B186 Time-Dependent Loss of Messenger RNA (mRNA) Transcripts From Forensic Samples Analyzed Using Next Generation Sequencing

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After attending this presentation, attendees will understand the relationship between mRNA degradation and age outside the body for body fluid stains and how this information may allow for estimating how long a biological sample has been at a crime scene.

This presentation will impact the forensic science community by providing tools with which to estimate how long evidence has been at a crime scene and by possibly improving interval-since-death estimates.

Forensic biology generally has a focus on DNA and the identification of the donor of a DNA sample recovered at a crime scene. Recently, RNA analysis has also demonstrated potential as a worthwhile analyte in the forensic biology laboratory. Applications for RNA analysis include the use of RNA to the identify the tissue source(s) of an evidentiary sample, perhaps assisting in determining the age of a biological sample and in a determining the cause of death through an analysis of expressed genes and how defects in gene expression may have contributed to the death (i.e., a molecular autopsy). Although recent research has indicated many possible forensic applications of RNA analysis, many questions remain concerning the behavior of RNA in degraded and limited samples. Specifically, the need remains for a thorough understanding of the differing patterns and rates of RNA degradation in postmortem and deposited samples.

The purpose of this research was to evaluate mRNA degradation in forensically relevant biological sample types (blood, saliva, semen, and vaginal fluid) utilizing next generation sequencing of fresh and aged samples. By incorporating a panel of synthetic well-characterized RNA sequences of known molar concentration with the initial mRNA preparation, it was possible to quantitatively compare samples stored for various lengths of time up to one year and measure the concentration of different transcripts over time. The mRNA transcripts from tissue-specific markers and those present in all tissue samples were examined. A significant number of tissue-specific transcripts were identified. Specifically, there were 1,449 blood-specific transcripts, 124 saliva-specific transcripts, 211 vaginal fluid-specific transcripts, and 1,712 semen-specific transcripts detected. In addition to the tissue-specific transcripts, there were 1,875 transcripts common to all of the sample types. Both the tissue-specific transcripts and the common transcripts provide an adequate population for the selection of transcripts that disappear over time in a predictable way. Transcripts of both types (tissue-specific and common) decreased in abundance over the one year of storage. Tissue-specific transcripts exhibited varying degradation rates, with times ranging from a rapid disappearance within 30 days of storage at room temperature to remaining stable over the course of one year at room temperature.

The mRNA degradation profiles obtained from this study can be used as a guide to gene expression patterns in different body fluid samples and to basal mRNA degradation rates in samples stored under non-hostile conditions (i.e., room temperature in low light). This guide can be compared to sample storage under conditions that may promote accelerated degradation. Once acceptable candidate genes with predictable degradation rates have been identified and characterized, real-time PCR assays can be developed and implemented more easily in the forensic biology laboratory for routine analysis of casework samples.

mRNA Sequencing, mRNA Degradation, Sample Age

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