HTG EdgeSeq miRNA Whole Transcriptome Assay identifies miRNA expression signatures in biofluids aiding in forensic identification

Problem: Forensic practice currently employs colorimetric and protein-based testing as well as microscopy for biofluid identification. However, those methodologies are often presumptive in nature and capable of detecting only one biofluid at a time. This current approach is inefficient—consuming time, labor, precious samples, and lab testing supplies in the pursuit of human biofluid differentiation.

One of the key tasks for forensic examiners at a crime scene is to detect and identify human biofluids which can yield important information about a crime. There are a range of biofluids that may be present including blood, urine, saliva, menstrual blood, and semen.

Biological specimens recovered from crime scenes may be complex, limited in size, and not always visually consistent with the biofluids of which they are comprised. The identification of specific biofluids can provide important contextual information, particularly in cases concerning sexual assault. Further, the presence or absence of specific biological material may help corroborate details, guide DNA analysis, or impact the probative value of certain evidence.

The limitations of colorimetric and protein-based testing suggest that a more specific and sensitive, multiplexed approach to forensic biofluid identification would be highly advantageous.

Solution: Recent studies indicate that biofluid types can be classified using miRNA data in conjunction with analysis software

MicroRNAs (miRNAs) are a class of small, non-coding ribonucleic acids that play an important role in regulating gene expression. Their hairpin shape allows for greater stability than messenger RNA. miRNAs are abundant in many mammalian cell types and over 2,000 have been identified in human tissue and biofluids.
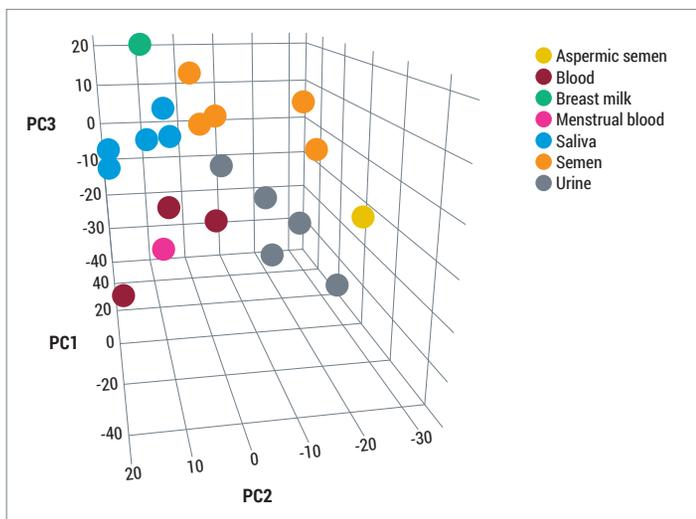
In a recent collaborative study, Amy Brodeur, Assistant Professor at Boston University School of Medicine, defined a novel application for the HTG EdgeSeq™ technology, which is widely used in translational medicine. The HTG EdgeSeq miRNA Whole Transcriptome Assay (WTA) uses an extraction-free protocol, PCR, and next-generation sequencing to identify and measure the expression of 2,000 miRNAs with a single, simple and robust assay.

This study used the HTG EdgeSeq miRNA WTA to assess if the HTG EdgeSeq system could differentiate common forensically relevant biofluids based on their miRNA expression profiles. For this research, seven human biofluid types were acquired fresh or from frozen storage. These were: peripheral blood, menstrual blood, semen containing sperm, sperm-free semen (aspermic), saliva, urine, and breast milk.

Fifteen samples were prepared by taking 15 µl of each liquid sample and combining it with an equal volume of proprietary HTG lysis buffer in a standard 2 mL microcentrifuge tube. The samples were then shipped frozen to HTG for processing and analysis. In the first round of testing, 13 biofluid samples submitted were successfully sequenced. Blinded to the composition of the samples, data analysis was performed showing a clear separation of the types of biofluids tested (data not shown). As an example, 187 miRNAs between saliva and aspermic semen, 92 miRNAs between saliva and semen and 77 miRNAs between aspermic semen and semen were found to drive this differentiation.

A further assessment of the testing data employed Principal Component Analysis (PCA) to assess the global distribution difference across the various miRNAs and specimen types. *Figure 1* clearly shows data point clustering across the various biofluid types, notably distinguishing menstrual blood from blood and aspermic semen from semen.

Significance: Gene expression profiling data from miRNA whole transcriptome analysis, in conjunction with software algorithms, suggests that human biofluids can be distinguished from one another

This case study showcases a novel potential of the HTG EdgeSeq miRNA multiplex assay in the differentiation of human biofluids.

Forensic science has long sought a reliable way to classify biofluids using low volume and often low quality specimens, this new approach shows great promise in that direction. Armed with the ability to distinguish subtle nuances in the composition of forensic specimens, scientists and investigators may soon be able to resolve key issues in criminal justice.

Figure 1. PCA 3D Plot A total of 22 samples were successfully sequenced,13 samples from this study plus an additional 9 samples from other studies, each dot represents one sample, with colors corresponding to the seven different biofluid types. Good separation of biofluid types was observed — illustrated by data point clustering.