SONICATION REMOVAL OF CELLULAR MATERIAL FROM NAIL CLIPPINGS AND DNA TYPING OF SEPARATED COMPONENTS

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Nail fragments are routinely submitted for PCR DNA analysis in violent crime investigations. Typing of the material beneath a nail is important in cases where victims have struggled in defense, while typing of a fingernail fragment can be used to associate an individual with a crime scene. Regardless of the forensic question at hand, separation of these components for separate typing is critical. Presented here are studies employing a method for efficient removal of foreign cellular material from fingernail clippings followed by a rapid method for washing the nail material, such that removed cellular material and the nail can be individually typed using PCR DNA technology.

Fingernail clippings from individuals were collected and treated with blood or semen originating from sources other than the fingernail donor to simulate forensic casework situations. Nails were also collected following vigorous scratching of a second individual. These nails, as well as untreated nail material, underwent the standard protocol for processing fingernail fragments. The method first removes cellular components adhering to the nail. The nail material itself is then subjected to a series of washes to remove any trace amounts of remaining foreign material that may adhere to the nail. Pellets and nail material can then separately undergo DNA extraction and typing. For nails treated with semen, the resulting pellets underwent a differential extraction designed to first isolate that portion of the sample containing “non-sperm” cells, and secondarily isolate DNA from all remaining cells including the sperm cells. Extracted DNA from all samples was quantified and amplified using the PowerPlex® 1.1 and Amelogenin Sex Identification Systems (Promega Corp., Madison, WI) and amplified products were detected by fluorescent imaging for genetic loci CSF1PO, TPOX, Amelogenin, TH01, vWA, D16S539, D7S820, D13S317, and D5S818 using the Hitachi FMBIO® II Fluorescent Imaging Device (MiraiBio Inc., Alameda, CA). Following initial protocol development and validation, the processing method was applied to a forensic case in which nail material was submitted for PCR DNA analysis.

Studies have indicated that when nails are treated with body fluids foreign to the nail donor, cellular material suitable for DNA typing was efficiently removed from the nail such that the contributors of foreign cells and the donor of the nail were easily differentiated. In cases where carry-over between fractions occurred, identification of a major contributor was possible, therefore alleviating the need for interpreting complex mixtures. Untreated nails yielded profiles consistent with the nail donor only; these findings were also true for nails extracted following scratching studies. This is likely due to the limited quantity of cellular material transferred to the nail during the scratching process relative to that already present beneath the nail from the donor. Regarding the forensic case in which a nail of unknown origin was submitted, a mixture of at least two individuals was obtained from the pellet fraction, while the nail fraction yielded a single source profile. The nail fraction profile could not be excluded from the pellet mixture profile, allowing for interpretation of obligatory alleles from other possible contributors present within the pellet fraction profile.

In conclusion, the method employed for separation of nail material from foreign components proved efficient for most samples where a sufficient quantity of cells from the foreign source were deposited on the nail. Because this method allows for the separate typing of nail and adhering cellular material, it can be applied in a standard manner regardless of the forensic question at hand.