ms. Walsh (the St. Louis County Police Crime Laboratory analyst on this case) also mentioned that E*rase* has been very helpful with male-male profiles in sexual assault cases where the victim has had relations with two men (either two rapists, or the rapist and a consensual partner). Because E*rase* removes so much

epithelial DNA, the crime lab obtains a mixed profile of just the two males, and not all three parties. She stated that this allows her to deconvolute the two male profiles. Using their traditional method in the past, the epithelial profile also appeared in the male fraction, and she was unable to deconfolute the profiles. Her conclusions are consistent with the results of the Mixture Studies performed at PTC for the developmental validation study.

(b) Comparison with Case Type Samples -- The St. Louis County Police Crime Laboratory performed some of the experiments for the E*rase* developmental validation study, including the comparison to current methods with case type samples. In this portion of the study, SLCPCL compared their traditional method of differential extraction with the selective degradation method, on postcoital swabs that were collected at two different intervals following coitus. These experiments are described in more detail in the attached Developmental Validation Study. The electropherograms for the sperm fractions in these comparisons are shown in Figure 2 of the Validation Study.

The 13-18 hour postcoital sample processed with the E*rase* Sperm Isolation Kit produced a single sperm fraction DNA contributor, with a well balanced male profile, ideal for identity matching. The sample processed with the standard differential extraction method produced a mixed donor profile in the sperm fraction, with the male as the major contributor. The sperm DNA profile is suitable for searching and matching.

The 31-36 hour postcoital sample processed with the E*rase* Sperm Isolation Kit produced a single contributor, well balanced male profile. The sample processed with the standard differential extraction method produced a mixed donor profile with the major profile being from a female contributor. Some alleles from the male donor are identified, but searching for a matching DNA profile would be difficult and may be impossible. In the event a potential match is identified, the statistical calculations necessary would be tedious and significantly less powerful than a single source DNA profile match.

The sensitivity studies that show how few sperm the selective degradation require in order to obtain a sufficient sperm DNA profile, are somewhat theoretical in the sense that in case work the crime lab doesn't know how many sperm they are starting with. However, comparisons with vaginal swabs collected at different intervals postcoitus are representative of actual casework situations. It is assumed that in general, the longer the interval before collection of the swab, the fewer sperm cells obtained. This study of case type samples is consistent with the findings of the sensitivity studies, and shows some of the advantages of the selective degradation method in situations routinely encountered in the forensic laboratory setting

(iii) Los Angeles County Sheriff's Department Crime Laboratory

The Los Angeles County Sheriff's Department Crime Laboratory performed experiments comparing their traditional method of differential extraction to E*rase*.

Side by side comparisons by the Los Angeles County Sheriff's Department produced data typical of standard differential/E*rase* comparisons.

Typical data from one of several experiments is shown. Two sets of swabs, one 12 hour postcoital swabs and the other a greater than 24 hour postcoital swabs were processed for a standard differential/E*rase* comparison. The data indicates that the 12 hour postcoital swab produced a single source sperm DNA profile with both methods, although there was some X/Y peak height imbalance (26%) noted for the standard differential extraction method. The standard differential method produced a mixed DNA profile for the >24 hour postcoital swab, while the E*rase* method produced a single source sperm DNA profile, with no indication epithelial DNA, but with some X/Y peak height (8%) imbalance noted.

The results for loci from the longer than 24 hour postcoital swab are shown in Figure 25.

Figure 25 Comparison of Methods at L.A. County Sheriff's Department

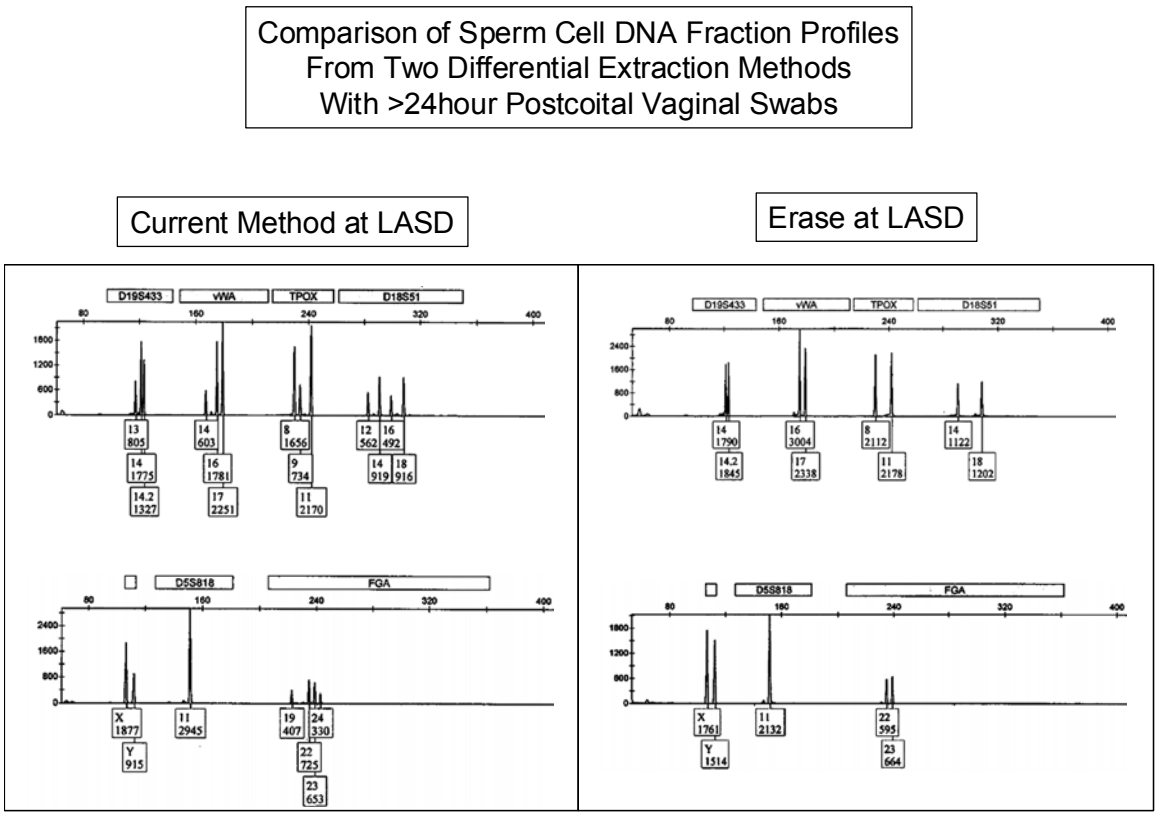


Figure 25 shows the results at a few loci in the sperm fraction from a 24 hour swab, under both methods, at the L.A. County Sheriff's Department Crime Laboratory.

Comparisons of another set of 12 and 24 hour postcoital swabs gave similar data, although there was less epithelial DNA in the subsequent >24 hour swab processed with the standard differential method than in the mixture in figure 25.

(iv) Differex Comparison

Differex is a product marketed by Promega Corporation as an alternative to the traditional method of differential extraction. It is likely the alternative to the standard differential extraction method that is most used by crime laboratories to process sexual assault casework.

A direct comparison of Erase with Differex was performed by a crime laboratory in Lausanne, Switzerland that has been using Differex on casework for a number of years. At the time of this study, Differex was the only differential extraction method used by the Lausanne crime lab for processing sexual assault cases. PTC was not familiar with the Differex product, and it was necessary to verify that the differences in results obtained in house were not due to inexperience with Differex. The advantage of this crime laboratory study was that both Differex and E*rase* could be tested by a laboratory with extensive experience using Differex.

Other details of the comparison are addressed in the separately attached study. In this section the comparisons between the Differex method and selective degradation method of differential extraction were made on samples containing varying numbers of sperm cells. The number of sperm for the four different sets of samples were 50,000 sperm; 16,700 sperm; 5,000 sperm and 1,670 sperm.

The CE data for these experiments is reflected in electropherograms in the attached Differex Comparison. The remainder of the discussion in this Section addresses the comparative results shown by that data.

The Differex results for samples containing 50,000 sperm have epithelial DNA profiles above the peak height threshold in the male fraction at several loci. However, the sperm DNA is clearly the major contributor and there appears to be much more sperm DNA than epithelial DNA in the sperm fraction. The Differex results with 50,000 sperm show a mixed profile, that profile will be easily deconvoluted, and will require little interpretation by the analyst.

The selective degradation method produced clean single-source sperm profile, with no indication of epithelial DNA.

The samples with 16,700 sperm have similar results to the samples with 50,000 sperm. Differex produced a mixture profile with more sperm DNA than epithelial DNA. Selective degradation produced a clean sperm profile with no indication of epithelial DNA.

The samples with 5,000 sperm cells demonstrate a greater difference between the two methods. Using 5,000 sperm, the male fraction of the Differex extraction generated a mixed profile, with the epithelial DNA profile as the major contributor and the selective degradation method produced a clean male profile, with no indication of epithelial DNA.

The comparison of the samples with 1,670 sperm cells are dramatically different. The Differex male fraction is mostly X at the Amelogenin locus and the Differex

male fraction profile is a mixed profile with the female as the major contributor. The selective degradation method continued to produce a clean male profile with no indication of epithelial DNA. The vWA locus has above peak height stutter, but those alleles are not consistent in size with the epithelial cell DNA profile.

The overall conclusion of the Differex comparison is that selective degradation provided a cleaner sperm profile than Differex in all differential extractions tested. With decreasing numbers of sperm cells the Differex method demonstrated an inability to isolate the sperm cells from the epithelial DNA producing mixed and/or partial sperm DNA profiles from the sperm fraction, while the selective degradation method continued to provide a clean and full sperm DNA profile.

The comparisons between the selective degradation method of differential extraction and other commonly used methods of differential extraction consistently provide similar results, whether performed by PTC or by public crime laboratories. The method of selective degradation effectively removes epithelial DNA from the sperm fraction of the differential extraction therefore providing better sperm DNA profiles than the methods compared. Selective degradation often results in single source sperm DNA profiles when other method produce mixed profiles.

2. Tables

The body of this Final Technical Report contains two tables, and the attached Developmental Validation Study contains three tables, as follows:

Table 1, Summary of Incubation Time Data, Section III-1-A-(iv)(j).

Table 2, Substrates Tested by Missouri State Highway Patrol Crime Laboratory

Validation Table 1, Sensitivity Study, in Tables section of Validation Study.

Validation Table 2, Mixtures, in Tables section of Validation Study.

Validation Table 3, Precision and Accuracy, in Tables section of Validation Study.

3. Figures

Certain figures are included in the body of this document, located where their content is being discussed in the text.

Figures that take a lot of memory, and could make it impossible to e-mail this document, are in separate attachments. The figures in the separate attachments include Figures 4, 6, 7, 9, 10, 14, 16, 17, 18 and 23. References to these figures in text also make the notation “supplemental” to indicate that they are in an attachment rather than in the text. The remaining figures are located in the text above.

Figure List

This list identifies figures in this report by number, title and location.

Figure 17, replaced by Table 1, Summary of Incubation Time Data, Section III-1-A-(iv)(j)

Figure 18 (in Supplement), Comparison of Methods on Vaginal Swab with Menstrual Blood, Section III-1-A-(iv)(k)

Figure 19, Effect of EGTA on Selective Degradation Sperm DNA Yield, Section III-1-A-(v)

Figure 20, Effect of Nuclease Heat Inactivation on Sperm Cell DNA Yield, Section III-1-A-(v)

Figure 21, Effect of Select Additives to Sperm Lysis Step on Sperm DNA Yield, Section III-1-A-(v)

Figure 22, Effect of Leaving the Substrate in the Extraction on Sperm DNA Yield, Section III-1-A-(vi)

Figure 23 (in Supplement), Profiles from Selective Degradation Extractions Performed in Column 1 of a 96 Well Plate.

Figure 24, Erase vs. Standard Method on a Difficult Sample, Section III-1-C-(ii).

Figure 25, Comparison of Methods by the L.A. County Sheriff's Department, Section III-1-E.

Validation Figure 1, Sensitivity Study, in the Figures section of Validation Study.

Validation Figure 2, Case Type Samples, in the Figures section of Validation.

Validation Figure 3, Precision and Accuracy, in the Figures section of Validation.

IV. Conclusions

1. Discussion of Findings

The major goals of this research were: (i) to optimize the technique of performing differential extractions using selective degradation to remove epithelial DNA from the sperm fraction, as originally published by Garvin [1]; so that it will work on most samples encountered in a forensic sexual assault case (ii) demonstrate that the selective degradation method is a valid method for use in forensic laboratories; (iii) demonstrate that by using the selective degradation method, the process of differential extraction may be automated, and design the process to be able to coordinate with whatever liquid handling equipment a crime laboratory is currently using; and (iv) compare the performance of the selective degradation method to other methods of differential extraction currently in use.

The following is a discussion of findings in the Results section of this paper, for each category of experiments (optimization, validation, etc.). It ties the findings and conclusions to the goals of the research.

A. Optimization of Selective Degradation

During the optimization experiments, even the shape of the microfuge tube that would best facilitate differential extraction was determined.

Experiments varying proteinase K concentration, incubation temperature and incubation time established optimal settings for lysing epithelial DNA, without reducing the yield of sperm DNA.

The findings regarding the elimination of epithelial DNA demonstrated that even after optimization of the selective degradation method, small amounts of epithelial DNA remain in the sperm fraction. But findings regarding the ability of the selective degradation method to obtain male profiles from samples with low amounts of sperm, when other methods cannot, suggests that the reduction in epithelial DNA is effective.

Experiments to optimize the nuclease treatment began with finding the optimal concentrations of Magnesium and Calcium necessary to activate the nuclease. Concentrations and sources of nuclease, as well as incubation times and temperatures, were optimized for maximum sperm DNA yield.

In a number of areas, the original protocol published by Garvin [1] was improved upon. As a result of these experiments, the selective degradation procedure for differential extraction has been optimized for use in the forensic laboratory, and is shown to be an improvement on current technology. This meets the first goal of this NIJ research grant.

B. Validation of the Selective Degradation Method

The developmental validation consisted of experiments designed to determine the efficacy and reliability of this method for forensic casework analysis, including the determination of the conditions and limitations of the new method.

The developmental validation studies included determinations of sensitivity, substrate storage stability, reproducibility, comparison to current methods with case type samples, mixtures, and precision and accuracy.

This research found that the selective degradation method was sensitive enough to identify a male profile from samples with as few as 166 sperm. Selective degradation was able to produce single source sperm DNA profiles after postcoital mock forensic samples had been stored at room temperature for nine months, and even on historical samples stored at room temperature for more than eleven years old.

Both the quantity of sperm DNA recovered from the selective degradation method, and the DNA profiles themselves, were found to be reliably reproducible in the hands of different analysts and when processed by different laboratories. It was also found that the selective degradation method worked well with samples that included a mixture of DNA from two sperm donors. The absence of DNA from the victim made deconvolution of the DNA profiles much easier than it would have been if the victims DNA was present.

The comparison to current methods using case type samples found that, as the interval between coitus and collection of the vaginal swab increased, the selective degradation method performed increasingly better than the traditional method. These findings were consistent with the sensitivity study findings showing the superior performance of the selective degradation method on samples with lower amounts of sperm cells.

The findings from the developmental validation study demonstrate that the selective degradation method of differential extraction is a robust method, with certain advantages over other current methods, and is capable of producing results reliably and repeatedly by multiple analysts and laboratories. This method is shown to be useful and usable for forensic casework analysis. This conclusion regarding the validity of the method meets one of the goals of this NIJ research.

C. Automation of Differential Extraction

The experiments regarding the ability to automate differential extractions by using the selective degradation method found that the method works well in a 96 well format, and can be automated using existing automation equipment in crime laboratories.

As each step of the selective degradation process developed, it was designed so that a robot could perform the same steps that would be performed by the analyst at the bench. This allowed for a very smooth transition from the single tube method to a 96 well plate format. All of the steps involved in automation of this process are steps that can easily be performed and programmed for very basic liquid handlers.

The more sophisticated robots may be able to handle a few more of the incubation steps than the most basic liquid handler, but all of the hands-on steps that would require more than a simple transfer of a plate by the analyst can be handled by the vast majority, if not all, of the liquid handling robots currently available in public crime laboratories.

The possibilities range from hands on moving of trays in and out of incubation steps, to a completely hands off approach with the probable exception of the centrifugation step after the initial lysis. However, Hamilton is introducing a liquid handler that also incorporates the centrifugation step, and that robot's overall mechanism for handling the DNA samples appears to be tailor made for the selective degradation process.

In most instances, the crime laboratories will be able to implement this methodology using existing equipment. Because most if not all of the standard liquid handlers currently employed by crime laboratories can be programmed to the parameters necessary for this differential extraction method, public crime laboratories will not have to invest in new equipment in order to use this methodology. A few hours of programming or executing a pre-prepared script and they will be ready to validate this method.

Demonstration of the ability to automate differential extractions using the method of selective degradation has accomplished another major goal of this NIJ research.

D. Comparison of Selective Degradation to other Current Methods

This section involves comparisons between the selective degradation method and the traditional method of differential extraction, as well as the Differex alternative. It draws together the results of experiments from various other portions of the study, to focus attention on the comparison between methods.

Comparisons between methods are documented here as performed by PTC, the St. Louis Count Police Department Crime Laboratory, the Los Angeles County Sherriff's Department Crime Laboratory, and the crime laboratory of Lausanne, Switzerland (for the Differex comparison).

These experiments consistently find that, no matter who performs the differential extraction, the selective degradation method of differential extraction consistently has significant performance advantages over other current methods.

Those advantages include:

(i) a clean, single source sperm DNA profile is developed in most cases, and mixtures with the epithelial DNA profile are generally avoided;

(ii) a single source sperm DNA profile is generally obtained even if the sample has so few sperm that the traditional method produces either a minor contributor partial sperm DNA profile, or no sperm profile at all. One practical application of the sensitivity of selective degradation is its superior performance on vaginal swabs collected at longer intervals following coitus;

(iii) comparison of selective degradation method to the Differex method of differential extraction produced results similar to the comparisons of selective degradation to the traditional method of differential extraction, with selective degradation producing far more instances of single source sperm profiles;

(iv) selective degradation's increased sensitivity also produced superior results when samples contained menstrual blood, because of the ability to eliminate the extraneous DNA contributor from the sperm fraction;

(v) the elimination of more epithelial DNA from the male fraction facilitates the deconvolution of the DNA profiles of multiple sperm donors;

(vi) the amount of analyst hands-on time to perform the differential extraction, as well as the overall time from start to finish of the differential extraction procedure, is much reduced using selective degradation (see chart at beginning of Methods section showing time savings for individual steps and overall); and

(vii) finally, there is the advantage of automation.

The comparisons performed in various laboratories repeatedly duplicate the same results obtained by PTC. Because of the ability to remove more epithelial DNA from the sperm fraction of the differential extraction, the selective degradation method of differential extraction provides substantially better results. Determining the relative performance of the selective degradation method compared to other current methods fulfills another goal of this grant.

Overall, this discussion of findings demonstrates that this research has accomplished all of the goals of the research grant, and provided a substantial benefit to law enforcement.

2. Implications for Policy and Practice

The implications for policy and practice are substantial. The results of this research mark the successful culmination of federal research expenditures and numerous efforts by the forensic community, researching various proposed mechanisms to eliminate the serious problem of mixed DNA profiles in sexual assault evidence.

Among the specific benefits of this technology, it will identify perpetrators of sexual offenses who could not previously be identified using DNA evidence. It will save time in the forensic laboratory and in court, and it can be expected to increase the rate of convictions of perpetrators of sexual assaults. It will also allow automation of the process of differential extraction, and consequently facilitate reduction of the backlog of sexual assault evidence.

The following is a list of some of the major impacts of this research on law enforcement practices and outcomes:

1. Mixtures in sexual assault evidence will be eliminated in almost all cases;

2. Time will be saved in the performance of differential extractions in the crime lab. Less hands on time and elimination of the rinsing steps to dilute the epithelial cells in the male fraction, as well as shorter incubation times, cut the overall processing time to roughly one third of the time needed for a standard differential extraction.

3. All of the time and effort previously devoted to mixtures in the laboratory involving the statistical analysis is eliminated in most cases. If the analyst would have previously obtained a mixture profile from the same sample and now obtains a single source male profile, then the hours or even days of mixture deconvolution, statistical calculation and review time for a single case may be reduced to minutes.

4. All of the time, effort and uncertainty resulting from mixtures at trial, possibly raising "reasonable doubt" and causing the criminal to be set free, is eliminated if the DNA profile is a single source profile.

5. In some cases, clean single-source male profiles will now be obtained where no profile was previously available. Already, using Er*ase* on a case in Missouri for which the crime laboratory was unable to detect any discernable sperm DNA profile in the mixed stain using a traditional differential extraction resulted in a full male profile in the sperm fraction.

6. Because of the ability to successfully profile smaller amounts of sperm DNA in mixed stains than previously possible, certain current and cold cases that had no identified suspect can now be solved.

7. For case work, the resulting ability to easily automate the differential extraction process will save additional time and manpower in crime laboratories that process larger volumes of sexual assault evidence. This method of differential extraction makes it possible for a single analyst to take a 96-well tray from the initial lysis step to DNA purification with only a few minutes of hands on time.

It will be possible for crime laboratories to automate whatever portion of the process is compatible with their existing equipment, without being forced to spend funds to obtain equipment just for this purpose.

Manufacturers of the most popular robots currently in use in crime laboratories in the United States and Europe, which are Tecan, Beckman-Coulter, and Hamilton, were contacted. Collaboration with those companies to develop scripts for the robots to be able to automate differential extractions using selective degradation is in progress. The scripts will be of major assistance to those crime laboratories choosing to automate.

The Er*ase* kit is now commercially available for the single tube method, and is available for custom ordering in the 96 well format.

8. The resulting ability to easily automate the differential extraction process will also be a tremendous benefit in reducing the backlog of sexual assault evidence.

Changes in Policy, Practice and Outcomes in the Forensic Laboratory

Since this technology makes it much more likely that an autosomal sperm fraction DNA profile will be produced, it changes the strategy and overall outcome of a

sexual assault case in many instances. If a sperm fraction autosomal profile can be produced then the profile can be searched in CODIS. If a CODIS search reveals the identity of the assailant then many more sexual assaults may be prevented, and an overall reduction in the number of sexual assaults results in a smaller caseload.

It is the policy or practice of some crime laboratories to eliminate the differential extraction step altogether if a minimum number of sperm are not identified before DNA extraction. This leaves only the option of Y Chromosome analysis in order to identify the rapist. This results in less certain identifications and the inability to perform productive CODIS searches. With selective degradation, it will be possible to obtain an autosomal profile in many or most of those cases and the overall outcome may be a change in policy that allows for differential extractions to be performed whenever sperm are present.

The policies and practices employed by crime laboratories for sexual assault evidence may change as a result of this product or there may be no need to change the policies and practices because using this product may change the nature of the evidence and therefore change the overall outcome of the case. If, for instance it is the policy of the crime laboratory to perform Y Chromosome testing any time that the quantitation data indicates that there is 4 times more epithelial DNA than sperm DNA in a sample, and a case has 5 times as much epithelial DNA as sperm DNA, then Y Chromosome testing is performed. If that same case, because of selective degradation, results in the laboratory finding virtually all male DNA, and it is the policy of the laboratory to therefore perform autosomal DNA testing, then the policy did not have changed, but the selective degradation method will have caused a change in the subsequent procedures and possibly the outcome. The policy and practices of the laboratory in this instance do not need to change in order to cause a change in the outcome of a case.

The impact on policies and practices for sexual assault cases will vary from one laboratory to the next. In some instances, there will be little or no impact on policy and practices. In other laboratories there may be a very significant impact. What will show significant impact in all crime laboratories is the outcome many cases, the overall time required to complete each case, and the likelihood that a DNA identification will be made.

The E*rase* kit has either been validated in, or is currently undergoing validation by, more than 20 U.S. crime laboratories, as well as several laboratories in Europe. Additional laboratories have expressed interest and, as others go online and report positive outcomes, it is expected that many more laboratories will also validate the kit. Several crime laboratories have completed validation of the single tube kit and are either on line or are completing competency exams. They are pleased with the results they are witnessing, and are anxious to take advantage of this technology as soon as possible.

Completing this Final Technical Report, and publishing the developmental validation study, should give all crime laboratories the necessary confidence in this technology to begin pursuing it as soon as they have the time and resources.

This successful NIJ grant has produced a tremendous benefit to law enforcement in the United States, and around the world.

3. Implications for Further Research

A. Time Sensitivity of Nuclease Step

Although this product is very reliable and easy to use there are areas that could benefit from improvement. The nuclease step is very time sensitive. Forgetting or leaving a sample in nuclease for too long could result in significant loss of sperm DNA. A product that has a very flexible incubation time would be beneficial. The analyst would not have to worry that if for some reason they could not get back to the extraction for a significant period of time that their DNA would be lost. More flexibility is always better.

B. Compromised Sperm Membrane

In cases where the sperm membrane is compromised the nuclease appears to penetrate the membrane readily and causes loss of sperm DNA. Therefore, in cases where microscopically the sperm membrane appears to be significantly damaged it is not advisable to use this product. Developing a product that can be used in all cases would be a significant value for crime laboratories.

Even in cases with no apparent damage to the sperm cell membrane there may be DNA loss using this method. Further research revolves around ways to employ this method without damaging DNA within the sperm cell. If it is possible to utilize this method without fear of damage to sperm DNA then the product becomes more useful to the DNA analyst. Several methods are being explored with one method giving very promising results. Future funding opportunities may be sought to develop this method further, and to explore other possibilities.

C. Sperm DNA Loss

Both the traditional method of differential extraction and the selective degradation method incur loss of some amount of sperm cell DNA during the differential extraction process.

Side by side comparison with the traditional method of differential extraction indicates no consistent difference in loss of sperm cell DNA between the traditional method and the E*rase* method. But differences in side by side comparisons of E*rase* samples processed with and without nuclease indicate that selective degradation causes a loss of sperm DNA during the nuclease step. Although selective degradation may not result in greater sperm DNA loss than the traditional method of differential extraction, improvements to the selective degradation method could result in a greater recovery of sperm DNA from forensic samples.

If the sperm DNA loss during the nuclease step of selective degradation can be eliminated then that would be another major improvement to the differential extraction process.

Comprehensive investigation into the causes of DNA loss would be beneficial. The amount of DNA evidence available from the perpetrator of the crime is often very small. Some minimum amount of DNA is necessary in order to obtain a DNA profile. Additional research may lead to improved methods for differential extraction that retain a larger portion of this critical evidence, leading to more criminal identifications and convictions.

D. Direct Amplification

Another area to be explored is direct amplification of the selective degradation sperm and epithelial cell DNA fractions. There are several advantages that would be realized from direct amplification. The cost and processing time associated with DNA purification would be virtually eliminated. The loss of DNA associated with DNA purification would be eliminated. There would be less chance of sample contamination because the sample would be handled less, and direct amplification would require less analyst hands-on time to complete.

PTC is exploring a number of possible ways to accomplish this goal. Ongoing research relating to direct amplification of differential extracts from selective degradation is needed.

V. References

[1] Garvin AM, Bottinelli M, Gola M, Conti A, Soldati G. Preparation from Sexual Assault Cases by Selective Degradation of Contaminating DNA from the Victim. Journal of Forensic Sciences 2009, 54 (6) 1297-1303.

[2] Gill P, Jeffreys AJ, Werrett DJ: Forensic Application of DNA Fingerprints. Nature 1985, 318:577-579.

[3] Wiegand P, Schurenkamp M, Schutte U. DNA extraction from mixtures of body fluid using mild preferential lysis. Int J Legal Med 1992;104:359-60.

[4] Dekairelle AF, Hoste B. Application of a Y-STR-pentaplex PCR (DYS19, DYS389I and II, DYS390 and DYS393) to sexual assault cases. Forensic Science International 2001;118:122-5.

[5] Delfin FC, Madrid BJ, Tan MP, De Ungria MC. Y-STR analysis for detection and objective confirmation of child sexual abuse. Int J Legal Med 2005;119:158-63.

[6] Hall A, Ballantyne J. Novel Y-STR Typing Strategies Reveal the Genetic Profile of the Semen Donor in Extended Interval Postcoital Cervicovaginal Samples. Forensic Science International 2003;136:58-72.

[7] Schoell WMJ, Klintschar M, Mirhashemi R, Pertl B. Separation of sperm and vaginal cells with flow cytometry for DNA typing after sexual assault. Obstetrics and Gynecology. 1999;94:623–627.

[8] Eisenberg A. Development of a spermatozoa capture system for the differential extraction of sexual assault evidence. Paper presented at: Cambridge Healthtech Institute Conference: Profiling PCR and Beyond; Washington, DC. June 27-28 2002.

[9] Chen J, Kobilinsky L, Wolosin D, Shaler R, Baum H: A physical method for separating spermatozoa from epithelial cells in sexual assault evidence. Journal of Forensic Sciences 1998, 43:114-118.

[10] Garvin AM: Filtration based DNA preparation for sexual assault cases. Journal of Forensic Sciences 2003, 48:1084-1087.

[11] Murray C, McAlister C, Elliott K: Identification and isolation of male cells using fluorescence in situ hybridisation and laser micro-dissection, for use in the investigation of sexual assault. Forensic Science International-Genetics 2007, 1:247-252.

[12] Sanders CT, Sanchez N, Ballantyne J, Peterson DA: Laser micro-dissection separation of pure spermatozoa from epithelial cells for short tandem repeat analysis. Journal of Forensic Sciences 2006, 51:748-757.

[13] Olson R and Cowan C: Automating the Differex™ System. Profiles in DNA 2007, 19-20

[14] Allery JP, Telmon, N, Mieussett R, Blanc A, Rouge D. Cytological Detection of
Spermatozoa: Comparison of Three Staining Methods. J. Forensic Sci. 2001;46(2):349-351.

[15] Hammadeh ME, Greiner S, Rosenbaum P, Schmidt W. Comparison Between Human Sperm Preservation Medium and Test-Yolk Buffer on Protecting Chromatin and Morphology Integrity of Human Spermatozoa in Fertile and Subfertile Men After Freeze-Thawing Procedure. Journal of Andrology 2001, 22 (6) 1012-1018.

[16] Wiame I, Remy S, Swennen R, Sági L. Irreversible Heat Inactivation of DN*ase* I without RNA Degradation. Biotechniques. 2000 Aug;29(2):252-4, 256.

[17] Arnaldo Soares-Vieira J, Correia Billerbeck AE, Sadayo E, Iwamura M, Andrade Zampieri R, Figaro Gattas GJ, Romero Munoz D, Hallak J, Bilharinho Mendonca B, Marmo Lucon A. Y-STRs in Forensic Medicine: DNA Analysis in Semen Samples of Azoospermic Individuals. Journal of Forensic Science, 2007 52 (3) 664-670.

VI. Dissemination of Research Findings

This research has resulted in the refinement and validation of a more effective method for performing differential extraction of sexual assault evidence. PTC and the co-investigators under this grant have been very active in publicizing the resulting method, along with examples and explanations of the improved results from this method. Some of the publicity was structured into the grant, and much of it was provided voluntarily.

Seminars

The selective degradation method of differential extraction was presented at the following seminars, either as a presentation to attendees, a poster or a vendor booth, or some combination of the three. Vendor booths made literature and additional information about selective degradation available to all attendees, in a setting that allowed all interested parties to ask questions and be given a full explanation, as well as to request a validation kit. For those unfamiliar with it, the Mid-America Forensic DNA Conference is an annual conference sponsored by PTC. It is attended by approximately one hundred DNA Criminalists each year, primarily from the Midwest.

1. 8th Annual Mid-America Forensic DNA Conference
   April, 2010 in Columbia, Missouri
   A. Presentation by Dr. Alex Garvin (co-PI)
      "Purification of Sperm DNA from Vaginal Swabs using DN*ase*"
   B. Vendor Booth by PTC

2. National Institute of Justice (NIJ) Conference 2010
   June, 2010 in Arlington Virginia
   A. Poster Presentation by Dr. Christian Carson (PI)
      "Purification of Sperm DNA from Vaginal Swabs using the Erase Sperm Isolation Kit"

3. 21st International Symposium on Human Identification by Promega
   October, 2010 in San Antonio, Texas
   A. Poster presentation by Dr. Alex Garvin (co-PI) and Kim Gorman
      "Purification of Sperm DNA from Vaginal Swabs using the Erase Sperm Isolation Kit"
   B. Vendor Booth by PTC

4. 9th Annual Mid-America Forensic DNA Conference
   April, 2011 in Columbia, Missouri
   A. Presentation by Kim Gorman, PTC
      "Erase: Better Differential Extractions"
   B. Validation Presentation by Kathy Press, Arizona Department of Public Service, Central Regional Crime Laboratory, Phoenix
      "Laboratory Validation of Erase for Differential Extractions"

C. Vendor Booth by PTC

5. National Institute of Justice (NIJ) Conference 2011
June, 2011 in Arlington Virginia
A. Poster Presentation by Kim Gorman, PTC
"Purification of Sperm DNA from Vaginal Swabs using the Erase Sperm Isolation Kit"

6. Green Mountain DNA Conference 2011
July, 2011 in Burlington, Vermont
A. Presentation by Michelle Beckwith, PTC
"Erase: Better Differential Extractions"

7. Midwestern Association of Forensic Scientists (MAFS), 2011 Meeting
September, 2011 in Lombard (Chicago), Illinois
A. Vendor Booth by PTC
(an Erase poster was also displayed, but not on the program)

8. American Society of Crime Laboratory Directors (ASCLD), 2011 Sympos.
September, 2011 in Denver, Colorado
A. Poster presentation by Michelle Beckwith
"Using Selective Degradation to Improve Differential Extraction Quality and Throughput"

9. 22nd International Symposium on Human Identification by Promega
October, 2011 in National Harbor, Maryland
A. Poster presentation by Kim Gorman
"Using Selective Degradation to Improve Differential Extraction Quality and Throughput"
B. Vendor Booth by PTC

10. California Association of Criminalists, Fall 2011 Meeting
October, 2011 in Sacramento, California
A. Presentation by Michelle Beckwith, PTC
"Using Selective Degradation to Improve Differential Extraction Quality and Throughput"
B. Vendor booth by PTC

11. Promega Users Working Group
October, 2011 in Vancouver, Canada
A. Presentation by Kim Gorman, PTC
"Erase: Better Differential Extractions"

12. Promega Users Working Group
November, 2011 in Ottawa, Canada
A. Presentation by Michelle Beckwith, PTC
"Erase: Better Differential Extractions"

13. 10th Annual Mid-America Forensic DNA Conference
    April, 2012 in Columbia, Missouri
    A. Presentation by Ruth Montgomery, Missouri State Highway Patrol, & Kyra Lienhop, St. Louis County Police Department
    "Erase: Validation and Casework"
    B. Vendor Booth by PTC

14. Louisiana Association of Forensic Scientists (LAFS)
    April, 2012 in Baton Rouge, Louisiana
    A. Presentation by Winnie Kurowski, Arcadiana Crime Laboratory, and Kim Gorman, PTC
    "Automation of Erase for Sexual Assault Cases"
    B. Poster
    C. Vendor Booth by PTC

Beta Testing by Crime Laboratories and Marshall University

PTC has provided Erase kits with tubes and reagents, as well as the complete protocol for this selective degradation method of differential extraction, to many crime laboratories, and to Marshall University. All of those institutions expressed interest in beta testing and ultimately using this method. Certain results from beta testing crime laboratories were reported above. A number of those laboratories are ready to go on-line with this method as their permanent method of differential extraction as soon as the developmental validation study is published.

Through the combination of publications, seminar presentations, posters and vendor booths, and bringing kits and instructions and support to crime laboratories and academic institutions in the United States and Europe, we have made a substantial start in introducing the results of this research to the entire forensic community. The hope is that this will accelerate the adoption of this method of differential extraction, and the value generated by this research, for the benefit of the entire law enforcement community and all Americans.

Thank you very much for the opportunity to perform this research and to present this Final Technical Report.