Final Technical Report for

"Sperm Capture Using Aptamer Based Technology"

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SomaLogic, Inc., Boulder, Colorado and Denver Police Crime Laboratory, Denver, Colorado

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Abstract

The Denver Police Department Crime Laboratory has continued the collaboration with SomaLogic, Inc. (Boulder, Colorado) to identify and develop a DNA aptamer-based system for sperm binding, isolation and purification for DNA analysis in sexual assault cases. Over the last several years, a class of DNA aptamer molecules called SOMAmers (**S**low **O**ff-rate **M**odified **A**ptamers) have been identified which bind specifically to sperm heads and tails. Several SOMAmer candidates have been further selected with the ability to bind sperm under very stringent and selective conditions. Conditions supporting both efficient sperm cell elution from cotton swabs and capture using SOMAmer-coated magnetic beads were identified by screening numerous combinations of detergents, salts and buffer additives. When tethered to magnetic beads using Biotin and Streptavidin linkers, selected SOMAmers demonstrated efficient affinity-based purification of sperm cells from eluates of mock forensic samples containing a mixture of HeLa cells and sperm cells or female buccal cells and sperm cells. This is the first aptamer based system used in whole cell sperm capture and adapted for use with forensic casework samples. Subsequent Short Tandem Repeat (STR) DNA analysis showed clean male DNA profiles from sperm cells eluted from cotton swabs, which are the preferred method used for rape kit evidence collection. SOMAmer-based sperm cell separation is comparable to existing differential extraction based methods, but more rapid sample processing would allow for sperm purification and DNA extraction to be completed in significantly less time. Further research is ongoing to optimize these SOMAmers under various stringent and forensically relevant conditions, with the goal of developing an inexpensive and rapid system for sperm cell capture adapted for automation using robotic workstations commonly found in forensic laboratories. As work continues on this project, a full forensic validation will be conducted that addresses reproducibility, sensitivity and reliability with both mock and adjudicated forensic case samples. This system will enable very high throughput systems for sperm capture and lysis based on magnetic beads, 96 well plastic plates, or other isolation techniques and is an improved method of forensic analysis for sexual assault evidence that will help to reduce the backlog of critical casework.

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Executive Summary

Evidence from sexual assault cases routinely consists of swabs collected during victim examinations at the hospital and from crime scenes. This evidence is commonly comprised of mixtures of body fluids from both a male perpetrator and female victim, whereby the separation of sperm containing the perpetrator's DNA from the victim's DNA is crucial in the analysis of evidence from sexual assault cases. Improved automation of sperm isolation and separation will enable more sexual assault cases to be processed in a timely manner and will populate the CODIS database with more DNA profiles from sexual assault perpetrators.

The goal of this research project was to develop a DNA aptamer-based system to specifically isolate and purify sperm cells for forensic applications, in partnership with SomaLogic, Inc. For this grant period, our work focused both on the development of the system and optimization of the conditions for effective separation of sperm cells from female epithelial cells in mixed sexual assault evidence. This system will allow for the amplification of male DNA from the perpetrator and subsequent STR DNA analysis in a rapid, cost effective, and high throughput format, amenable to robotic workstations that are becoming more common in forensic laboratories. Aptamers are short DNA molecules that have the molecular specificity and affinity recognition properties of antibodies, but are more stable than antibodies for uses involving "affinity" chromatography since their complex, three-dimensional structures are capable of reforming after denaturation. Aptamers are reusable molecules that can routinely be chemically modified to increase their stability. They are used as diagnostic reagents in biosensors for the detection of environmental contaminants, for monitoring carcinogen or drug levels in the blood of patients and can be used as therapeutic agents for the treatment of disease. Aptamer targets may include intracellular proteins (such as transcription factors), extracellular proteins (such as growth factors or coagulation factors) or more complex targets such as viruses or bacteria.

The specific objective for this phase of the project was to further characterize lead SOMAmer candidates that bind sperm with the highest affinity and optimize the conditions for efficient immobilization and purification of sperm cells from mock forensic samples. The long term vision for this research is the development of a cost-effective aptamer-based assay suitable for high throughput analysis that can easily be implemented in forensic laboratories. A system using SOMAmer-coated magnetic beads has been evaluated for sperm binding and separation, which will likely be the platform for development of a sperm

capture procedure to isolate DNA for subsequent STR analysis. Later stages of this research are focused on development of the sperm-capture assay as a commercial product at SomaLogic.

Results, Findings and Conclusions:

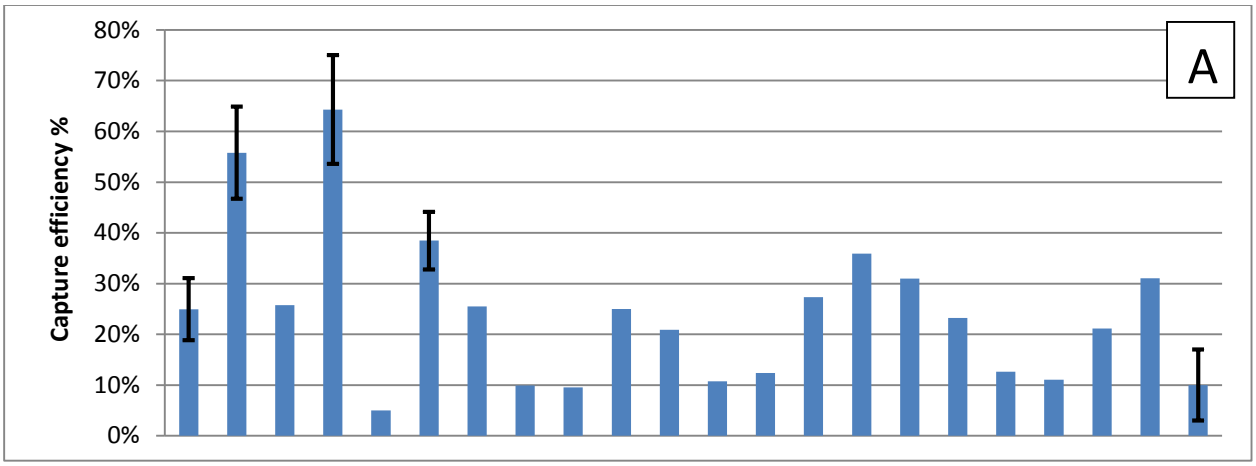
SOMAmer localization on sperm cells: SOMAmers were characterized for their binding and localization on sperm cells. We targeted the highly conserved and stable proteins that remain after elution from swabs, and we chose to purify those SOMAmers specific to sperm that had been stripped of plasma membranes with detergent. Magnetic beads coated with different SOMAmers localize on different cell surface epitopes and for development of the sperm purification assay, we chose three SOMAmers which bind both sperm heads and tails. These selected SOMAmer clones were tested in a sperm cell pull down assay in solution to identify SOMAmers suitable for sperm isolation from mixed swabs. The results of the pull down assay demonstrate that several SOMAmers support nearly 100% capture efficiency when appended to magnetic beads

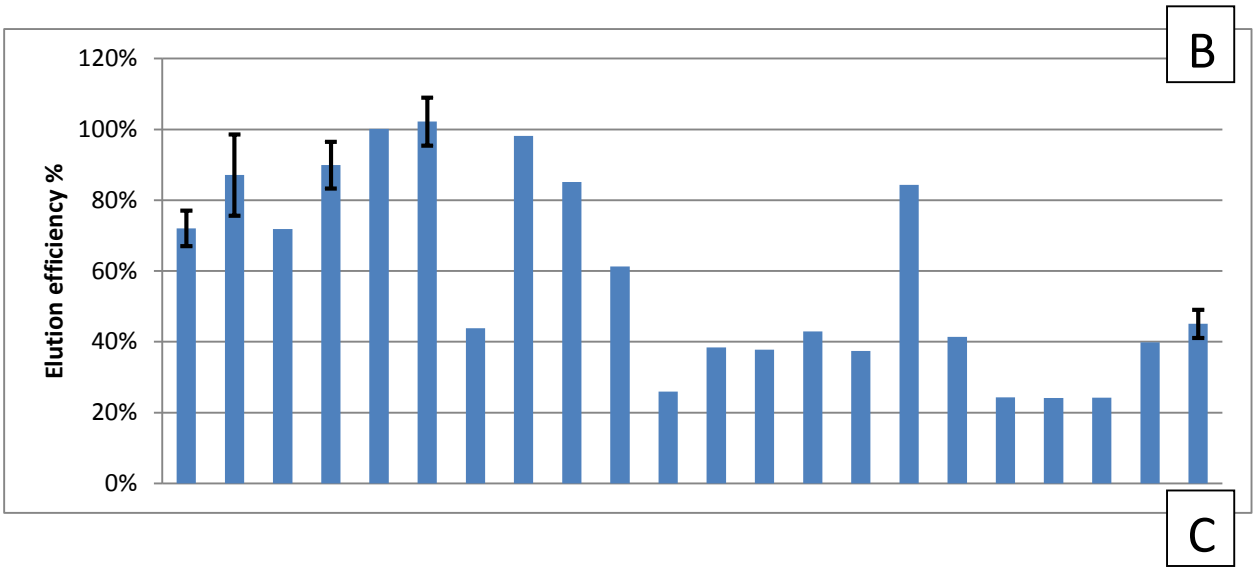
Sperm elution and capture from cotton swabs: Elution of cellular material from forensic evidence swabs is typically performed using a 1-2% solution of sodium dodecyl sulfate (SDS), which is an efficient medium for cell recovery. However, because we anticipated that SDS would be incompatible with SOMAmer binding, selection buffer containing the non-ionic detergent Triton X-100 was intended to support efficient SOMAmer binding as well as efficient elution from cotton swabs. We compared the efficiency of sperm elution from cotton swabs to which semen had been adsorbed using selection buffer at various concentrations versus a solution containing 1% SDS (to mimic elution buffer commonly used in differential extractions). Here, we found that cellular recovery with selection buffer containing Triton X-100 compared favorably to SDS buffer, with recoveries for selection buffer ranging from 44% to 78% of that seen with SDS buffer.

Swab elution condition screening: We evaluated ~500 conditions with different detergents, salt concentrations, pH's and various additives to identify the optimal parameters for both elution and subsequent sperm capture by SOMAmers. Sperm cell elution and capture was performed using magnetic beads coated with a mixture of three lead SOMAmers and the overall efficiency was assessed by DNA quantitation from lysed sperm cells using the Picogreen assay. While the majority of tested conditions did not support effective sperm elution or capture by SOMAmer-coated magnetic

beads, several solution conditions identified supported efficient sperm elution and capture, all of which contained anionic detergents, such as Triton X-200, sodium deoxycholate or lithium dodecyl sulfate (LDS).

A detailed analysis of sperm cell elution from cotton swabs in buffers containing various detergents is presented in Figure 1. Testing was performed in a standard buffer supplemented with various detergents and sperm capture by SOMAmer-coated magnetic beads was evaluated for each distinct elution buffer. For a majority of the buffer conditions, less than 30% of the total sperm cells were captured (Figure 1B), whereas sperm were effectively isolated in buffers containing 0.1% sodium deoxycholate and 1% Triton X-200 detergents. Although somewhat lower, reasonable sperm capture efficiency was achieved in buffer containing 1% lithium dodecyl sulfate. This was surprising, given that these are all relatively harsh anionic detergents that would not be expected to support specific SOMAmer binding to native epitopes. Figure 1C shows the efficiency of SOMAmer-specific sperm recovery from cotton swabs. Efficient overall sperm cell recoveries were achieved with buffers containing either Triton X-200 or sodium deoxycholate detergents.





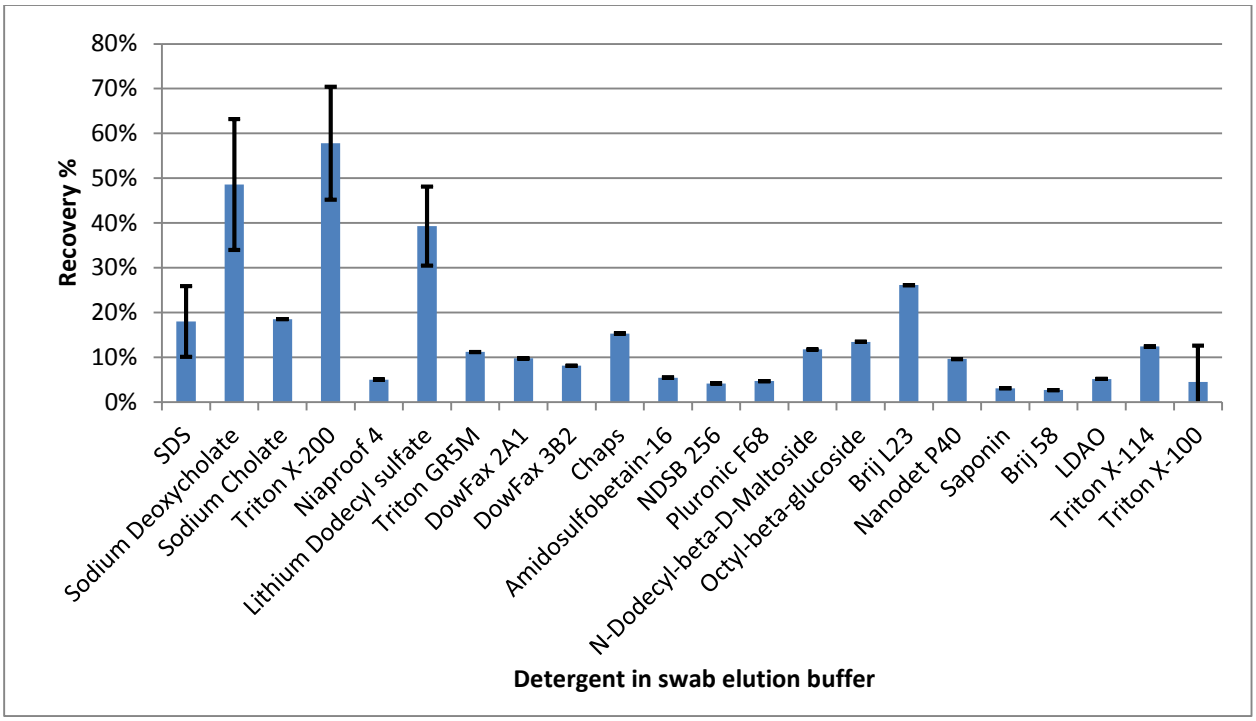


Figure 1. *Elution, capture, and overall recovery efficiency of sperm cells from cotton swabs in buffers containing different detergents. All detergents were at 1% w/v in 40 mM Hepes pH 7.5, 350 mM NaCl and 0.5 mM EDTA, with the exception of Sodium Deoxycholate and Triton X-100, which were at 0.1%, and octyl-beta-glucoside, which was at 1 mM concentration.*

Sperm purification from samples eluted from mock forensic swabs: We evaluated the purification of sperm cells from mock forensic samples by preparing cotton swabs with a mixture of HeLa cells and semen. For initial tests, HeLa cells derived from human cervical cancer cells were chosen as a surrogate for human vaginal epithelial cells. These experiments were aimed at identifying the optimal buffer and detergent conditions for sperm cell isolation from samples containing a mixture of sperm and epithelial cells. Swabs bearing a 3-fold excess of HeLa cells over sperm cells were eluted in buffer containing either Triton X-200, deoxycholate or lithium dodecyl sulfate. Sperm purification was most efficient in buffer containing Triton X-200, whereas sodium deoxycholate and LDS based buffers were less effective, with apparent carryover of HeLa cell DNA. Overall, sperm capture in solutions containing Triton X-200 were also considerably more efficient than in other detergents, suggesting that this detergent is the most resistant to background interference from eluted HeLa cells or their debris. We also investigated how excess HeLa cells can impact sperm cell purification and determined that a 10-fold HeLa cell excess was tolerated by the assay.

SOMAmer-coated magnetic beads were used to isolate sperm cells eluted from mock forensic swabs consisting of female buccal swabs prepared with semen. The resulting STR profiles illustrate that clean sperm DNA fractions were isolated from mock forensic swabs using SOMAmer-coated magnetic beads (Figure 2). Minimal carry over from the epithelial cell fractions were observed in the sperm fractions (see Figure 2, panels A and C) allowing for straightforward determination of the major source male profiles without contaminating alleles from the female. Similarily, the epithelial cell fractions presented in Figure 2, panels B and D show nearly non-detectable levels of male alleles, demonstrating that the sperm cell DNA component has been almost entirely separated from the epithelial cell DNA fraction.

C

D

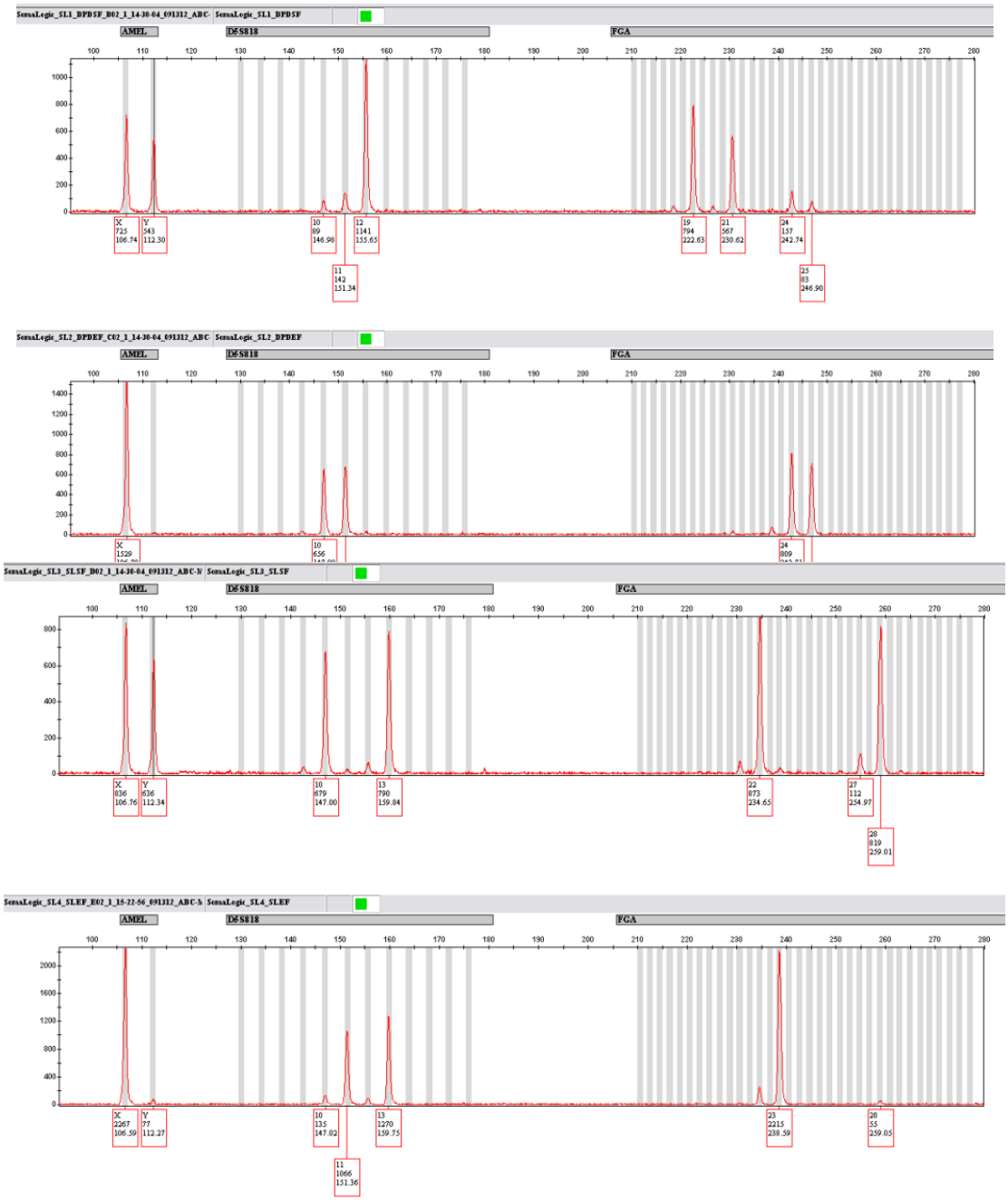


Figure 2. *STR profiles (showing three genetic loci – Amelogenin, D5S818 and FGA) from mock forensic samples treated with SOMAmer coated magnetic beads. Sperm fraction profiles are shown in panels A and C while panels B and D show the respective epithelial cell fractions from two separate sets of mock forensic swabs.*

However, while the separation achieved using SOMAmer coated magnetic beads is effective, we have not yet achieved complete separation of male DNA from female DNA that is observed with either a traditional DE or with Differex. By comparing the DNA profiles generated from the identical samples purified using

the Differex system, we see no carry-over of female alleles in the sperm DNA profile (compare Figure 3 to Figure 2, panel A). With further optimization, we hope to achieve the same level of sperm cell separation from female epithelial cells that will result in male DNA profiles lacking any female carry-over. Current efforts are focused on optimizing the immobilization of SOMAmer molecules on magnetic beads to eliminate the protein (biotin and streptavidin) components thought to bind non-specifically to the female epithelial cells during separation. This, along with further optimization of the buffer conditions and possible additives and/or inhibitors, will help to minimize the amount of carry-over female DNA and will facilitate the generation of a clean male DNA profile.

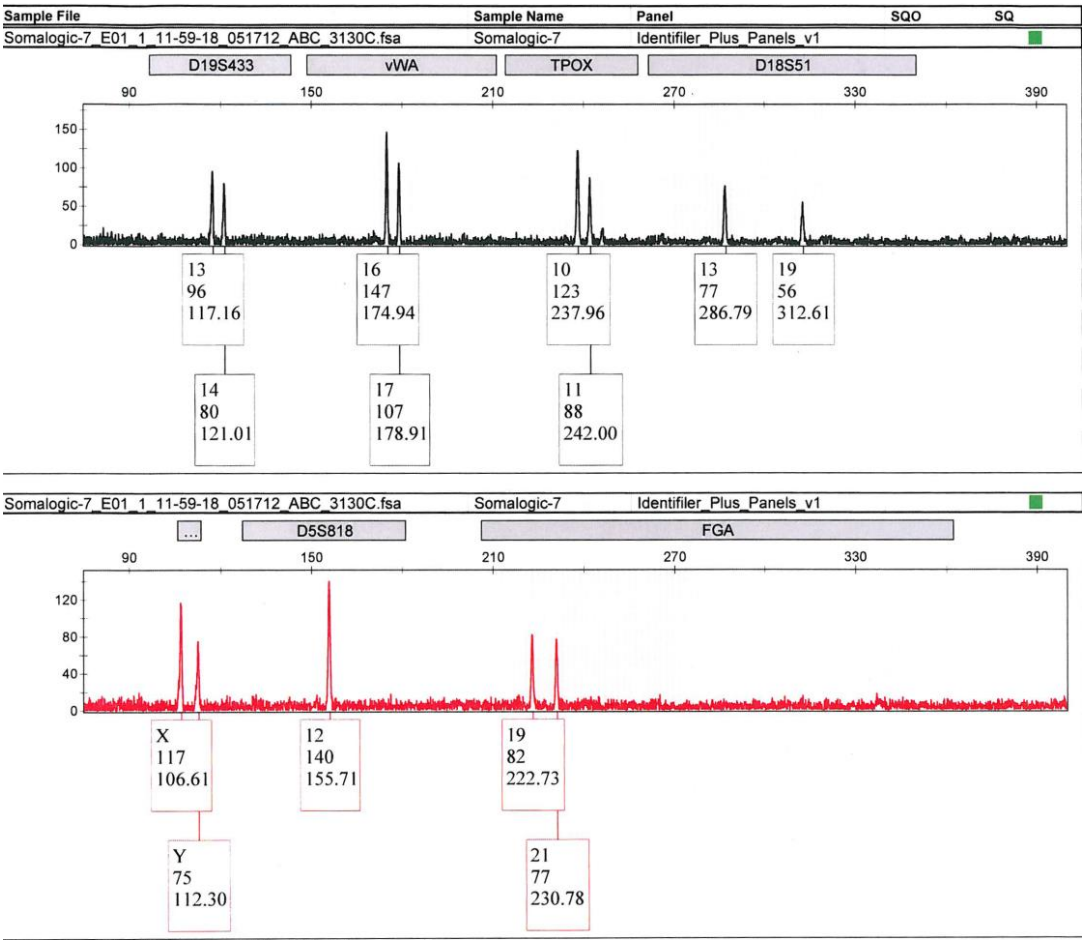


Figure 3. *Sperm fraction STR profile (showing 7 genetic loci) from mock forensic sample purified using Differex. Profile is from identical sample as that shown in Figure 2A (sperm fraction) and 2B (epithelial fraction)*

Further Research Beyond the Scope of this Project and Future Development:

Several SOMAmer candidates have been identified that preferentially bind sperm cells with high affinity. By linking the synthesized SOMAmer molecules to magnetic beads, we have established the matrix that will likely form the basis for development of a commercially available sperm-capture assay. Magnetic bead-bound SOMAmers can be adapted to one of several high-throughput formats, including coating the surface of a tube, a 96-well plate, slide, column, or other suitable matrix. Further experiments will concentrate on testing the SOMAmer-coated magnetic beads with mock forensic samples containing low levels of sperm cells to more closely mimic the types of samples routinely seen in casework. This will challenge the SOMAmers for their ability to bind to small numbers of sperm in mixtures with a vast excess of female epithelial cells in order to generate DNA profiles that are devoid of any female carry-over into the male DNA profile.

In the course of this project, we have developed a sperm capture assay that is amenable to automation using robotic workstations that are becoming more commonly used in forensic casework. Automation can further decrease the amount of time required to process sexual assault cases and will help decrease the backlog of casework in forensic laboratories. During development of these protocols, the suitability for high throughput processing was evaluated as well as the potential for automation of this sperm-capture system. The sensitivity of this sperm isolation technology could allow for an increase of the post-coital interval in which sperm can be detected beyond the traditional 24-72 hour time interval. Additionally, the coating of a potential matrix could be manipulated to enable the binding of sperm cells at a specific density. This would allow for a constant amount of DNA being isolated in the sperm-capture assay and would potentially make DNA quantitation steps obsolete, thereby further increasing the processing speed of sexual assault evidence. This would improve capacities for other casework that does not involve mixed evidence samples and will also allow laboratories to revisit cold sexual assault cases in an attempt to identify perpetrators.

Implications for Policy and Practice:

The potential impact of this proposed project could be very significant for the forensic community in terms of sexual assault case backlog reduction. Due in part to the large backlog of rape cases in the United States, DNA laboratories would greatly benefit from a cost effective assay that is fast, easy, and reliable for processing sexual assault evidence. Effectively, this will reduce the time required to obtain a DNA

profile from a putative perpetrator and will potentially result in both faster identification and prosecution of suspects, possibly preventing additional crimes. Furthermore, more DNA profiles will be generated that are suitable for entry in the CODIS database, which in turn can help to link and potentially solve further crimes. In cases without a suspect and no immediate identification of the putative perpetrator, a DNA profile can also lead to a John Doe warrant which halts the Statute of Limitations.

Final Technical Report: Main Body

I. Introduction

A. Statement of the Problem

There is a critical need for improved forensic methods to recover purified sperm DNA from samples in sexual assault cases. A key type of evidence in these types of cases is a DNA profile originating from cells left by a perpetrator. This evidence is generally obtained from swabs collected in the course of the forensic examination of victims and from crime scenes. These swabs are typically mixtures of epithelial cells from the victim as well as varying amounts of sperm cells from the perpetrator. The vast excess of released DNA from the victim's epithelial cells can interfere with generation of a clean and unambiguous DNA profile from the perpetrator, thereby requiring a purification step to separate contaminating epithelia from sperm cells. Improved methods of automation to detect and separate sperm cells from female epithelial cells will enable crime laboratories to process more sexual assault cases in a timely manner and populate the CODIS database with more evidentiary samples from sexual assaults.

B. Literature Citations and Review

At present, the most common protocol for sperm separation from epithelial cells is the differential extraction (DE) procedure, which relies on differential lysis of sperm and epithelial cells in sodium dodecyl sulfate (SDS) and proteinase K [1, 2]. This treatment results in preferential lysis of epithelial cells, while sperm cells remain largely intact. Following a series of centrifugation and wash steps, sperm cell DNA is then released by lysis using proteinase K in the presence of DTT, which reduces and dissociates the highly disulfide-bonded proteins comprising the sperm nucleus. However, despite the relative simplicity of the differential extraction process, it is time consuming, labor intensive, and can result in possible DNA mixtures when sperm cell counts are low.

A variety of methods described in the literature have attempted to improve or modify the DE procedure to achieve more rapid, more efficient, and/or cleaner separation of sperm cells from victim epithelial cells. For example, cellulose-digesting enzymes have been used to improve the release of sperm cells from cotton swabs [3], track-etch filters with micrometer size holes have been developed for differential filtering of sperm cells away from the lysed female epithelial cell debris and DNA [4], and controlled

digestion of contaminating female DNA using deoxyribonuclease (DNAse I) has also been described [5]. This method relies on the disruption of the disulfide-bonded protamines protecting the sperm nucleus which is shielded by the perinuclear theca or calyx [6]. In addition, microfluidic devices have been created which exploit the differential physical properties of sperm cells versus epithelial cells and allow for direct silica-based DNA extraction [7, 8]. Several publications describe the use of laser microdissection to selectively capture and isolate sperm cells [9-11]. These methods use an optical microscope fitted with a laser to capture sperm cells from cell smears. Although this method is highly specific and requires only limited numbers of spermatozoa on microscope slides for DNA extraction and subsequent STR analysis, it is expensive, time-consuming, labor-intensive and not easily amenable to automation.

Affinity purification of sperm cells is in principle one of the simplest and most accessible methods for processing forensic samples and has been tested using antibodies to various sperm cell surface antigens [12]. However, this approach has suffered from low sperm recoveries, as captured sperm cells tend to be lost in wash steps [12]. While photocrosslinking of antibody and antigen has been utilized to improve complex retention, this approach has not gained popularity in actual forensic applications due to its complexity.

C. Rationale for the Research

Aims for this grant period were directed at the development of a practical prototype system for the isolation of sperm cells from forensic samples. The goals were:

1. Identify SOMAmer(s) suitable for isolation of sperm cells from forensic samples.
2. Develop an overall scheme (including identification of a suitable matrix and SOMAmer immobilization scheme) that supports isolation of sperm cells from forensic samples.
3. Develop solutions and protocols that support SOMAmer-dependent isolation of sperm from forensic samples bearing significant burdens of contaminating epithelial cells.
4. Render the protocol compatible with downstream STR DNA analysis.
5. Create a system amenable to automation and high-throughput methods.

Here we demonstrate an affinity-based method for sperm purification using synthetic, DNA-based affinity reagents called SOMAmers. SOMAmers (**S**low **O**ff-rate **M**odified **A**ptamers) are aptamer-like DNA molecules that contain chemical modifications on some DNA bases [13, 14]. We selected SOMAmers that specifically bind to sperm cells *in vitro* and these were used as the basis for an affinity capture system that

effectively separates sperm cells from mixtures with female epithelial cells and cellular debris eluted from cotton swabs.

II. Materials and Experimental Methods

Purchased materials: All chemicals were purchased from Sigma Aldrich unless noted otherwise. Purified Sperm cells (research vials prepared by density gradient centrifugation and subsequent washing) were obtained from California Cryobank. Semen samples were obtained from Lee Biosolutions. MyOne T1 magnetic streptavidin beads were purchased from Life Technologies.

SOMAmer preparation: SOMAmer selection has been described previously in the report for the preceding grant period. Selected clone sequences were synthesized using standard solid-state DNA synthesis methods. Sequences were synthesized containing a 5' biotin tag. Synthetic SOMAmer stocks were HPLC purified and the sequence identity was verified using mass spectrometry.

For assays, selection buffer consisted of 40 mM Hepes pH 7.5, 350 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, and 0.1% Triton X100 detergent. Before use, a heat-cool step was performed where the SOMAmer solution was heated to 95 °C for at least 5 minutes and allowed to cool to room temperature in order to refold SOMAmers into their active conformations. Libraries containing modified deoxyuridine base were prepared by enzymatic primer extension reactions using a biotinylated anti-sense oligonucleotide library consisting of 40 random deoxynucleotides flanked by constant regions (ggcagtccgtccgtc at the 5' end; gccagaagcagaaggacg at the 3' end, containing two biotin molecules at 3' end). Antisense libraries were immobilized on the streptavidin coated agarose beads. A 5' primer was added with a reaction mixture containing dA, dC and dG as well as modified dU deoxynucleoside triphosphates (0.8 mM each) and KOD exo$^-$ DNA polymerase (0.1 unit/µL) in appropriate buffer. The reaction mix was incubated for two hours at 68 °C. Extended DNA molecules were eluted with 20 mM NaOH and the solution was neutralized to pH 7 with HCl. The resulting SOMAmer libraries were concentrated using YM10 centrifugal ultrafiltration units, quantified, and analyzed by gel electrophoresis.

Sperm cells were prepared for selection by washing three times with selection buffer supplemented with Triton X100 detergent and NaCl (final concentrations of 1% and 600 mM, respectively) followed by suspension in selection buffer. Libraries containing approximately 1.5×10^{15} molecules in selection buffer were heated to 95 °C, slowly cooled to 37 °C and then combined with an equal volume of competitor solution composed of 2 mg/ml herring sperm DNA, 2 mg/ml yeast tRNA, 200 µM dextran sulfate, 2 µM prothrombin, 2 µM casein, and 2 mg/ml human serum albumin in selection buffer. The high-salt conditions and high competitor concentrations were necessary to limit non-specific binding of SOMAmer

library DNA to sperm cells. 10^7 HeLa cells were added and the mixtures were incubated for ten minutes, after which the cells and debris were pelleted by centrifugation. The remaining library DNA was recovered in the supernatant and sperm cells (10^7) were added to the depleted library. After incubation for one hour with library DNA, cells were washed with selection buffer 8 times. Washes were performed by centrifugation at 6000xg for 5 min to pellet the cells and removing the supernatant. Bound SOMAmers were eluted from sperm cells by addition of buffer (40 mM Hepes pH 7.5, 0.025% SDS, 5 mM EDTA, and 50 µg/ml proteinase K) followed by incubation at 55 °C for 15 minutes and removal of sperm cells by centrifugation. Eluted SOMAmers were purified by hybridization to magnetic streptavidin beads substituted with a biotinylated 3' primer. After a wash step, SOMAmer DNA was recovered by elution in 20 mM NaOH. Eluted SOMAmer solutions were neutralized and PCR-amplified using biotinylated 5' and 3' primers.

To prepare modified SOMAmer DNA pools, amplified DNA was captured on magnetic streptavidin beads and the non-template DNA strand stripped from the immobilized anti-sense DNA with 20 mM NaOH. A 5' DNA primer, KOD exo$^-$ DNA polymerase and deoxynucleoside triphosphates (dA, dC, dG and modified dU each at 0.8 mM) were added and the mixture incubated at 68 °C for 1 hour with shaking. The extended products were recovered by elution in 20 mM NaOH, neutralized using HCl, and evaluated by gel electrophoresis. Subsequent rounds of selection were performed in a similar fashion, using amplified SOMAmer pools from the previous round. In all, seven rounds of selection were performed. The progress was monitored at the end of each round by measuring the rate and extent of reassociation of the thermally denatured SOMAmer pools. These C0t curves reflect the complexity of a DNA population and thus serve as a measure of the selection progress. Rounds 3-7 included incubations for increasing periods of time in a 10 mM dextran sulfate solution in selection buffer, designed to pose a "kinetic challenge" for removing SOMAmers with high off-rates. After round 7, eluted SOMAmers were amplified, cloned and sequenced to determine the SOMAmer sequences. Individual SOMAmers were generated by primer extension using PCR amplified templates from isolated plasmid DNA, and tested for binding to both sperm cells and HeLa cells.

Binding assays with radiolabeled SOMAmers: SOMAmers were radiolabeled with ^{32}P using adenosine triphosphate and T4 polynucleotide kinase. Radiolabeled SOMAmers were separated from free label by gel filtration on size exclusion spin columns (ProbeQuant G50). Radiolabeled SOMAmer preparations (~10,000 cpm/reaction) were combined with sperm cells in selection buffer supplemented with 1 mg/ml

herring sperm DNA, 1 mg/ml tRNA, 100 µM dextran sulfate, 100 µg/ml human serum albumin, and 1 µM casein. Reactions were incubated for 1 hour at 37 °C and filtered through filter plates under vacuum. Plates were then washed with 200 µL of selection buffer, dried, and exposed to phosphor screens for several hours. Phosphor screens were imaged using a Fuji Bioanalytic scanner and images were analyzed and quantified using Image Gauge v4.0 software (Fujifilm).

SOMAmer immobilization on magnetic beads: 1 mg of magnetic beads was washed using 1 mL of selection buffer. 1 mL of 500 nM SOMAmer solution in selection buffer was mixed with the magnetic beads and was allowed to bind for 30 minutes at room temperature with constant mixing. The beads were then washed three times with 1 mL of selection buffer before final resuspension in 1 mL of selection buffer. SOMAmer-coated magnetic beads stocks were stored at 4 °C until use.

SOMAmer binding to sperm cells: SOMAmer binding localization on sperm cells was observed by optical microscopy. SOMAmer-coated magnetic beads (1 µg) were mixed with purified sperm cells (10^4 cells) in 25 µL of selection buffer. Beads were allowed to bind to cells for 15 minutes at room temperature before being partitioned on magnets and washed once with 100 µL selection buffer. The bead pellet containing captured cells was resuspended in 20 µL of selection buffer and 10 µL of the resuspended bead/cell mixture was placed in a cell counter chamber and imaged using a Nikon Eclipse Ti-S microscope. Imaging was conducted using the bright field mode with a 20X Plan Fluor microscope objective and images were captured using a Nikon DS-Fi1 digital camera.

Sperm capture assays: Sperm capture assays were performed using SOMAmers synthesized with a biotin moiety attached to the 5' end. Biotinylated SOMAmers were immobilized on MyOne T1 magnetic streptavidin-coated beads via standard methods and washed with assay buffer (40 mM Hepes pH 7.5, 350 mM NaCl, 1 mM EDTA, 0.1% Triton X-100) several times prior to assay. For pull-down assays, purified sperm cells were suspended in assay buffer supplemented with 50 µM dextran sulfate, which served as an inhibitor of non-specific binding. SOMAmer-coated MyOne T1 beads were added to the solution (typically 10-20 µg of beads) and the solution incubated with mixing for 30 minutes to allow bead binding to cells. After incubation, SOMAmer-substituted magnetic beads were washed with assay buffer twice. Captured cells were lysed by addition of a buffer consisting of 100 µg/ml of proteinase K and 40 mM DTT in assay buffer, followed by incubation at 55 °C for 1 hour. DNA yield in the cell lysate was quantified using the Picogreen assay (Life Technologies) according to the manufacturer's protocol.

Elution condition screening: A large number of buffer conditions (~500), including different detergents, salt concentrations, pH's and various buffer additives were evaluated to determine the optimal conditions for effective sperm elution from cotton swabs and subsequent capture using SOMAmer-coated magnetic beads. Sperm cell elution and capture was assessed by DNA quantification from lysed sperm cells using fluorescence-based methods (Picogreen, Life Technologies). Standard curves were prepared using diluted human genomic DNA. Sperm capture was performed using magnetic beads coated with a mixture of three lead SOMAmers (sequence IDs 4105-10, 4105-257 and 4105-365). Cotton swabs were prepared with 10 µL of human semen and elution was performed under various conditions in a SlicPrep plate (Promega). A portion of the swab eluate was removed and the sperm DNA isolated using the DNA IQ kit (Promega). Recovered DNA was quantified using Picogreen assay. 100 µL of the swab eluate was transferred from the SlicPrep plate into a separate 96-well plate and 20 µg of SOMAmer-coated magnetic beads were added to each well. The plate was then shaken at room temperature for 30 minutes to allow for binding between sperm cells and the magnetic beads. SOMAmer-coated magnetic beads were washed once with buffer, the captured cells were lysed and DNA yield in the lysate was quantified by Picogreen assay. Sperm elution efficiency was defined as the ratio of DNA eluted from the swab to the amount DNA in the 10 µL semen sample. Capture efficiency was defined as the ratio of DNA in the captured cell eluate to the amount of DNA present in the swab eluate.

Mock forensic swabs: To demonstrate the ability to purify sperm cells from mixed samples, buccal swabs collected from female volunteers were prepared with a small amount of semen (5 µL or less). Swabs were prepared at Denver Police Department Crime Laboratory using typical cotton swabs used for rape kit evidence collection. Swabs were allowed to dry at room temperature for several hours and then stored at -20 °C until use.

Mock forensic swab analysis: Prior to assay, swabs were kept at room temperature for about 5-10 minutes. Elution of cellular material from the swabs was performed in 1 mL of elution buffer (1% Triton X-200, 40 mM Tris-HCl pH 9.0, 350 mM NaCl, 1 mM EDTA, 0.5% BSA) in 1.7 mL Eppendorf tubes by incubating the swabs for ~10 minutes with occasional swirling. The solution was recovered by pressing the swab on the side of the tube. To isolate sperm cells, mixed SOMAmer-coated magnetic beads were added to the eluted samples and incubated for 20 minutes with continuous mixing. Magnetic beads were then washed three times with wash buffer (40 mM Hepes pH 7.5, 350 mM NaCl, 1 mM EDTA, 0.02% Triton X-100). Following the wash steps, beads were resuspended in 0.5 mL of epithelial cell lysis solution (40

mM Hepes pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.02% Triton X-100, 200 µg/ml proteinase K), incubated at 55 °C for 30 minutes and washed with wash solution three times. Sperm lysis solution (40 mM Hepes pH 7.5, 350 mM NaCl, 1 mM EDTA, 0.02% Triton X-100, 200 µg/ml proteinase K, 60 mM DTT) was then added and the captured sperm cells were lysed at 55 °C for at least 30 minutes with shaking. Sperm and epithelial cell DNA from crude lysates were further purified using DNA IQ kit (Promega) according to the manufacturer's instructions. DNA was quantified using either Picogreen or Quantifiler Human DNA quantification kit (Life Technologies). STR profiling was conducted using Identifiler Plus Short Tandem Repeat PCR kit (Life Technologies).

Sperm DNA purification using Differex: For comparison purposes, sperm DNA was purified from mock forensic swabs using Differex (Promega). Sperm cell purification and DNA extraction were performed according to the manufacturer's protocol, with a slight modification to use of the Differex Magnet. Swabs were incubated in Digestion Solution for 2 hours at 37 °C. The saturated swab was filtered through a spin basket and the recovered eluate was then applied to the Separation Solution. Sperm cells were pelleted by centrifugation at 14000 RPM for 10 minutes in a microcentrifuge tube. 3.5 µL of DNA IQ resin was added to fully cover each sperm pellet followed by three washes with nuclease free water. The cells were again pelleted by centrifugation for 10 minutes at 14000 RPM. 7 µL of DNA IQ resin was added and cell pellets were washed again with nuclease free water. DNA was isolated using the DNA IQ kit, quantified using Quantifiler Human DNA quantification kit and STR profiling was done using Identifiler plus Short Tandem Repeat PCR kit.

III. Results

SOMAmer localization on sperm cells: SOMAmers were characterized for their binding and localization on sperm cells. We targeted the highly conserved and stable proteins that remain after elution from swabs, and we chose to purify those SOMAmers specific to sperm that had been stripped of plasma membranes with detergent. This treatment exposes the "perinuclear calyx", which is the cytoskeletal structure that remains intact after treatment with detergents. In the previous grant report, we demonstrated that SOMAmer-coated magnetic beads bind to sperm cells in solution and can be used to magnetically separate bead-cell complexes. Here, we show more detailed micrographs demonstrating that magnetic beads coated with different SOMAmers localize on different cell surface epitopes (Figure 1). Microscopic images of sperm cells with bound magnetic beads were evaluated to determine the location of SOMAmer binding. We discovered that most SOMAmers bound the sperm tails, while a few clones showed either exclusive binding to the head or binding to both head and tail. Although beyond the scope of this current report, efforts were made to identify the precise epitopes on the sperm cell surface bound by the lead SOMAmer candidates. Preliminary results from this study suggest that the SOMAmers bind to conserved structural proteins typically arranged in repeating patterns on the sperm cell surface, thereby presenting multiple epitopes for SOMAmer recognition and binding. For development of the sperm purification assay, we chose three SOMAmers which bind both sperm heads and tails. SOMAmers with a scrambled control sequence (used as a negative control) did not effectively bind to sperm cells and similar results were obtained using beads with no SOMAmers attached.

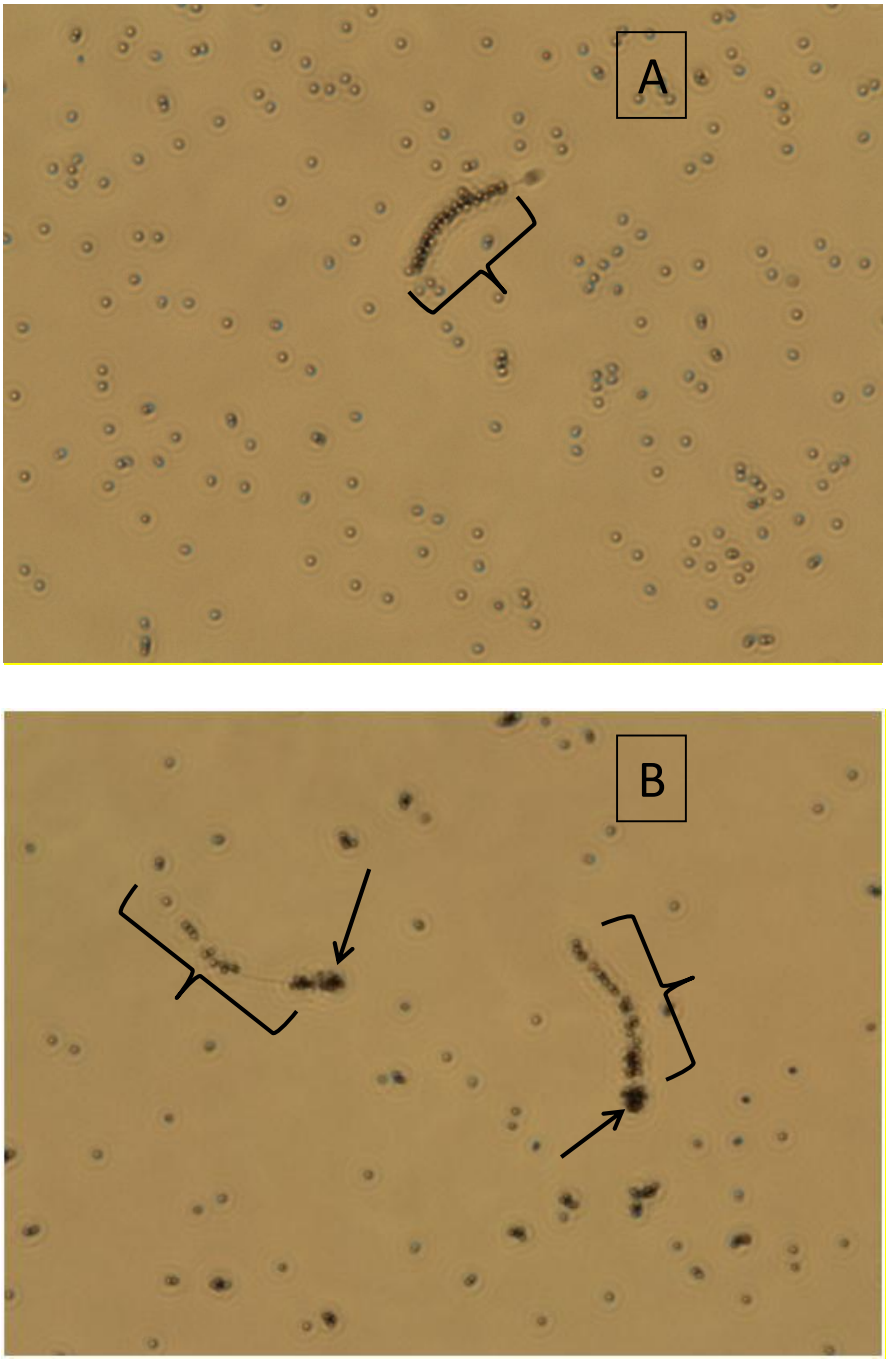


Figure 1. *Light micrographs of SOMAmer-coated magnetic beads bound to sperm cells. Preferential localization of beads coated with different SOMAmers is observed. Panel A shows magnetic beads coated with SOMAmer ID 4105-10 binding preferentially to sperm tails. Panel B shows beads coated with SOMAmers ID 4105-257 and 4105-365 binding to both tails and heads of sperm cells, respectively. Arrows identify sperm heads and tails are denoted by brackets. Sperm cells were not bound by SOMAmers with a scrambled control sequence or by beads only.*

Sperm pull down assays: Selected SOMAmer clones were tested in a sperm cell pull down assay in solution to identify SOMAmers suitable for sperm isolation from mixed swabs. The results of the pull down assay, shown in Figure 2, demonstrate that several SOMAmers support nearly 100% capture efficiency when appended to magnetic beads.

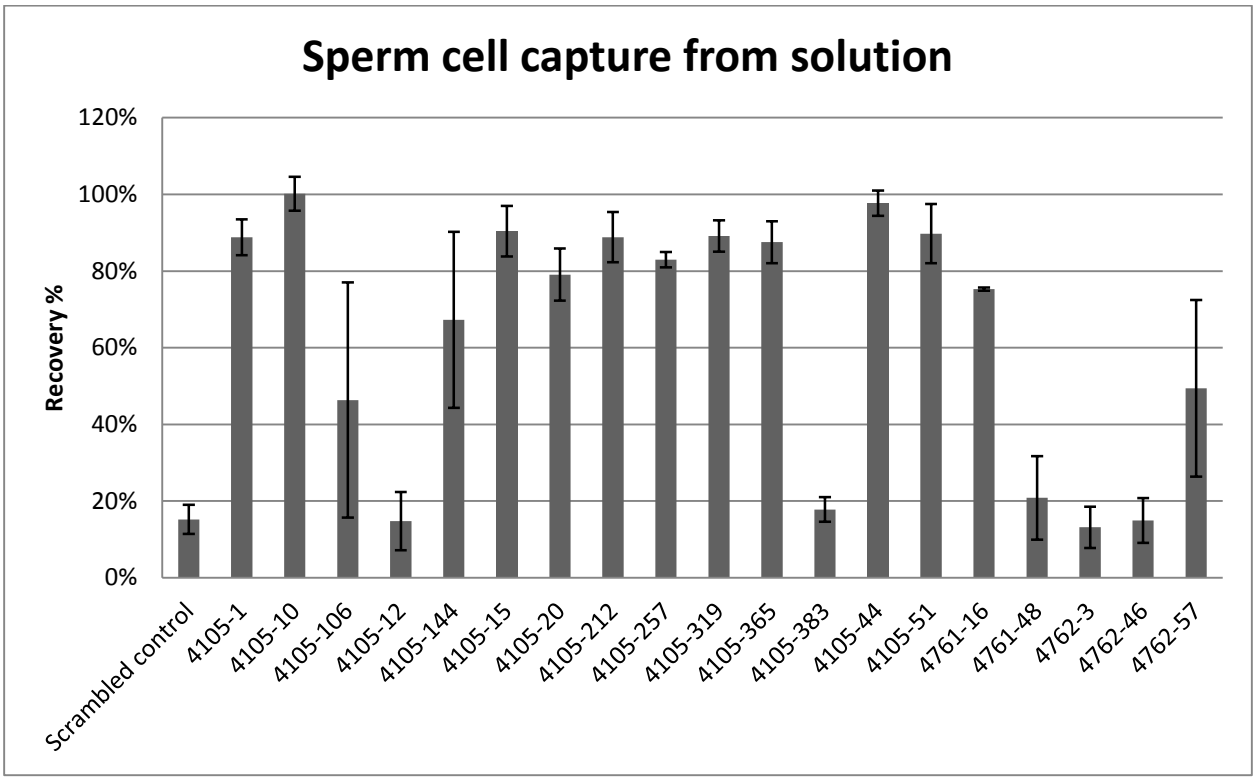


Figure 2. *Sperm pull down efficiency in solution using magnetic beads coated with different SOMAmers.*

Sperm binding assays: Several lead SOMAmer candidates were evaluated for sperm cell binding affinity using a binding assay with radiolabeled SOMAmers. Apparent binding dissociation constants were measured for three lead SOMAmers that had been incubated with serial dilutions of sperm (Figure 3A). All three lead SOMAmers all show specific binding to sperm cells when compared to a random DNA library. Furthermore, the lead SOMAmer candidates demonstrate preferential binding to sperm cells as none of the lead candidates bind HeLa cells with any measurable affinity in this assay (Figure 3B).

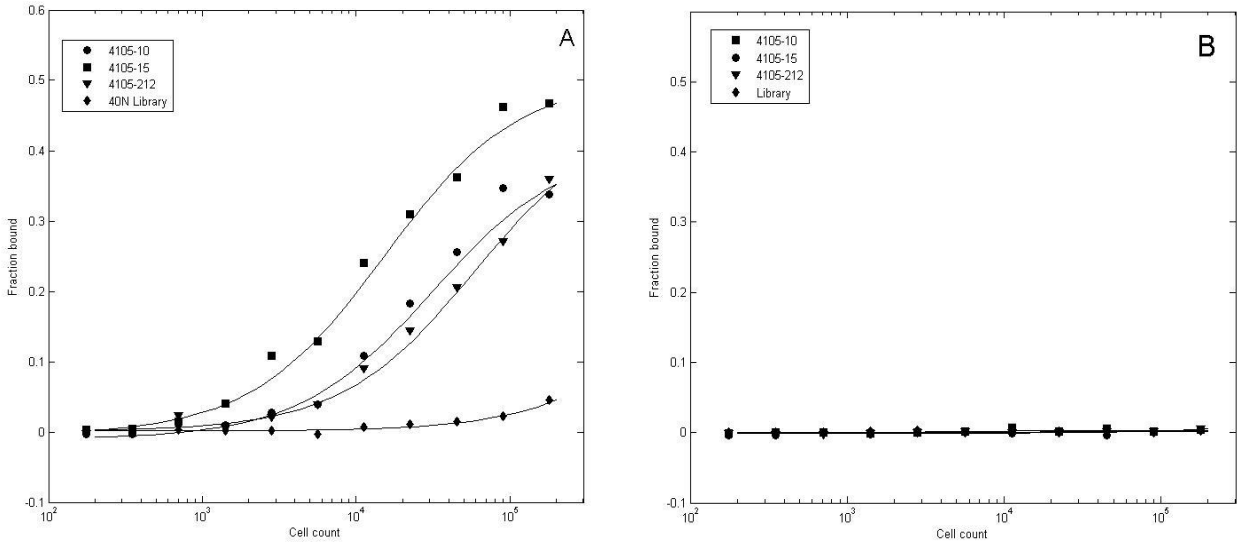


Figure 3. *SOMAmer affinity characterization. Selected SOMAmers were characterized for their binding affinity and specificity to human sperm cells. 2-fold serial dilutions of sperm cells were used to evaluate apparent dissociation constants for three different SOMAmers and a random library (panel A). Sperm binding specificity was verified by testing binding of the same SOMAmers to HeLa cells (panel B). Binding dissociation constants were determined by fitting the data to the Hill equation.*

Sperm elution and capture from cotton swabs: Elution of cellular material from forensic evidence swabs is typically performed using a 1-2% solution of sodium lauryl sulfate (sodium dodecyl sulfate - SDS), which is an efficient medium for cell recovery (defined as the amount of sperm cells bound by SOMAmer coated magnetic beads after elution from the swabs). However, we anticipated that SDS would be incompatible with SOMAmer binding because of its capacity to denature proteins and disrupt protein-protein and protein-DNA interactions. Our selection buffer containing the non-ionic detergent Triton X-100 was intended to support efficient SOMAmer binding as well as efficient elution from cotton swabs, although our measurements of cell recovery do not account for differences in elution efficiency from the swab material. We compared the efficiency of sperm elution from cotton swabs to which semen had been adsorbed using selection buffer at various concentrations versus a solution containing 1% SDS (to mimic

elution buffer commonly used in differential extractions). Here, we found that cellular recovery with selection buffer containing Triton X-100 compared favorably to SDS buffer, with recoveries for selection buffer ranging from 44% to 78% of that seen with SDS buffer (set at 100% for comparison). The highest recovery (78%) was observed with selection buffer containing 2-fold higher Triton X-100 concentration, while a 4-fold concentrated selection buffer yielded no obvious improvement, with 54% recovery relative to the SDS control.

We investigated the efficiency of sperm capture from samples eluted from swabs made of cotton, Dacron, or nylon prepared with either purified sperm cells or semen. As seen in Figure 4, sperm cells that were spotted on cotton swabs and eluted in Triton X-100 containing buffer were not efficiently captured using SOMAmer-coated magnetic beads when compared to Dacron or nylon swabs using selection buffer. Based on this observation, we performed extensive screening of different buffer conditions in order to find an alternative elution buffer that would support efficient binding of sperm cells eluted from cotton swabs.

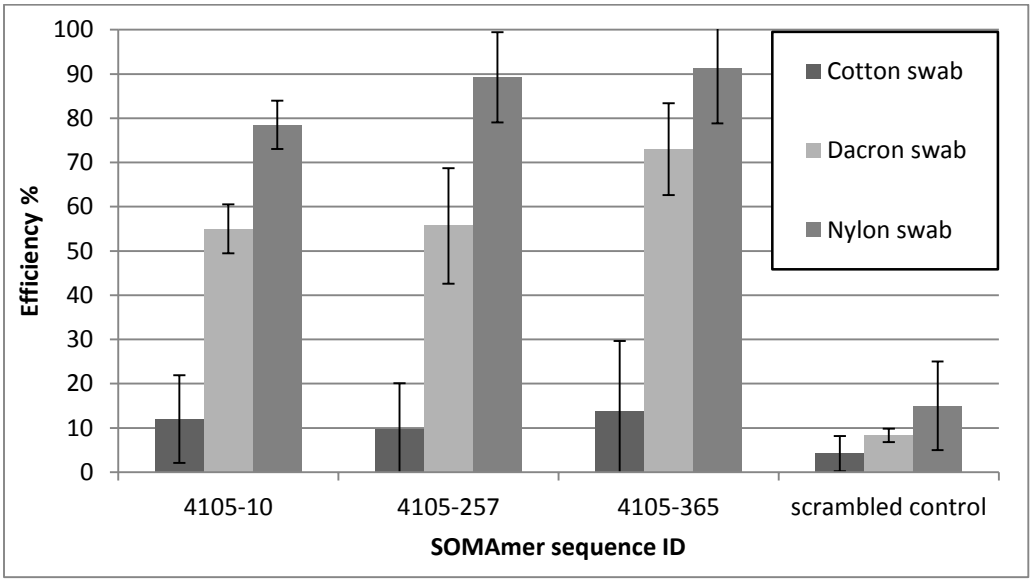


Figure 4. *Sperm capture from different swab materials. Several SOMAmers were compared to the scrambled control sequence.*

Swab elution condition screening: The reduced efficiency of sperm cell capture from cotton swab eluates posed a significant problem, as forensic evidence is most often collected using cotton swabs. To address this problem, we evaluated approximately 500 conditions with different detergents, salt concentrations, pH levels and various additives to identify the optimal parameters for both elution and subsequent sperm

capture by SOMAmers. Sperm cell elution and capture was performed using magnetic beads coated with a mixture of three lead SOMAmers and the overall efficiency was assessed by DNA quantitation from lysed sperm cells using the Picogreen assay. A global overview of SOMAmer-specific sperm recovery under these different conditions is shown in Figure 5. While the majority of tested conditions did not support effective sperm elution or capture by SOMAmer-coated magnetic beads, several solution conditions were identified that supported reasonably efficient sperm elution and capture. These solutions all contained anionic detergents, such as Triton X-200, sodium deoxycholate or lithium dodecyl sulfate (LDS) (Figure 5).

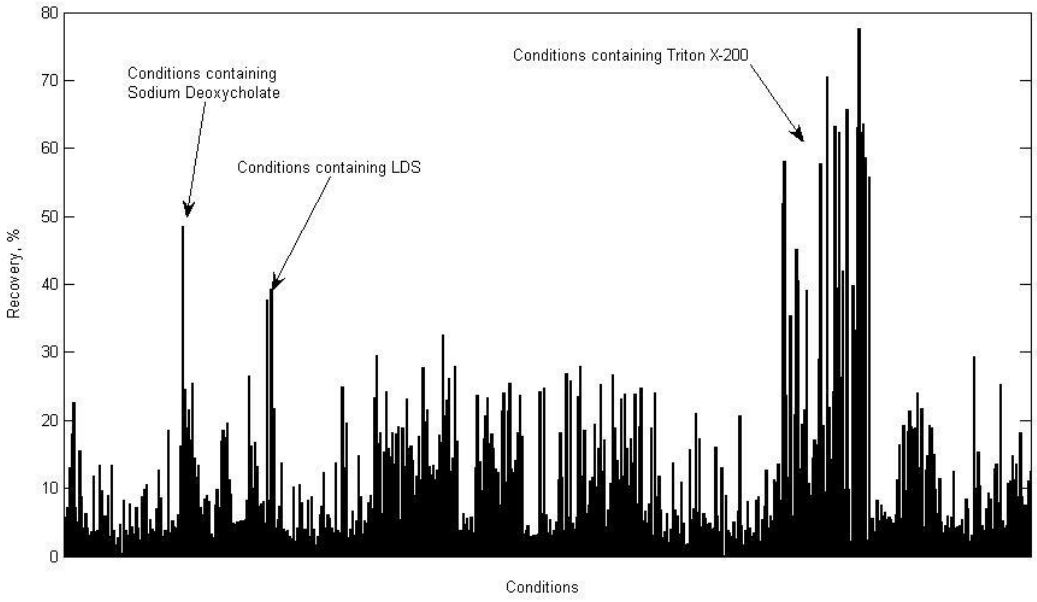


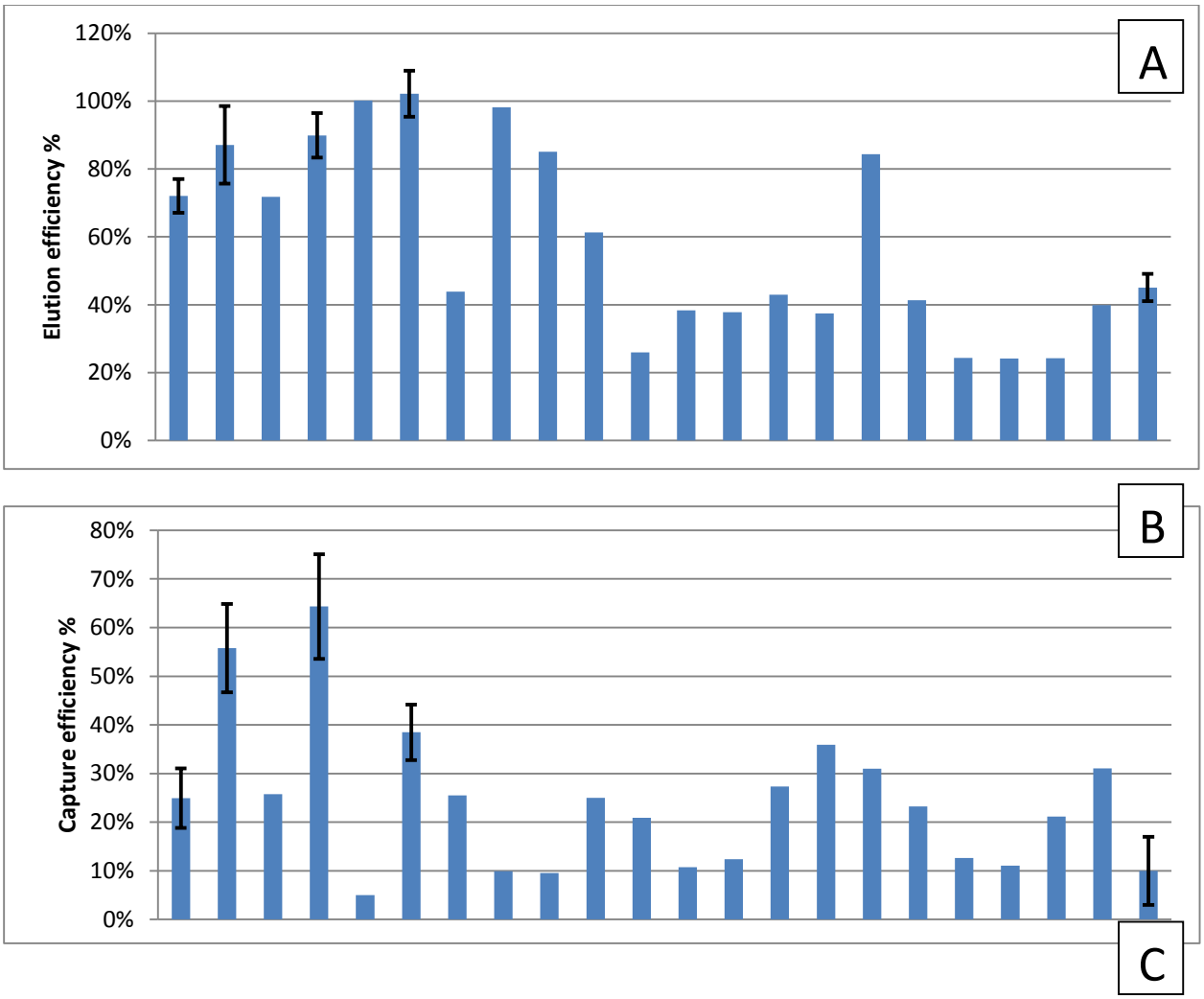
Figure 5. *Sperm recovery from cotton swabs. ~ 500 conditions were evaluated with a variety of buffer conditions for elution and subsequent capture using SOMAmer coated magnetic beads. Each individual bar represents a unique solution condition.*

A more detailed analysis of sperm cell elution from cotton swabs in buffers containing various detergents is presented in Figure 6. Testing was performed in a standard buffer (40 mM Hepes pH 7, 350 mM NaCl) supplemented with various detergents. Although the most efficient sperm cell elution is achieved in SDS containing buffer, our results indicate that comparable sperm elution efficiencies are achieved when swabs are treated with buffer containing anionic detergents, while somewhat lower efficiencies are obtained using zwitterionic and non-ionic detergents.

Sperm capture by SOMAmer-coated magnetic beads was evaluated for each distinct elution buffer. For a majority of the buffer conditions, less than 30% of the total sperm cells were captured (Figure 6B),

whereas sperm were effectively isolated in buffers containing 0.1% sodium deoxycholate and 1% Triton X-200 detergents. Although somewhat lower, reasonable sperm capture efficiency was achieved in buffer containing 1% lithium dodecyl sulfate. This was surprising, given that these are all relatively harsh anionic detergents that would not be expected to support specific SOMAmer binding to native epitopes. Screening results with detergent additives presented in Figure 6 represents a subset from the larger data set (Figure 5) where approximately 500 different buffer conditions were screened. Due to the complexity of this screen, not every condition was analyzed in replicates, meaning measurements of variability are not available for every buffer condition tested. Optimal conditions were chosen and several replicates using these conditions were performed to ensure reproducibility.

Figure 6C shows the efficiency of SOMAmer-specific sperm recovery from cotton swabs. Efficient overall sperm cell recoveries were achieved with buffers containing either Triton X-200 or sodium deoxycholate detergents. Overall recovery was calculated by multiplying the elution efficiency by the capture efficiency to generate a value that represents the final amount of DNA obtained after bead capture and lysis compared to the initial concentration of DNA in the semen sample used for swab preparation. The data does not account for potential differences in lysis efficiency, although lysis conditions were evaluated and it was established that nearly 100% lysis efficiency was achieved (data not shown). The efficiency of sperm cell capture by our lead SOMAmers from cotton swab eluates (>80%, data not shown) was slightly diminished when compared to capture of sperm cells from solution, suggesting that samples eluted from cotton swabs may be subject to epitope loss/degradation during drying, freezing, or elution. Additionally, it is possible that inhibitory substances from cotton swabs are released upon elution that are not present in either Dacron or nylon swabs.



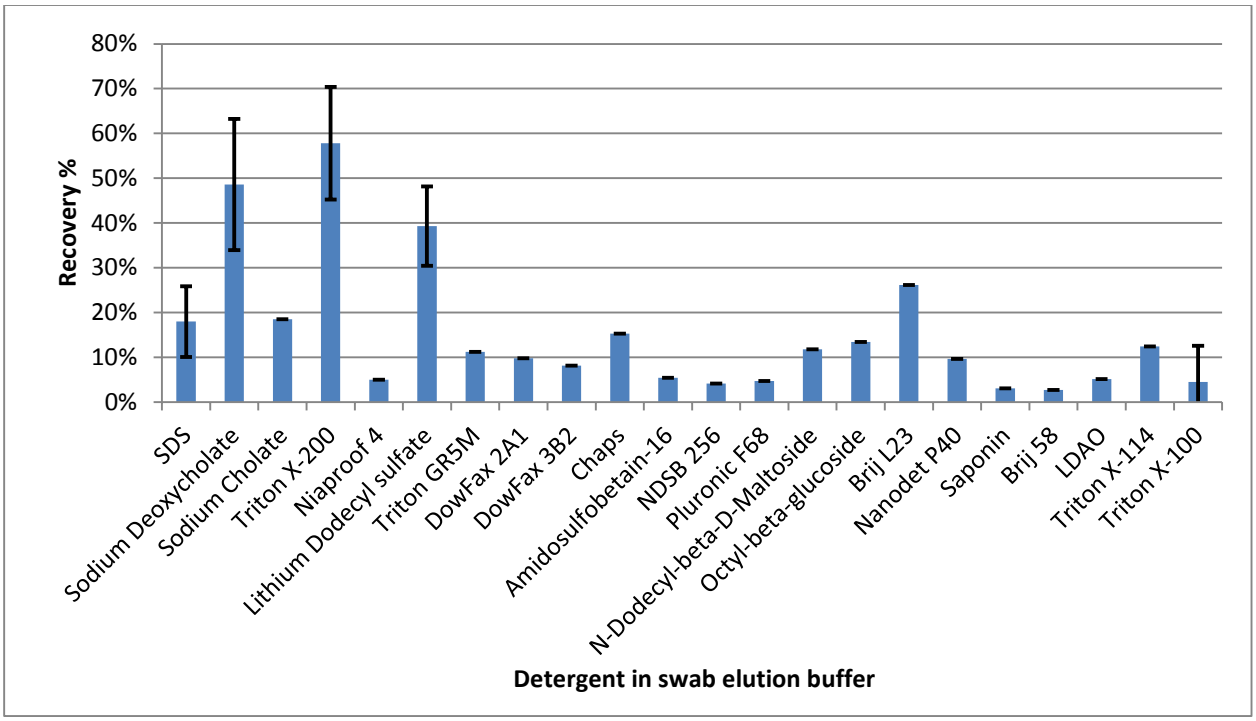


Figure 6. *Elution, capture, and overall recovery efficiency of sperm cells from cotton swabs in buffers containing different detergents. All detergents were at 1% w/v in 40 mM Hepes pH 7.5, 350 mM NaCl and 0.5 mM EDTA, with the exception of Sodium Deoxycholate and Triton X-100, which were at 0.1%, and octyl-beta-glucoside, which was at 1 mM concentration. Overall recovery calculated by multiplying elution efficiency by capture efficiency.*

Sperm purification from samples eluted from mock forensic swabs: We evaluated the purification of sperm cells from mock forensic samples prepared on cotton swabs with a mixture of HeLa cells and semen. For initial tests, HeLa cells derived from human cervical cancer cells were chosen as a surrogate for human vaginal epithelial cells. These experiments were aimed at identifying the optimal buffer and detergent conditions for sperm cell isolation from samples containing a mixture of sperm and epithelial cells. Duplicate swabs bearing a 3-fold excess of HeLa cells over sperm cells were eluted in buffer containing either Triton X-200, deoxycholate or lithium dodecyl sulfate. DNA yields from isolated cells were quantified using Quantifiler Total Human DNA and Y Male DNA kits to compare relative amounts of X and Y chromosomes. The C_t values, calculated DNA concentration and relative ratio of Y to X chromosomes are displayed in Table 1.

Table 1. *Sperm purification from mock forensic swabs.*

	Y chromosome		X chromosome		
Detergent used for swab elution	**C_t**	**DNA conc. ng/uL**	**C_t**	**DNA conc. ng/uL**	**Ratio Y to X**
Sodium Deoxycholate	31.3±0.4	0.05±0.03	30.0±0.2	0.07±0.02	74%
Triton X-200	29.5±0.03	0.40±0.01	28.4±0.02	0.47±0.01	85%
Lithium Dodecyl Sulfate	33.0±0.7	0.007±0.003	30.9±0.9	0.02±0.01	31%
Triton X-100	30.5±0.7	0.14±0.02	26.9±0.9	2.0±0.7	5%
Sperm DNA control	27.9±0.1	2.6±0.4	26.9±0.1	3.1±0.5	90%
HeLa DNA control	ND	ND	27.6±0.1	1.3±0.2	0%

ND - not determined (C_t value below the kit quantification range).

As shown in Table 1, sperm purification was most efficient in buffer containing Triton X-200. The ratio of Y DNA to X DNA in purified samples with Triton X-200 containing elution buffer was nearly 100%, indicating near-complete sperm purification. Sodium deoxycholate and LDS based buffers were less effective, with possible carryover of HeLa cell DNA. Overall, sperm capture in solutions containing Triton X-200 were also considerably more efficient than in other detergents, suggesting that this detergent is the most resistant to background interference from eluted HeLa cells or their debris. We also investigated how HeLa cell excess can impact efficient sperm cell purification and determined that a 10-fold HeLa cell excess was tolerated by the assay (results not shown).

To demonstrate the effective separation of DNA profiles from male and female contributions in mock forensic samples, we generated STR profiles from swabs containing a 5-fold excess of HeLa cells versus sperm. Four loci were amplified (D16S539, D7S820, D13S317 and D5S818) and short tandem repeat (STR) profiles generated from DNA fractionated on 6% polyacrylamide-urea gels (data not shown). DNA from the eluted samples was profiled without further purification on SOMAmer-substituted magnetic beads. The DNA profiles are mostly representative of the female (HeLa) contribution, as might be expected for swabs prepared with an excess of HeLa cells. Eluates subject to sperm capture using SOMAmer coated magnetic beads prior to DNA purification have resulting profiles that closely match the profile of the semen DNA control. From this, we conclude that purification on SOMAmer-coated magnetic beads was

sufficient to generate a clean sperm STR profile from a mixture of HeLa cells and sperm adsorbed to cotton swabs.

As HeLa cells were used during the initial assay development, the lead SOMAmer candidates may be de-sensitized to HeLa cells due to counter selection steps. For that reason, SOMAmer-coated magnetic beads were then used to isolate sperm cells eluted from mock forensic swabs consisting of female buccal swabs prepared with semen. The resulting STR profiles illustrate that clean sperm DNA fractions are effectively isolated from mock forensic swabs using SOMAmer-coated magnetic beads (Figure 7). Minimal carry over from the epithelial cell fractions are observed in the sperm fractions (see Figure 7, panels A and C) allowing straightforward determination of the major source male profiles without contaminating alleles from the female. Likewise, the epithelial cell fractions seen in Figure 7 panels B and D show almost non-detectable levels of male alleles, demonstrating that the sperm cell DNA component has been almost entirely separated from the epithelial cell DNA fraction.

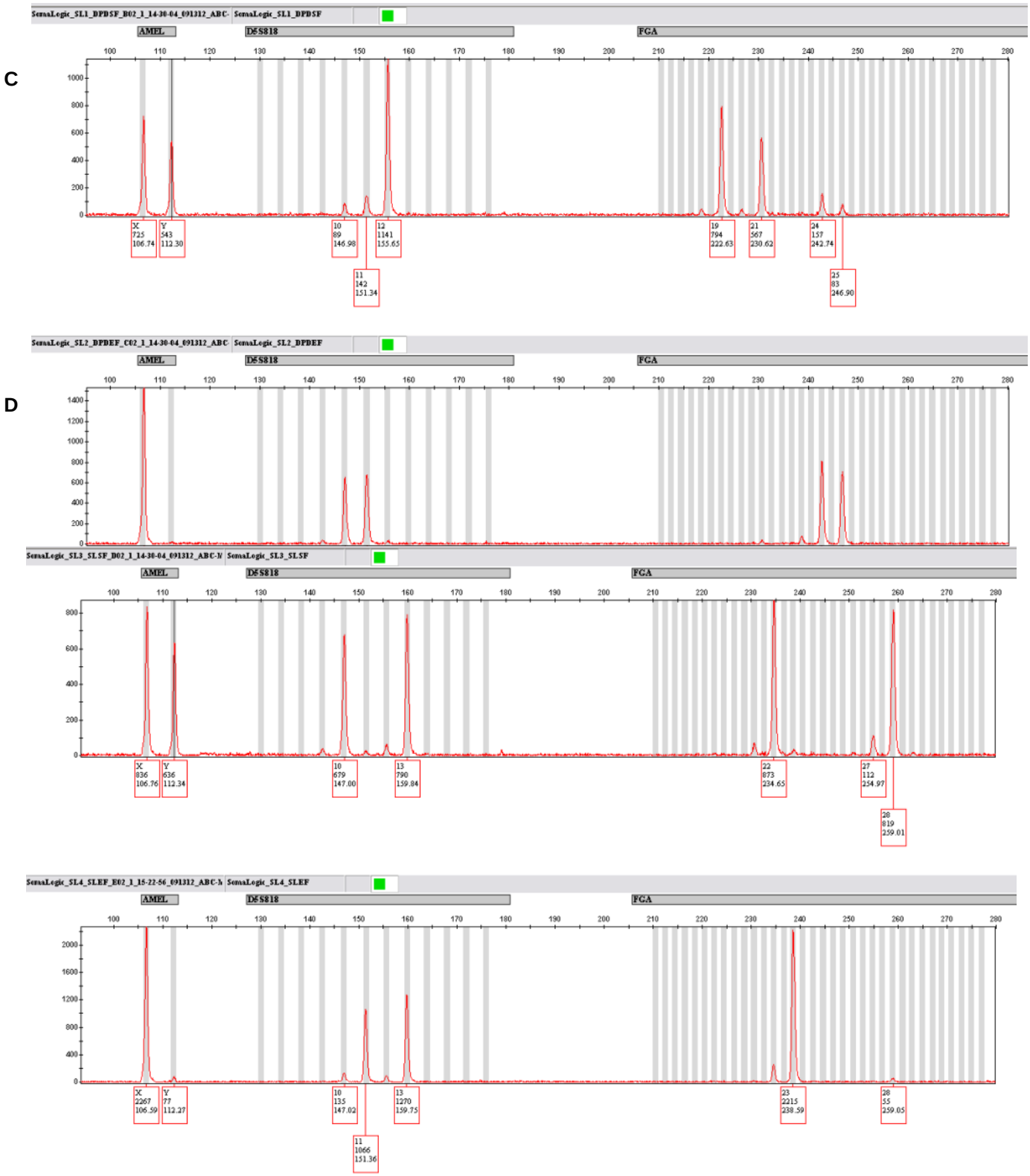


Figure 7. *STR profiles (showing three genetic loci – Amelogenin, D5S818 and FGA) from mock forensic samples treated with SOMAmer coated magnetic beads. Sperm fraction profiles are shown in panels A and C while panels B and D show the respective epithelial cell fractions from two separate sets of mock*

forensic swabs. Essentially no DNA was recovered using beads only or a scrambled control sequence. No STR profiles were generated from these samples.

However, while the separation achieved using SOMAmer coated magnetic beads is very effective, we have not yet achieved complete separation of male DNA from female DNA that is observed with either a traditional DE or with Differex. By comparing the DNA profiles generated from the identical samples purified using Differex, we see no carry-over of female alleles in the sperm DNA profile (compare Figure 8 to Figure 7, panel A). With further refinement of our SOMAmer bead binding assay, we hope to achieve the same level of sperm cell separation from female epithelial cells as is achieved with the differential extraction. The aim is to minimize the amount of carry-over female alleles, which will facilitate generation of an entirely clean male DNA profile.

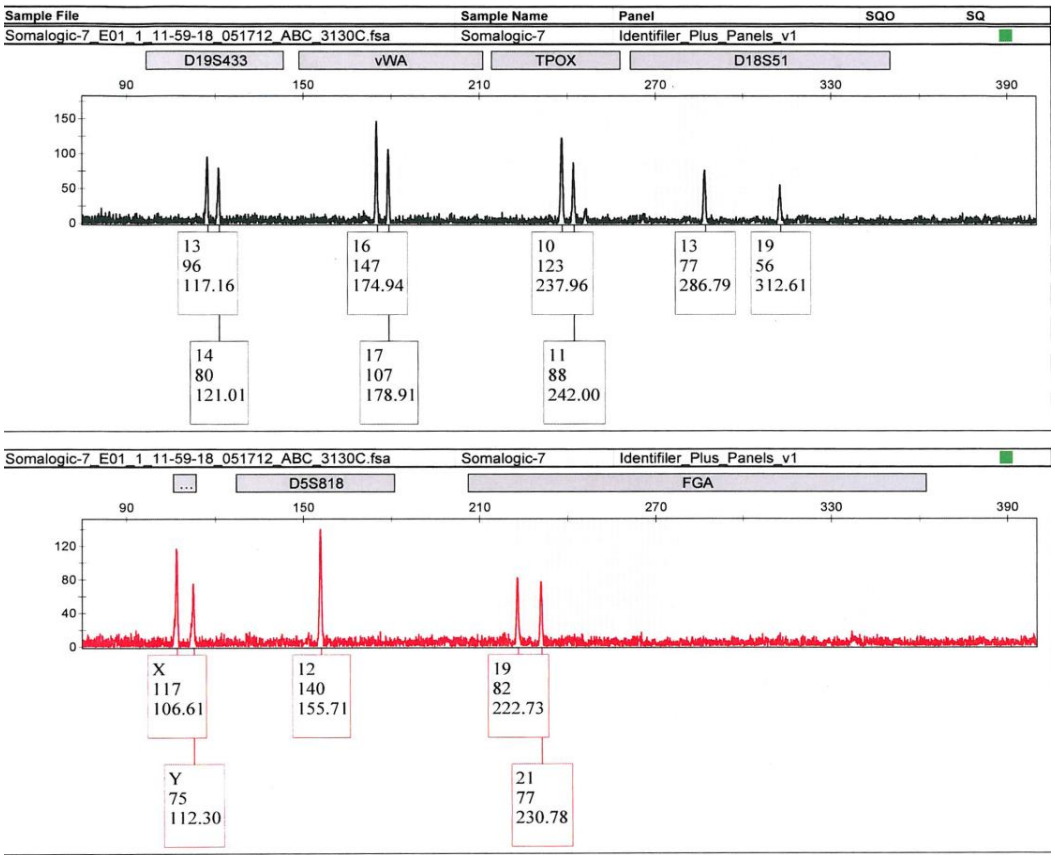


Figure 8. *Sperm fraction STR profile (showing 7 genetic loci) from mock forensic sample purified using Differex. Profile is from identical sample as that shown in Figure 7A (sperm fraction) and 7B (epithelial fraction)*

IV. Concluding Remarks

A. Discussion of Findings

It is generally acknowledged that the DE procedure, which is widely used for analysis of forensic sexual assault evidence swabs, is a time consuming and labor intensive process. Given this, a number of different methods have attempted to improve or circumvent this process. For example, an automated format for the DE process has been developed by Promega called Differex, which allows for processing up to 48 samples in parallel using 96 well microtiter plates and a robotic pipetting system such as the Biomek 2000 from Beckman Coulter. However, this platform has not gained popularity in the field, perhaps due to the high cost of an appropriate automation platform. Moreover, the need to minimize the risk of sample cross-contamination essentially precludes the use of an open 96-well plate. Improved methods that allow forensic analysts to process rape kit samples individually are clearly needed.

One approach that bypasses the selective lysis and extensive cell washing and centrifugation steps used in the differential extraction involves physically separating sperm cells from intact epithelial cells. Cell sorting using flow cytometry is one method where effective separation has been demonstrated [16, 17]. However, it is unlikely that this technique would be widely used on casework samples due to the high cost of cytometry instruments containing the necessary cell sorting capacity and the resulting difficulty in operating them. Sperm cell separation from epithelial cells can also be achieved using size filtration, whereby sperm can be physically separated from much larger epithelial cells using a filtering device with a 10 micrometer filter [18]. With this technique, sperm can be retained using a 2 micrometer filter after the epithelial cells have been preferentially lysed [4, 19]. These filtration methods still require several centrifugation steps and filters are susceptible to clogging and inefficient cell recovery. Additionally, they do not typically result in male DNA fractions that are as pure that those generated by the standard differential extraction method. Laser microdissection of sperm cells from slides has been also demonstrated [9-11]. While this method allows for highly specific isolation of cells, it is unlikely to be widely adopted for forensic casework analysis due to the high cost of the necessary instrumentation.

Previous efforts with antibodies tethered to magnetic beads have been used to demonstrate affinity-based sperm purification via binding to several sperm-specific antigens [12]. However, this method suffered from inadequate antibody affinity resulting in high sample losses from mixed samples containing epithelial cells. Here, we established that sperm cells can be effectively purified from samples eluted from

swabs containing a mixture of female and male cells using SOMAmer coated magnetic beads. In contrast to antibodies, SOMAmers attached to magnetic beads bind sperm cells with high affinity and specificity, likely mediated by multivalent interactions, and these complexes are sufficiently robust to survive proteinase K treatment performed at 55 °C used to lyse buccal cells non-specifically bound to the magnetic beads. With this simple magnetic bead-based method, we have effectively purified sperm cells from solution in a pull-down assay with mixtures of several SOMAmers that bind to tail and head regions of the sperm cells. By employing a whole-cell SELEX-based procedure to select sperm-specific SOMAmers, we developed a highly heterogeneous pool of SOMAmers that bind to distinct targets on the sperm cell surface. Imaging experiments were used to visualize the localization of SOMAmer-coated magnetic beads on the sperm cells which facilitated the identification of several lead SOMAmers that bind sperm cell tails, heads or both. Because sperm cells undergo relatively rapid degradation resulting in the loss of their tails, these might be absent from sperm cells in forensic casework evidence depending on the time at which the evidence is collected. Hence, we chose lead SOMAmers that exhibit binding to both sperm tails and heads to mitigate the potential loss of the binding surface in samples where tails have been degraded.

We have further demonstrated that SOMAmer-based assays yield sperm DNA fractions that are nearly comparable to those typically obtained using standard DE methods [15], including the results achieved using commercially available DE kits such as Differex [20]. At the same time, SOMAmer-based methods can offer more rapid processing of samples that allow sperm purification and DNA extraction to be completed in substantially less time. These experiments demonstrate that SOMAmer-based affinity purification is a practical and efficient method for casework in the forensic laboratory setting.

B. Implications for Policy and Practice

Further experiments will concentrate on testing aptamers for their ability to bind small numbers of sperm in mixtures with female epithelial cells using either mock samples that are more representative of samples typically seen in casework or actual casework samples from adjudicated cases. Non-sperm cells will be completely removed by cellular digestion and wash steps while retaining immobilized sperm cells. The development of a formal procedure to isolate DNA from the immobilized spermatozoa will follow, potentially involving either lysis with a chaotropic salt (e.g. guanidine isothiocyanate) and a reducing agent, or, conceivably, an enzymatic digestion step to lyse the sperm cell wall or with addition of DTT as with current protocols. Also, the potential use of DNA isolation using magnetic beads will be tested further with PCR amplification and STR analysis on the separated male DNA being performed at

the Denver Police Department Crime Laboratory to ensure that the recovered DNA is viable for DNA profiling.

We have developed a novel system for sperm cell isolation and purification using SOMAmer-based sperm capture technology as an alternative to differential extractions for efficiently separating sperm from victim epithelial cells. When conjugated to magnetic beads, SOMAmers selectively bind and immobilze sperm cells allowing wash steps to remove female DNA and other cellular debris prior to sperm lysis. We envision the development of an automated "differential extraction" procedure with SOMAmer-coated magnetic beads added to a conventional kit-based DNA purification system and adapted for use in a multi-well plate format with liquid handling robotic workstations. With an automated high-throughput sperm capture and DNA purification system, the need to perform conventional differential extractions will be eliminated. Ultimately, this will result in reduced time necessary to process sexual assault evidence samples, which will in turn reduce case backlogs by allowing for more rapid and efficient processing of evidence samples from sexual assault cases.

The potential impact of this proposed project could be very significant for the forensic community in terms of sexual assault case backlog reduction. Due in part to the large backlog of rape cases in the United States, DNA laboratories would greatly benefit from a cost effective assay that is fast, easy, and reliable for processing sexual assault evidence. Effectively, this assay will reduce the time required to obtain a DNA profile from a putative perpetrator and will potentially result in both faster identification and prosecution of suspects, possibly preventing additional crimes. Additionally, more profiles will be created for entry in the CODIS database, which in turn can help to link and potentially solve further crimes. In cases without a suspect and no immediate identification of the putative perpetrator, a DNA profile can lead to a John Doe warrant which halts the Statute of Limitations.

In the course of this project, we have developed a sperm capture assay that is amenable to automation using robotic workstations that are becoming more common in forensic casework. Automation can further decrease the amount of time required to process sexual assault cases and will help decrease the backlog of casework in forensic laboratories. This would improve capacities for other casework that does not involve mixed evidence samples and will also allow laboratories to revisit cold sexual assault cases in an attempt to identify perpetrators.

C. Implications for Further Research

To make the product commercially available we will develop a partnership with a supplier of forensic products and employ their existing distribution and marketing networks. We therefore expect the finished sperm-capture assay to be cost-effective and affordable for forensic laboratories. Considering that the majority of evidentiary samples in crime laboratories arise from sexual assault cases, this system will have a significant impact on the forensic community by decreasing the amount of time required to handle this evidence in a cost-effective way.

The continuing goal of this research is the development of a cost-effective assay suitable for high throughput analysis that can easily be implemented in forensic laboratories. However, a developmental validation must be conducted in order to prepare this assay for use in forensic casework. According to the Revised Validation Guidelines issued by the Scientific Working Group on DNA Analysis Methods (SWGDAM) in 2004, the developmental validation must demonstrate the accuracy, sensitivity, precision and reproducibility of a novel methodology and provide the forensic community with the necessary information to assess the abilities, required conditions and limitations of this method.

The viability of this assay will be tested using various sperm concentrations and varying ratios of sperm to epithelial cells. The samples will be extracted and purified using the SOMAmer-based sperm capture assay protocol, the DNA concentrations determined by real-time PCR analysis and the resulting STR profiles will be developed using any one of the major STR analysis kits used in the forensic community (AmpFℓSTR Identifiler or Identifiler Plus PCR Amplification Kits from Life Technologies or the PowerPlex 16 System from the Promega Corporation). This will evaluate the feasibility of using the sperm-capture assay in the context of forensic casework samples.

Later research will focus on evaluation of known inhibitors, non-human sperm samples, mixed samples, as well as non-probative casework samples. Additional testing will be used to demonstrate the accuracy, precision, and reproducibility, as well as the sensitivity and specificity of the assay and identify possible limitations of the procedure. Following the successful completion of these developmental validation studies, the product will be useful for forensic DNA casework and be made available to the forensic community.

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VI. Dissemination of Research Findings

2013 - Sperm Cell Purification from Mixed Swabs Using SOMAmers. Manuscript in preparation for publication in PLOS One Genetics.