

## **MULTIPLEX HIGH RESOLUTION MELT (HRM) MESSENGER RNA PROFILING ASSAYS FOR BODY FLUID IDENTIFICATION**

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Current body fluid identification methods use a variety of labor intensive and technologically diverse techniques that do not permit the identification of all frequently encountered body fluids such as saliva, vaginal secretions, menstrual blood or skin. Proper identification of the biological material present might be crucial to the investigation and prosecution of a criminal offense and a misrepresentation of the nature of the evidence can have undue influence on the perception of the circumstance of the crime. Therefore it is critical that novel strategies for the conclusive identification of forensically relevant biological fluids be developed.

Messenger RNA (mRNA) profiling is an example of such a molecular based approach. Terminally differentiated cells become such during a developmentally regulated program in which certain genes are turned off (i.e. are transcriptionally silent) and others are turned on (i.e. are actively transcribed and translated into protein). This produces a pattern of gene expression that is unique to each cell type not only evidenced by the specific mRNAs present but also their relative abundance. Thus a determination the type and abundance of mRNAs in a stain or tissue sample recovered at the crime scene provides the ability to definitively identify the tissue or body fluids present. This approach offers a number of advantages over conventional methods for body fluid identification including: (i) the ability to perform parallel tests for numerous markers of a single body fluid in a single assay format, (ii) the ability to perform parallel tests for different body fluids in a single assay format, (iii) a definitive identification of body fluids for which presently no specific tests exist.

Current mRNA body fluid identification assays typically involve either capillary electrophoresis (CE) or quantitative RT-PCR (qRT-PCR) platforms, each with its own limitations. Both platforms require the use of expensive fluorescently labeled primers or probes. CE-based assays require separate amplification and detection steps thus increasing the time required for analysis. For qRT-PCR assays, only 3 or 4 markers can be included in a single reaction since each requires a different fluorescent dye. To simplify mRNA profiling assays and to reduce the time and cost of analysis, we have developed rapid multiplex high resolution melt (HRM) assays that provide an identification of all of the commonly found forensically probative biological fluids and tissues. The HRM assays require only the use of unlabeled PCR primers and a single intercalating fluorescent dye (Eva Green). Each body-fluid specific marker can easily be identified by the presence of a distinct melt peak.

Here, we describe the development of singleplex, duplex (blood/menstrual blood, semen/saliva, vaginal secretions/skin), triplex (epithelial cells) and hexaplex HRM assays for body fluid identification as well as an initial performance evaluation (specificity, sensitivity, mixtures and mock casework samples). The initial results demonstrate the potential use of HRM assays for the rapid screening of biological evidence.