H117 On Combining MicroRNA Analysis With DNA STR Profiling in a Single Stream Process

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The goal of this presentation is to explain the benefits of microRNA analysis in forensic samples in order to perform body fluid identification. Newly identified controls will be demonstrated using a novel approach, combining them with DNA profiling in a single reaction. It will emphasize the opportunities arising from co-extraction of microRNAs with genomic DNA. The presentation contains state-of-the-art research that is yet largely unpublished and will update attendees on some current developments in the upcoming field of microRNA research in forensic science.

This presentation will impact the forensic science community by expanding on a novel approach where microRNA analysis is combined with DNA Short Tandem Repeat (STR) profiling to identify a human and the tissue of origin, simultaneously. New markers and controls will be revealed to work using a novel capillary electrophoresis-based approach. The presented analysis method is fully compatible with current DNA profiling workflows and allows analysis of cold-case extracts. It has the potential of becoming a versatile and cost-effective method of learning more from biological samples.

MicroRNAs have a potential to be ideal forensic markers due to their small size (~22nt), high abundance per cell, and sensitive and specific Polymerase Chain Reaction (PCR) -based detection. Thousands of microRNAs are present in biological material and they are rich in information due to their tightly regulated and cell type-specific expression. Their advantageous properties increase the chances of successful analysis from challenged crime scene samples. In addition, it has been demonstrated previously that informative microRNA expression levels can be obtained from common DNA extracts without a change in protocol and will likely be present in cold-case extracts, too.

Following an earlier pilot project on a single stream process with the integration of microRNA analysis into a DNA profiling multiplex, progress on this line of research is now presented. The small nucleolar RNAs SNORD7, SNORD44, SNORD47, and the microRNA hsa-miR-93-5p have been identified as endogenous controls. These endogenous controls have been used real-time PCR experiments — in combination with results from other research groups — to determine a larger panel of microRNAs that allow differentiation between blood, saliva, vaginal material, and mixtures thereof.

With the markers identified, the transition has been made to analysis by capillary electrophoresis. Here the analysis of the endogenous controls using capillary electrophoresis on ABI's® 3130 genetic analyzer is presented and the effects of combining their analysis with genomic DNA human identification STR markers in a single reaction are explored. The endogenous control markers are reverse transcribed using a multiplex stem-loop reverse transcription, followed by multiplex PCR with labeled primers for the cDNA and genomic DNA markers simultaneously. This approach was demonstrated before, when it was shown that blood and saliva can successfully be distinguished by amplifying hsa-miR-451a and hsa-miR-205 cDNA during DNA profiling. This will now be expanded with the newly identified endogenous controls. Future work will include the incorporation of the additional body fluid-specific markers, working toward a single reaction that can provide a DNA profile and body fluid identification on single source and mixed samples.

MicroRNA, Body Fluid Identification, Capillary Electrophoresis