

# **AUTOMATING THE DIFFERENTIAL DIGESTION PROCESS IN THE ANALYSIS OF SEXUAL ASSAULT EVIDENCE USING SELECTIVE DEGRADATION**

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The goal of this presentation is to describe the methods in automating the differential digestion process using selective degradation of the non-sperm DNA. The effects of using a degradative agent on sexual assault evidence samples will be discussed.

This presentation will demonstrate that the selective degradation differential digestion process can produce high yield and high quality DNA profiles given the optimal parameters. Successful application of this process allows the ability to process more sexual assault evidence in a shorter amount of time, aiding in decreasing the number of backlogs in many crime laboratories.

Forensic laboratories are faced with an increase in demand for sexual assault evidence analyses. Lacking sufficient analysts to perform the time consuming, labor intensive work required for DNA analysis, the large amount of requests quickly become an overwhelming backlog. This high demand has led laboratories to transition to automation for processing cases in a highly efficient manner. While many areas of DNA analysis have adopted automation, the differential digestion process remains a time consuming, manual task.

An automated differential digestion protocol was developed using selective degradation. The current differential digestion process requires multiple wash and centrifugation steps to remove residual epithelial DNA from the sperm fraction. The selective degradation technique replaces these labor intensive steps by using a degradative agent, DNase I, to digest the remaining epithelial DNA. The use of DNase on evidence samples and its effect on DNA yield and DNA typing quality was assessed. Studies were performed on semen stains stored for an extended period of time (up to 60 years) and on semen samples subjected to heat, humidity and multiple freeze/thaw cycles to evaluate the effects of DNase on environmentally compromised sperm samples. Sensitivity, reproducibility and contamination studies were performed on a robotic liquid handler used to automate the differential digestion process. The automated protocol utilized 96-well plates for high efficiency and incorporated microscope slide preparations for the confirmation of the presence of sperm.

Initial evaluations of the selective degradation process resulted in lower DNA yield. Samples digested with the selective degradation process recovered approximately 30% of the sperm fraction male DNA when compared to the same sample digested with the conventional method. An explanation for the loss of sperm DNA was incomplete deactivation of DNase prior to sperm lysis. DNase activity is directly related to the divalent ion concentration so multiple experiments were performed to optimize the  $Mg^{2+}$  and  $Ca^{2+}$  concentrations. Results showed that decreasing the  $Ca^{2+}$  concentration from 125mM to 5mM significantly increased the DNA yield comparable to the conventional method.

STR DNA typing for samples subjected to the initial, non-optimized selective degradation process resulted in poor DNA typing quality. 1.5 ng DNA sample input produced profiles with low peak height levels ranging from ~100-500 RFUs and peak height imbalances. The poor typing data may have been caused by inhibition and degradation. Inhibition was eliminated as a possible cause through controlled experiments. Degradation cause by DNase was tested by decreasing the amount of DNase used from 360 units to 18 units. Decreasing the amount of DNase resulted in significantly improved STR DNA typing data. The peak height levels were approximately three times that of the same sample digested with 360 units of DNase.

Environmentally compromised sperm samples were digested using the conventional method and the selective degradation method. DNA quantitation showed no statistical difference in the DNA yield for the samples prepared using both methods. No difference in the STR DNA typing data between both sets of samples was observed. Most samples resulted in full DNA profiles with satisfactory peak heights. A few samples yielded partial profiles with low peak heights. These results were observed using both conventional and selective degradation methods, indicating that compromised sperm samples were able to withstand DNase treatment.

Sensitivity, reproducibility, and contamination studies were performed on an automated robotic liquid handler. Sensitivity studies showed that full DNA profiles were obtained from samples with DNA yield as low as 150 pg (~2 sperm/3 $\mu$ L). In samples where no sperm was observed microscopically, the STR DNA typing data resulted in either no or few alleles detected. Quantitation data from the reproducibility studies demonstrated that the robot was very consistent in the manipulation and preparation of the samples. Contamination studies showed no signs of contamination in the automated selective degradation differential digestion process.

Through the use of the selective degradation method, automation of the differential digestion process was achieved without having to compromise on the quantity and quality of the DNA obtained.