B174 Experimental Approaches to Study the Degradation of Messenger RNA (mRNA) in Dried Bloodstains Subjected to Storage in the Laboratory

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The goals of this presentation are: (1) to help define the useful roles for the molecular analysis of RNA in evidentiary body fluid stains; (2) to help attendees understand the role of stochastic effects on quantitation of RNA species; and, (3) to educate attendees regarding the molecular mechanism by which RNA degrades once outside the environment of the body.

This presentation will impact the forensic science community by explaining that, when a criminal act is discovered, the role of the investigative process is to answer the "who," "what," "where," and "when" of the crime. Of these questions, the "when" is often the most difficult to pinpoint, especially in identifying how long a sample has been at the scene or when someone has died.

Studies on the degradation of transcripts in dried body fluid stains hold potential for correlating transcript disappearance with the passage of time. If such a relationship could be established confidently, RNA degradation could be used to estimate the length of time since deposition of a body fluid at a crime scene or help estimate the time of death. Quantitative Polymerase Chain Reaction (qPCR) assays directed against one transcript whose abundance changes with time and a second that is stable presumably allows for normalization of results from different qPCR reactions and the delta Ct results (i.e., Ct for changing transcript minus Ct for the stable transcript) can be plotted as a standard curve relating transcript degradation with time. Transcripts from housekeeping genes as well as ribosomal RNA (rRNA) species have served the role of the "stable transcript" for comparative purposes; however, housekeeping transcripts and rRNA also slowly disappear over the time course and, moreover, stochastic effects that manifest in results from one or the other, or both, transcripts during reverse transcription and subsequent qPCR amplification cause unacceptable levels of variability in the quantities of all but the most abundant transcripts.

RNA sequencing data, produced using Ion Torrent™ Next Generation Sequencing (NGS) technology, from the transcriptomes of dried body fluid stains stored for varying periods of time identified collections of transcripts that disappeared from RNA-seq data with differing rates and also revealed the sequencing read depth of nucleotides in the 5' ends of numerous transcripts decreased from sequencing results faster than the read depth for nucleotides at the 3' end.[1] This observation raised questions of how mRNA transcripts degrade in dried body fluid stains and suggested that assessing transcript degradation based on the apparent differential disappearance of the 5' and 3' ends of **a single transcript** would allow for the results to be expressed as ΔCt (i.e., 5' Ct minus 3' Ct) and also perhaps minimize stochastic effects on data reproducibility, thereby improving the overall reliability of time estimates using the transcript degradation curves. Thus, rather than comparing Ct values produced during qPCR for two different transcripts (either or both susceptible to stochastic effects during reverse transcription), this method quantifies the opposite ends of a single transcript. Reported here are findings of this approach with several transcripts present in bloodstains that were aged at room temperature for periods of up to two years. Results suggest that mRNA in dried bloodstains degrades from the 5' end of the transcript and the difference in amplicon abundance from the 5' and 3' ends of several transcripts produces degradation curves that are more consistent and reproducible and thus may more accurately estimate the age of the stain.

Reference(s):

1. Weinbrecht Katelyn D., Fu Jun, Payton Mark, Allen Robert W. Time-Dependent Loss of mRNA Transcripts from Forensic Stains. *Research and Reports in Forensic Medical Science.* 2017;7:1-12.

RNA Degradation, RNA Quantitation, Sample Age

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