B4 The Student Experience in Participating in a Collaborative Exercise for Methylation-Based Body Fluid Identification

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Learning Overview: After attending this presentation, attendees will better understand forensic epigenetics and the potential of using DNA methylation for the identification of biological fluids. Attendees will also gain a better understanding of the student experience in implementing an established Multiplex SNaPshot™ assay and interpreting body fluid identification results via methylation profiles for the purposes of an interlaboratory collaborative exercise.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing results from a student evaluation of a novel methylation-based Multiplex SNaPshot™ assay for body fluid identification. The assay has strong potential to be implemented in a general forensic laboratory as a confirmatory test. This would prevent the need to perform time-consuming serological testing to identify semen and offer a confirmatory assay for the identification of saliva for which there is currently not a test commonly used by crime labs in the United States. This assay also has the potential to be automated using equipment well-established in forensic laboratories and to be used for the identification of biological fluid mixtures.

Epigenetics is defined as reversible heritable changes in gene expression that do not alter the DNA sequence itself. Epigenetic changes play an important role in gene expression and can be altered due to a person's age, lifestyle, and environment.[1] DNA methylation, a DNA base modification, is the process by which methyl groups are added to cytosines on the DNA. These sites are termed CpG sites. DNA methylation patterns are established through cell division, giving various cells and tissue types a specific DNA methylation profile, and these patterns have been thoroughly studied and are regarded as a promising tool for forensic science research, including age prediction and body fluid identification.[2] DNA methylation can be quantified to produce methylation profiles via a process known as bisulfite conversion. In this process, un-methylated cytosine residues are converted to uracil, and during Polymerase Chain Reaction (PCR) are then converted to thymine. Following PCR amplification and single-base extension, the resulting amounts of cytosine and thymine correspond to the amount of methylation present at a specific CpG site. Bisulfite conversion is considered the gold standard in assessing DNA methylation, proving to be successful using small amounts of DNA (50–100pg) and result in 80% DNA recovery.[3] The accurate identification of various body fluids using a methylation-based assay will aid forensic investigators in linking evidence and acts of crime where current assays may yield a false-positive or false-negative result.

In this collaborative study, samples from Seoul National University College of Medicine were sent to various labs, including The George Washington University Department of Forensic Science, to be analyzed using an established Multiplex SNaPshot™ assay for body fluid identification.[3] Samples include swabs of unknown tissue source, genomic DNA of known and unknown tissue source, bisulfite-converted DNA of known tissue source, and reference Single Base Extension (SBE) products. Methylation profiles were comprised of a panel of nine CpG sites (cg17610929, cg26763284-138d, cg06379435, cg08792630, cg09765089-231d, cg26079753-7d, cg09652652-2d, cg18069290, and cg09696411). These CpG sites were chosen based on their specificity to blood, semen, and saliva.[2] The panel includes two CpG sites for semen, two for blood, one for saliva, two for vaginal fluid, and two for menstrual blood. Appropriate samples were extracted on the QIAGEN® QIAamp® DNA Investigator Kit, quantified on the Quantifiler® Trio, then processed via bisulfite conversion on the QIAGEN® EpiTect™ Fast DNA Bisulfite Kit, multiplex PCR, and multiplex SNaPshot™. SBE products were then analyzed on the Applied Biosystems® 3500 Genetic Analyzer to produce methylation profiles. Each profile shows peaks for each of the nine CpG sites, indicating methylation status indicative of either semen, saliva, or blood.

All samples produced reliable results using 40ng of input DNA for the bisulfite conversion process. Methylation profiles produced results consistent with blood, semen, and saliva from the corresponding samples. In addition, three genomic DNA samples and three swabs of unknown tissue source produced a methylation profile consistent with blood, semen, and saliva. Observed peak heights for CpG sites cg06379435 and cg08792630, which correspond to blood, and site cg09652652-2d, which corresponds to saliva, were present but lower than expected. Preliminary results indicate the multiplex SNaPshot™ assay for body fluid identification can be successfully performed by analysts familiar with the protocol and carried out using instrumentation that is well-established in general forensic laboratories.

Reference(s):

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2. Lee H.Y., Park M.J., Choi A., An J.H., Yang W.I., Shin K.J. Potential forensic application of DNA methylation profiling to body fluid identification. *International Journal of Legal Medicine*. 126 (2012) 55-62.
3. Vidaki A., Keyser M. Recent progress, methods and perspectives in forensic epigenetics. *Forensic Science International: Genetics*. 37 (2018) 180-195.

Biological Fluid Identification, DNA Methylation, Bisulfite Conversion

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