Forensic body fluid identification using epigenetic markers based on multiplex PCR and pyrosequencing

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Background and aim of the research

Determination of the source of biological materials found at crime scenes can provide important clues for forensic investigations. The presence of certain type of body fluid can be an indicator of the type of events which occurred (1), which is particularly important in some cases such as sexual assault or child abuse.

Since DNA methylation has proven to have great potential for forensic body fluid identification, numerous DNA methylation markers and their profiling methods have been introduced for body fluid identification. Pyrosequencing is a simple, cheap and robust platform that is able to yield rapid quantitative results and be implemented in forensic laboratories. Current methods based on pyroseqeucning are mostly for analyzing one type of tissue from a single DNA source at a time (2). However, it would be much more useful to type multiple markers of different tissues at a time with a single sample of DNA where the DNA quantity for analysis is often limited in forensic casework. Therefore, the aim of this study was to develop a multiplex PCR assay capable of amplifying all proposed DNA methylation markers for body fluid determination in one test using pyrosequencing.

Outcome and impact of this research in Forensic Science

A set of markers for identifying saliva (BCAS4), blood (cg06379435), vaginal epithelia (VE_8), and semen (ZC3H12D) have been adopted from the literature (3), and then optimized for amplification with balanced concentration of primers in the multiplex PCR system. PCR products generated from a single amplification with four primer sets were placed into a pyrosequencer, PyroMark Q48 Autoprep (Qiagen, CA, USA), for analyzing methylated cytosine loci. In pyrosequencing, some technical strategies were applied to minimize interferences such as the random binding of sequencing primers to non-target amplicons by modifying sequences of primers. DNA methylation levels of each CpG loci measured on the developed system were evaluated for differentiation of different body fluids. For sensitivity of this system, 10, 5, 2.5, 1, 0.5, 0.25, 0.1, and 0.05 ng of DNA were tested in the multiplex PCR.

In the results, the distinctive methylation patterns were reproducible among different body fluids tested; the hyper-methylated patterns of loci in BCAS4 and cg06379435 in saliva and blood, respectively, and the hypo-methylated patterns of loci in VE_8 and ZC3H12D in vaginal epithelia and semen, respectively. These patterns were able to discriminate among different body fluids clearly, and this system was sensitive to 0.5-1ng of total input DNA in the multiplex PCR for all four markers. Currently, our multiplex PCR-based pyrosequencing assay system is on statistical evaluation, and in preparations for the U.S. patent and publication. It is believed that this assay system can save valuable forensic evidence and time, as well as permit faster analysis and more comprehensive results.

Reference

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2. Vidaki A, Giangasparo F, Syndercombe Court D. Electrophoresis 2016;37(21):2767-2779.
3. Silva DSBS, Antunes J, Balamurugan K, Duncan G, Alho CS, McCord B. Forensic Sci Int Genet. 2016;23:55-63.