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IDENTIFICATION AND SEPARATION OF MALE/FEMALE MIXTURES OF VARIOUS CELL TYPE USING INTERPHASE FISH TECHNIQUES AND LASER-CAPTURE MICRODISSECTION METHODS

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We present a protocol to produce single DNA profiles from a mixture of male and female epithelial cells. The protocol incorporates the use of Fluorescent In Situ Hybridization, Laser Capture Microdissection, and DNA typing using the ABI AMPFISTR Identitifiler® ®system. Fluorescent In Situ Hybridization (FISH) is a standard cytogenetic technique used to detect the presence or absence of specific chromosomes and/or sequences of an individual's genome. This method utilizes fluorescent probes which are designed to bind to the targeted conserved sequences of individual chromosomes. The absence of membrane rupture during interphase FISH techniques represents a distinct functional advantage over metaphase methods for the purposes of forensic operations. These methods would allow for the visual identification of male and female cells of similar morphology from sexual assault evidence using Vysis CEP X® alpha satellite and CEP Y® satellite III probes. A working protocol for the hybridization of these probes was developed. Processing samples with this technique has allowed us to visually identify the male and female contribution to each sample mixture. Sex chromosomes were easily identified from epithelial/epithelial, white blood cell/white blood cell, and epithelial/white blood cell mixtures. Following fluorescence processing, intact sample cells were removed from the slides via laser-capture with the Arcturus Pixcell II system and extracted for further STR interpretation. Currently, profiles have been generated from 500 FISH treated male epithelial cells. Cells were extracted using QIAGEN QIAamp® DNA Micro Kit and amplified for 28 cycles in a 25 µl Identifiler® reaction. These initial trials have demonstrated successful protocol design, probe hybridization specificity, cell type compatibility, and the ability to generate full profiles from cells hybridized with X and Y probes. Future examinations will include the laser-capture of 100 and lower counts of FISH processed cells (white blood and epithelial) with the Arcturus Pixcell II instrument and amplification with the ABI Identifiler® amplification system.