FINAL SUMMARY OVERVIEW

Optimized, Semi-Automated Differential DNA Extraction

Award - 2015-DN-BX-K005

INTRODUCTION AND STATEMENT OF PURPOSE

DNA testing methods for evidence in cases of sexual assault focus on the isolation of sperm cells. The differential DNA extraction method [1, 2] involves the differential lysis of epithelial and sperm cells resulting in two separate DNA sample fractions. This method allows for the separation of epithelial DNA and sperm cells prior to their lysis. The traditional differential DNA extraction method is labor intensive, time consuming, not amenable to automation, and can yield mixed DNA profiles in the resulting fractions due to incomplete separation of the different cell types.

Modified differential extraction methods and manufactured kits (Promega Corporation, QIAGEN, Life Technologies) are available that accelerate epithelial cell lysis by skipping the removal of all intact cellular material from the sample substrate prior to initiating epithelial cell lysis. However, these methods still retain the steps necessary to separate and identify intact sperm cells prior to their lysis and are still labor intensive and time consuming. Multiple publications describe modern methods to selectively capture and isolate sperm cells such as laser microdissection [3-5], micro-fluidic devices [6, 7], and track-etch filters [8]. Laser microdissection can result in high specificity and capturing of limited numbers of spermatozoa on microscope slides for DNA extraction and STR analysis; however, it is time-consuming, labor-intensive, requires expensive equipment, and is not easily amenable to automation. The filtration of intact sperm cells from epithelial cells [9, 10] has been evaluated with mixed results dependent on centrifugation parameters and inefficient cell recovery due to filter clogging.

The goal of this project is to develop and evaluate a modified differential DNA extraction method expedited by the Costar® Spin-X® centrifuge tube filter from Corning Life Sciences in conjunction with the Hamilton MICROLAB® STARlet® and the Promega DNA IQ™ System to increase sexual assault evidence processing and throughput.

The goals of changing from the current Chelex differential extraction procedure to one utilizing the Spin-X® columns are to decrease the hands-on analyst time required to complete an extraction batch as well as to decrease the number of tube manipulations. By evaluating the use of the Hamilton MICROLAB® STARlet® as an automated extraction robot for the laboratory's differential extraction needs, the hands-on analyst time should be minimized. The testing of a decreased number of sperm pellet washes should limit the tube manipulations, thereby decreasing the potential for sample switches and contamination events.

The DNA section of the Denver Crime Laboratory has been processing sexual assault evidence using a male screen procedure followed by a traditional differential DNA extraction for samples suspected of containing semen. The male screen procedure is a highly sensitive assay and enhancements to the procedure have made it highly effective for detecting male DNA and identifying samples suitable for male-specific Y-chromosome STR DNA analysis. The traditional differential DNA extraction, however, is a manually performed Chelex extraction and is a labor intensive and less sophisticated DNA extraction procedure than an automated magnetic bead extraction. The manual differential DNA extraction requires post-extraction processing to achieve similar sample quality as samples processed using the Promega DNA IQ™ System on the Hamilton STARlet during the male screen procedure. This study is designed to evaluate the use of the Spin-X tube filter for an optimized differential DNA extraction method that will combine the male screen and traditional differential DNA extraction procedures used at the Denver Crime Laboratory.

The following research objectives guided the experimental design:

Objective 1: Reduce the amount of hands-on user input and sample transfers.

Objective 2: Reduce the possibility of human error and sample contamination.

Objective 3: Increase sample throughput leading to a reduction in sexual assault evidence processing time.

Objective 4: Prevent a backlog of untested sexual assault examination kits.

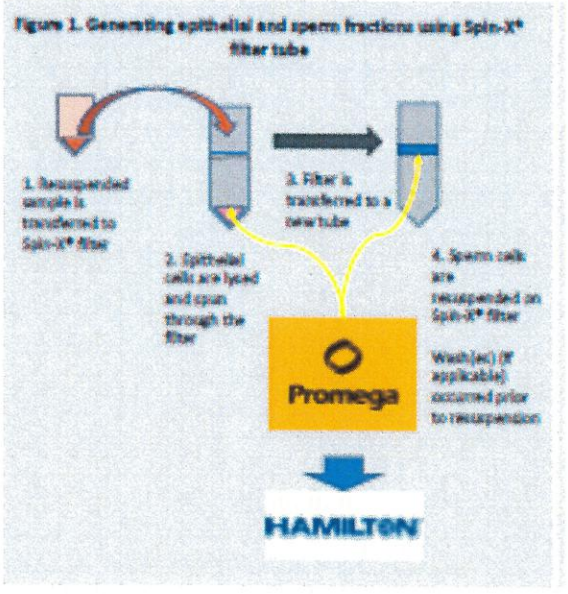
PROJECT DESIGN AND METHODS

Spin-X® tube filters have a 0.45 micrometer pore cellulose acetate membrane which provides fast flow rates of liquids. This product is intended to filter bacteria, particles, or cells from liquids, as well as DNA removal from agarose or acrylamide gels. Preliminary testing of the Spin-X® tube filter has shown no clogging of the filter during the centrifugation step to separate epithelial DNA from an abundance of sperm. For this study the Spin-X® filter was used to differentially separate the epithelial fraction and the sperm fraction prior to robotically extracting these samples on a Hamilton MICROLAB® STARlet® using the Promega DNA IQ™ System.

Substrates were cut into 1.5mL conical tubes. 1mL of water was added and the sample was incubated for 30 minutes at room temperature. The substrate was stick twirled for 2 minutes and then removed. The sample was spun for 2 minutes at 13,000 x g to form a cell pellet. All but 50uL of the supernatant was removed and the pellet was resuspended prior to transfer to the Spin-X® filter for differential separation of the epithelial and sperm fractions from each sample.

500uL of TE Buffer and 15uL of Proteinase K were added and the sample was incubated at 56°C for 2 hours to lyse the epithelial cells. The sample was spun for 2 minute at 13,000 x g to filter the epithelial fraction through to the bottom of the tube, while the un-lysed sperm cells remain on the filter. The filter was transferred to a new tube. 50uL of TE Buffer was added to the filter to resuspend the sperm cells. (Figure 1). The epithelial and sperm fractions then underwent pretreatment with the DNA IQ™ System and prepared for extraction on the Hamilton MICROLAB® STARlet®

Samples were quantitated with Quantifiler Trio™, amplified with Promega PowerPlex® Fusion 5C (29 cycles), run on a 3130 and analyzed with GeneMarker HID Software v2.9.0 using the laboratory's color specific analytical thresholds (B-26 RFU, G-28 RFU, Y-34 RFU, R-34 RFU).



DATA ANALYSIS AND FINDINGS

To test the sensitivity of the proposed assay, a thirteen-point dilution series of sperm-containing semen (1:20 to 5000) was prepared (Table 1). Samples for each of the series were prepared by spotting 50uL of each sample, along with 50uL of 1:20 diluted female saliva onto clean swabs. One replicate was extracted using the current Chelex differential extraction protocol. A second replicate was extracted using the Spin-X® filter tube / DNA IQ™ / Hamilton STARlet®.

Comparison of the quantitation values obtained from the sperm fractions indicates similar recovery of male DNA for both methods. The Chelex method had all 'neat male' male: female ratios, compared to the Spin-X® which ranged from 'neat male' to 1:48.23. This indicates a cleaner separation with less epithelial crossover into the sperm fractions for those samples generated using the Chelex method. Additionally, comparison of the percent of unique alleles called for each contributor in each of the sperm fraction samples (Chelex 1:20-1:1750 were not amplified) along with the average RFU of those alleles also indicates a cleaner separation. 100% of the male contributor's unique alleles were recovered as a major contributor to the DNA profile in all of the sperm fractions generated via the Chelex method. Conversely, the male contributor's unique alleles recovered using the Spin-X® method are present as a major contributor in the 1:20-1:250 dilution points and become present as a minor contributor at the 1:1500 dilution point with drop out beginning at the 1:3000 dilution point.

The DNA IQ™ System protocol has three wash steps to assist in the isolation of the DNA recovered during the extraction process. The current Chelex protocol also uses three washes (digest buffer and/or water). To test if the number of washes needed in the Spin-X® method could be reduced and still maintain similar recovery of human and male DNA, four dilution point replicates (1:20 to 1:500) prepared in the same

fashion as in the sensitivity series were processed using the Spin-X® method with either three, two, one, or zero washes occurring prior to the resuspension of the sperm cells step.

Comparison of the quantitation data (not shown) obtained from the sperm fractions indicated similar recovery of both human and male DNA. Additionally, 87.5% of the samples had a male:female ratio of 'neat male' indicating a clean separation irrespective of the number of washes prior to extraction.

Prior to casework implementation, additional work is needed and includes: 1) an evaluation of the sperm and epithelial cell ratings for the samples that are processed on the Hamilton MICROLAB® STARlet® to determine the actual time needed to process one batch of differential extraction samples, 2) repeating the sensitivity study, particularly at the higher dilution points, with different donors and replicates, 3) determining if the decrease in sensitivity can be minimized to a tolerable level of loss and balanced by the increase in efficiency, 4) determine a quantitation threshold for the suitability of this method for male screen samples types and, 5) evaluate larger batch sizes that may increase the already noticeable differences in efficiency that the automation of Spin-X® columns in conjunction with the Hamilton MICROLAB® STARlet® provides.

DELAYS IMPACTING THE PROJECT

There were significant difficulties that arose due to Finance and Purchasing issues. Discussions between the key players has resulted in changes to City procedures and increased communication between stakeholders. These discussions will have an impact of future grant solicitations and limit the effects of City processes on the grant execution process. Additionally, the work completed by the Laboratory on the CEBR grants did not allow staff to mix grant funded overtime with a pay period due to City policies. As a result, much of the work for this project was completed as part of the staff's normal work week. This was not the

original goal of the project. The laboratory is actively working within the constraints of City policy to limit this impact. For future research projects, the laboratory will change their model in order to place less emphasis on overtime allotment and more emphasis on the needs of the project itself in order to purchase reagents and equipment. This shift in philosophy will result in less funds for de-obligation.

IMPLICATIONS FOR CRIMINAL JUSTICE POLICY

The timeliness for the processing of sexual assaults is a hot-button issue in the forensic DNA workplace. Any techniques that can optimize procedures already in place or find significant improvements must be considered. The procedure from this study has shown to be more efficient and require less hands-on analyst time. Differential extractions are still the gold standard for the separation of sperm cells collected in sexual assault evidence. The results of this research project indicate that any laboratory that conducts testing on sexual assault kit evidence could implement a SpinX column protocol to optimize their differential extraction workflow. The number of washes required in the sperm pellet processing could be reduced to zero and the cost of implementation for this procedure is minimal as the cost of the SpinX columns is not significant. Depending on the workflow in place, a laboratory could either process their samples manually or incorporate an automated workflow on a liquid handling robotic platform.

The potential impact of this proposed project could be very significant for the forensic community in terms of sexual assault case processing and backlog reduction and prevention. Forensic DNA testing laboratories could very strongly benefit from the time savings and cost effectiveness that this expedited method possesses. Reduced processing time facilitates the submission of perpetrator DNA profiles from sexual assault cases into DNA databases where

investigative leads can be made, and criminal suspects identified. Any DNA testing laboratory can implement the proposed DNA extraction method and the automated purification of DNA extracts can be performed on any DNA extraction/purification robot currently available on the market.

Semen Dilution Ratio	Sample Sperm Rating (SP/EPI)	Differential Extraction Method	SF Sperm Rating	SF Human Quant (ng/uL)	SF Male Quant (ng/uL)	Ratio M:F	% Unique Alleles Called in SF		Average RFU* of Unique Alleles Called in SF	
							Male Contributor	Female Contributor	Male Contributor	Female Contributor
1:20	+4 / +3	Chelex	+4 / 0	3.5894	3.7309	Neat Male				
		Spin-X/DNA IQ		5.2318	4.6572	Neat Male	100	65.6	2312	53
1:100	+3 / +3	Chelex	+2 / 0	0.3096	0.3827	Neat Male				
		Spin-X/DNA IQ		0.5585	0.4143	1 to 0.35 (STRs)	100	96.9	1668	159
1:250	+3 / +4	Chelex	+1 / 0	0.1807	0.2137	Neat Male				
		Spin-X/DNA IQ		0.4344	0.3430	Neat Male	100	100	1701	270
1:500	+3 / +4	Chelex	<+1 / 0	0.0607	0.0709	Neat Male				
		Spin-X/DNA IQ		0.1582	0.0757	1 to 1.09 (STRs)	100	100	782	519
1:1000	+2 / +3	Chelex	<+1 / 0	0.0427	0.0436	Neat Male				
		Spin-X/DNA IQ		0.1296	0.0463	1 to 1.80 (STRs)	100	100	626	661
1:1250	+2 / +3	Chelex	+1 / 0	0.0433	0.0514	Neat Male				
		Spin-X/DNA IQ		0.1240	0.0456	1 to 1.72 (STRs)	100	100	588	749
1:1500	+2 / +3	Chelex	4 heads / 0	0.0165	0.0205	Neat Male				
		Spin-X/DNA IQ		0.1245	0.0157	1 to 6.92 (STRs)	100	100	263	1310
1:1750	+2 / +3	Chelex	9 heads / 0	0.0171	0.0217	Neat Male				
		Spin-X/DNA IQ		0.1323	0.0203	1 to 5.50 (STRs)	100	100	213	980
1:2000	<+1 / +2	Chelex	0 / 0	0.0097	0.0127	Neat Male	100	90.6	1736	151
		Spin-X/DNA IQ		0.1583	0.0203	1 to 6.79 (STRs)	100	100	222	1270
1:2500	+1 / +3	Chelex	1 head / 0	0.0136	0.0129	Neat Male	100	100	973	181
		Spin-X/DNA IQ		0.0904	0.0099	1 to 8.14 (STRs)	100	100	194	1092
1:3000	+1 / +3	Chelex	2 heads / 0	0.0154	0.0199	Neat Male	100	100	959	162
		Spin-X/DNA IQ		0.1690	0.0070	1 to 23.03 (STRs)	87.1	100	110	1274
1:4000	5 heads / +3	Chelex	2 heads / 0	0.0080	0.0077	Neat Male	100	100	515	188
		Spin-X/DNA IQ		0.1270	0.0026	1 to 48.23 (STRs)	74.2	100	67	1173
1:5000	7 heads /+3	Chelex	0 / 0	0.0096	0.0071	Neat Male	100	100	927	313
		Spin-X/DNA IQ		0.1433	0.0076	1 to 17.87 (STRs)	77.4	100	95	1091

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