A11 Dual Extraction of DNA and mRNA From Human Body Fluids for Forensic Analysis

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After attending this presentation, attendees will be presented with results suggesting that a dual extraction protocol is realistic for isolation of DNA and RNA for downstream forensic analysis of evidentiary-type body fluid samples.

This presentation will impact the forensic science community by showing that how DNA and mRNA can be isolated and analyzed from a single evidentiary-type body fluid stain yielding sufficient quality and quantity of nucleic acids in order to obtain DNA short tandem repeat (STR) typing. It will also demonstrate how to perform mRNA profiling to identify person of origin and tissue of origin from a forensic sample, respectively.

DNA evidence allows for the identification of the person from whom a sample was derived by STR typing but does not provide information concerning the tissue origin. Identification of the tissue origin of a stain may aid in the investigation. Currently, reverse transcription polymerase chain reaction methods have been developed for definitive identification of body fluids including blood, saliva, semen, menstrual blood, and vaginal secretions. These methods utilize tissue-specific mRNA markers to identify fluid origin in a rapid manner with minimal amounts of sample. Many of the stains encountered at crime scenes involve heterogeneous mixtures and, in many cases, involve small amounts of body fluids. Separate samplings of mixed stains to isolate DNA and RNA are less desirable than performing one extraction on a single sample. Therefore, a pre-requisite to the use of mRNA expression profiling in forensic analysis is the ability to co-extract DNA and RNA from the same sample that yields sufficient sensitivity for downstream forensic applications.

The purpose of this study was to identify the best method to co- extract DNA and RNA from a single sample that yields sufficient quality and quantity for molecular typing. Various dual extraction kits for DNA and RNA were tested for their ability to extract DNA and RNA from multiple sized body fluid stains. A phenol-chloroform method for DNA extraction and a silica spin-column based kit for RNA extraction was used for each sample set to serve as a control to compare the dual extraction kits. These studies were performed on various amounts of blood, saliva,

and semen stains along with menstrual blood and vaginal secretion stains on cotton swabs. All of the dual extraction kits successfully yielded DNA and RNA based on quantification utilizing real-time quantitative polymerase chain reaction (qPCR) assays. The RNA yield obtained from the dual extraction kits was similar to that of the silica spin-column based RNA extraction kit. In contrast, the DNA yield obtained from these kits was significantly lower than the phenol-chloroform extraction for all of the body fluids tested. The DNA isolated from the body fluid stains were analyzed by STR typing, and the mRNA was converted to cDNA and analyzed by PCR utilizing tissue-specific primers for mRNA profiling. Despite lower DNA yields compared to phenol-chloroform extraction, all of the dual extraction kits analyzed produced DNA and mRNA of sufficient quantity and quality to generate full STR profiles from the DNA and to obtain positive results for mRNA profiling utilizing tissue-specific primers. In conclusion, dual extraction of DNA and RNA from forensic- type samples appears feasible for the use of STR typing and mRNA profiling.

DNA, mRNA Profiling, STR Analysis

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