B79 Identification of Body Fluid Using Multiplex Polymerase Chain Reaction (PCR)

Quentin T. Gauthier, MSFS, 16 Longacre Court, Hockessin, DE 19707; Joana Antunes, MS, Florida International University, 11200 SW 8th Street, Lab OE294A, Miami, FL 33714; and Bruce R. McCord, PhD, Florida International University, Dept of Chemistry, University Park, Miami, FL 33199*

After attending this presentation, attendees will better understand the development of a novel trace method for the determination of body fluids based on epigenetic markers and sequencing techniques. The procedure involves a multiplex amplification of tissue-specific methylation sites followed by pyrosequencing and/or massively parallel sequencing to determine the identity of the tissue.

This presentation will impact the forensic science community by providing results from an optimization study and evaluation of a multiplex reaction that combines previously developed singleplex reactions determined in prior studies. This presentation will contribute to efforts being made worldwide to expand the information that can be gleaned from an individual's DNA for use in a forensic investigation.

When investigating a crime, it can sometimes be more important to determine the origin of a DNA extract in order to prove criminal intent. For example, in situations such as child abuse, it is the type of body fluid present that is important and not the presence of the suspect. In recent years, multiple DNA methylation markers have been developed for use in the prediction of whether a DNA sample originated from blood, saliva, vaginal epithelia, or semen.[1,2] These loci, known as tissue-specific Differentially Methylated Regions (tDMRs) can exhibit hypomethylation in one body fluid type while exhibiting relatively high methylation levels in other body fluids. Additionally, methylation markers have been found for the prediction of biological age, but demonstrate that the body fluid can influence the results.[3,4] Therefore, it is necessary to first determine from which body fluid the DNA originated. The goal of this project is the development of a multplexed amplification that would permit the analysis of a single sample of genomic DNA for all body fluid types. Such a procedure can save valuable evidence and time, as well as permitting a quicker and more comprehensive result.

In this experiment, a multiplex of previously published tDMRs, found in BCAS4, CG06379435, PFN3A, and ZC3H12D, was created and the amplification process was optimized for further analysis. This optimization was achieved by careful design of the primers to minimize mispriming events, as well as altering the concentrations of primers and Magnesium Chloride ($MgCl_2$) in order to attain roughly equal representation of each amplicon in the final PCR product. Next, a set of 16 samples of extracted DNA — consisting of saliva, blood, vaginal epithelia, and semen — were treated with bisulfite in order to convert unmethylated cytosines to uracil.[5] Methylated cytosines are not converted, permitting determination of relative levels of methylation based on sequence analysis. A multiplex amplification of the above tissue's specific methylation sites was then performed and the resultant DNA sequenced using a pyrosequencer. The results of pyrosequencing were performed using four separate pyrosequencing runs of the amplified samples. An additional subset of samples was also analyzed simultaneously using massively parallel sequencing. The results of the pyrosequencing and massively parallel sequencing were compared to ensure that the same results were being attained in either method.

The 16 DNA samples were correctly identified as belonging to one specific body fluid type. In either sequencing method, the percent methylation at each of the amplified regions was found to be consistent with the results of these methylated regions when amplified individually.[6] Going forward, it would be advantageous to build upon this multiplex by adding in age-specific methylation markers. These markers would rely on the identification of the body fluid in order to increase the accuracy of the age prediction model.

Reference(s):

1. Madi T, Balamurugan K, Bombardi R, Duncan G, McCord B. The determination of tissue-specific DNA methylation patterns in forensic biofluids using bisulfite modification and pyrosequencing. *Electrophoresis*. 2012:33(12):1736-45.
2. An JH, Choi A, Shin KJ, Yang WI, Lee HY. DNA methylation-specific multiplex assays for body fluid identification. *Int J Legal Med*. 2013:127(1):35-43.
3. Zbieć-Piekarska R, Spólnicka M, Kupiec T, Parys-Proszek A, Makowska Ż, Pałeczka A, Branicki W. Development of a forensically useful age prediction method based on DNA methylation analysis. *Forensic Sci. Int. Genet*. 2015:17:173–179.
4. Weidner CI, Lin Q, Koch CM, Eisele L, Beier F, Ziegler P, Zenke M. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biology.* 2014:15(2):1-12.
5. Frommer M, McDonald LE, Millar DS, Collis CM, Watt F, Grigg GW, Molloy PL, Paul CL. A genomic sequencing protocol that yields a positive display of 5--methylcytosine residues in individual DNA strands. *Proc Natl Acad Sci USA*. 1992:89:1827–1831.
6. J. Antunes, D.S. Silva, K. Balamurugan, G. Duncan, C.S. Alho, B. McCord. High-resolution melt analysis of DNA methylation to discriminate semen in biological stains. *Anal. Biochem.* 2016:494:40–45.

Body Fluid Identification, tDMR, Massively Parallel Sequencing

*Presenting Author