G162 Application of the IPCRp Method for Genotyping of Male DNA Obtained by Pressure Cycling Differential Extraction

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After attending this presentation, attendees will understand some principles and methods used in comprehensive approach to the problem of obtaining a male genotype from sexual assault evidence where the major issue is the mixture of body fluids from the victim and the suspect. Application of the standard methods on these mixtures often produces an incomprehensible genotype of the suspect.

This presentation will impact the forensic science community by providing attendees with a better understanding of how the small quantities of a male DNA material obtained by the pressure-cycling differential extraction method can be efficiently amplified and detected by IPCRp method (Isolation of PCR products).

The IPCRp method has been previously described to be a fast and efficent way to amplify the low copy number DNA. The method itself increases the sensitivity of the PCR reaction several-fold by highly concentrating a dye-labeled DNA target that is ready to be loaded on the genotyping platform. Therefore, only a few copies of the DNA are sufficient to obtain the genotype. The method utilizes the PCR reaction in which the reverse primer is labeled with biotin acting as a probe for capturing the fluorescently-labeled targeted DNA strand during the amplification. After the PCR, the biotin is attached to the streptavidin-coated plates. Following the washing step, the single-stranded dye-labeled targeted DNA is released by denaturation and loaded for detection on a genotyping instrument. Results are almost free of background noise due to the washing step, in which all of the unincorporated labeled and labeled primers, dNTP's, PCR-reaction buffer, and polymerase are separated and do not interfere with detection.

Control DNA samples prepared by mixing the epithelial and sperm cells and differentially extracted by using pressure cycling technology yielded small quantities of male genomic DNA. The pressure cycling technology followed by the DNA extraction manipulation resulted in a degradation of the female epithelial cells (female DNA). The male DNA was amplified by modified Y-STR and autosomal PCR-multiplex amplification kits. To increase the sensitivity of the PCR, the IPCRp method was applied. The isolation of the PCR products method required modification of the standard multiplex PCR genotyping kits. The amplification kits were designed with the forward and reverse primers fluorescently and biotin labeled, respectively. Following the PCR, performed under standard conditions, the amplified products were captured on the streptavidin-coated, 96-well plates and following the washing step of the unincorporated labeled and unlabeled primers, dNTP's, polymerase, and other material, only the fluorescently-labeled PCR-targeted products in a single stranded DNA configuration were released by denaturation, and loaded into a capillary electrophoresis instrument. Full profiles of the DNA samples were obtained by both kits, although the results from the modified Y-STR kit were with less background noise than the autosomal kit, presumably due to the combined effects of both differential extraction and differential PCR amplification (capturing).

This simple and robust approach of using the pressure cycling technology for differential extraction and decomposition of the epithelial cells, followed by the IPCRp amplification of the male DNA, can improve the genotyping of the male DNA obtained from the mixtures of body fluids from the victim and suspect. Although the IPCRp procedure requires modification of the standard DNA-identification kits, it added only a few extra steps (capturing and washing) to the standard post-PCR protocol which increased the additional time of the post-PCR manipulation by 20 minutes; this is an insignificant accommodation for the standard forensic laboratory practice compared to the benefits of obtaining a genotype of the suspect.

IPCRp, Pressure Cycling, Sexual Assault

 ** Presenting Author*