B134 Selective Degradation Using the Erase™ Sperm Isolation Kit and PrepFiler® Purification

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After attending this presentation, attendees will understand the benefits of using selective degradation in place of the standard differential extraction method when processing sexual assault evidence. Attendees will learn how selective degradation can be used to replace the standard differential extraction method to obtain a single-source male DNA profile and how the process can be partially automated by integrating the Erase™ Sperm Isolation Kit with the PrepFiler® DNA Extraction Kit.

This presentation will impact the forensic science community by introducing a reliable procedure that can be successfully used for differential extraction of DNA from very challenging forensic samples in a shorter time and with less intensive labor.

This presentation discusses how selective degradation can be used to replace the standard differential extraction method to obtain a single-source male DNA profile from post-coital vaginal swabs containing sperm. Differential extraction is traditionally used to separate and purify the sperm cell DNA from the epithelial cell DNA. Differential extraction is time consuming and requires intensive work by the analyst. With the high number of sexual assault cases and increasing backlog of sexual assault kits, it is necessary to implement a simpler method to separate sperm cell DNA from epithelial cell DNA.

Selective degradation is accomplished by selectively destroying epithelial DNA using a nuclease, while sperm DNA remains intact inside the sperm cell. The Erase™ Sperm Isolation kit provides crime laboratories with the components necessary to perform selective degradation on sexual assault evidence.

Once the epithelial cell DNA and sperm cell DNA are separated using selective degradation, the DNA sample must be purified. The Erase™ protocol states that the DNA sample can be purified using ethanol precipitation, size filtration, or QIAGEN® EZ1 DNA purification. This study determined that the PrepFiler® DNA Extraction kit can also be used to purify DNA samples previously digested with the Erase™ Sperm Isolation kit. Although the selective degradation portion of this method is performed manually, the DNA purification portion of this method can be performed automatically using the Tecan Freedom EVO® 150, which is a robotic liquid handler. The usage of a robotic liquid handler greatly reduces the amount of analyst hands-on time, variability, and exposure to harmful chemicals, such as phenol-chloroform. Once purified, the DNA sample can be used in further downstream applications.

Vaginal swabs were first collected zero to one hour after intercourse and for the purpose of this study are considered high-sperm samples. Vaginal swabs were collected again between 21 and 25 hours after intercourse and considered low-sperm samples. Stained microscope slides were prepared to identify the presence of spermatozoa. Microscopic identification of spermatozoa confirmed that each of the four samples tested contained a low amount of sperm.

The low-sperm post-coital swabs collected at 21 to 25 hours were digested using the Erase™ Sperm Isolation kit protocol and purified using the automated PrepFiler® DNA Extraction kit. After purification, the sperm and non-sperm fractions of the DNA sample were quantitated, amplified, and typed using Identifiler Plus®. The sperm fraction DNA profiles resulted in single-source male DNA profiles 87.5% of the time. For the purposes of this study, a DNA profile was considered single-source when all loci were identified as originating from one individual and where the minor peak represented was less than 20% at each locus. If the sperm fraction DNA profile had female signals between 20% and 80% of the total signal at a locus, it was considered a mixed profile.

Experimental findings show that the Erase™ Sperm Isolation kit can successfully separate epithelial cell DNA from sperm cell DNA located on post-coital vaginal swabs containing a low amount of sperm. Results indicate that the Erase™ Sperm Isolation kit can be integrated with the PrepFiler® DNA Extraction kit to separate and purify epithelial cell DNA from sperm cell DNA.

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This study shows that selective degradation combined with automated DNA purification can be used in place of the standard differential extraction method on sexual assault evidence containing a mixture of DNA from the victim, in the form of epithelial cells, and from the perpetrator, in the form of spermatozoa. Using selective degradation as opposed to the standard differential extraction method when processing sexual assault evidence can conserve time and effort and result in better sperm fraction DNA profiles.

Differential Extraction, Selective Degradation, Sexual Assault Evidence

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