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Short Communication

A capillary electrophoresis method for identifying forensically relevant body fluids using miRNAs

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ABSTRACT

Body fluid identification (BFID) can provide crucial information during the course of an investigation. In recent years, microRNAs (miRNAs) have shown considerable body fluid specificity, are able to be co-extracted with DNA, and their small size (18–25 nucleotides) make them ideal for analyzing highly degraded forensic samples. In this study, we designed a preliminary 8-marker system for BFID including an endogenous reference gene (let-7g) to differentiate between venous blood (miR-451a and miR-142-3p), menstrual blood (miR-141-3p and miR-412-3p), semen (miR-891a and miR-10b), and saliva (miR-205) using a capillary electrophoresis approach. This panel uses a linear primer system in order to incorporate additional miRNA markers by forming a multiplex system. The miRNA system was able to distinguish between venous blood, menstrual blood, semen, and saliva using a rudimentary data interpretation strategy. All STR amplifications from co-extracted DNA yielded complete profiles from human identification purposes.

1. Introduction

Body fluid identification (BFID) can be of importance during the course of an investigation and in the courtroom. Determining the origin of a stain may provide probative information about the events that took place during the commission of a crime. Current methods for BFID such as chemical tests, microscopy, enzymatic activity, and immunological tests do not offer the level of specificity or sensitivity required by the forensic field, and results from these methods are often subject to doubt [1–5].

Recently, microRNAs (miRNAs) have been suggested as a potential biomarker for conclusive BFID, and research to date indicates that these markers may meet the desired specificity and sensitivity for forensic use [6–10]. miRNAs are short, non-coding RNA sequences (18–25 nucleotides) that participate in post-transcriptional gene regulation [11]. These molecules are thought to be stable and resistant to degradation due to their association with proteins that provide protection from hydrolysis and RNase activity [12]. Though there has been little research on the stability of miRNAs in challenging conditions, extracted miRNAs have shown considerable stability at room temperature for one year and remained detectable in storage at −20 °C for up to ten years [8,13].

A common strategy for miRNA profiling systems is to use reverse transcription quantitative PCR (RT-qPCR) to determine the relative differences in expression of various miRNAs. Although this method has generated successful interpretation strategies [14,15], it requires multiple reactions, which increases sample consumption, the risk of contamination, cost of reagents, and time of analysis [16]. To address these issues, Li et al. developed a four marker miRNA BFID profiling system using capillary electrophoresis based on a set of linear primers [17]. The system was originally developed using one dye channel with the intent of expansion in order to incorporate additional markers.

In this study, we designed additional primers for the linear primer set to generate an eight marker miRNA multiplex to distinguish between venous blood, menstrual blood, semen, and saliva. A co-extraction strategy was utilized to generate DNA for STR analysis and miRNA for BFID profiles from a single sample.

2. Materials and methods

2.1. Sample collection and extraction

Five samples each of venous blood, menstrual blood, semen, and saliva were collected from volunteers in accordance with Sam Houston State University Institutional Review Board approval (#2015-09-26124). Menstrual blood was collected with cotton swabs. Venous blood was collected via venipuncture and semen provided by donors in specimen containers. Saliva was collected in plastic tubes. Aliquots

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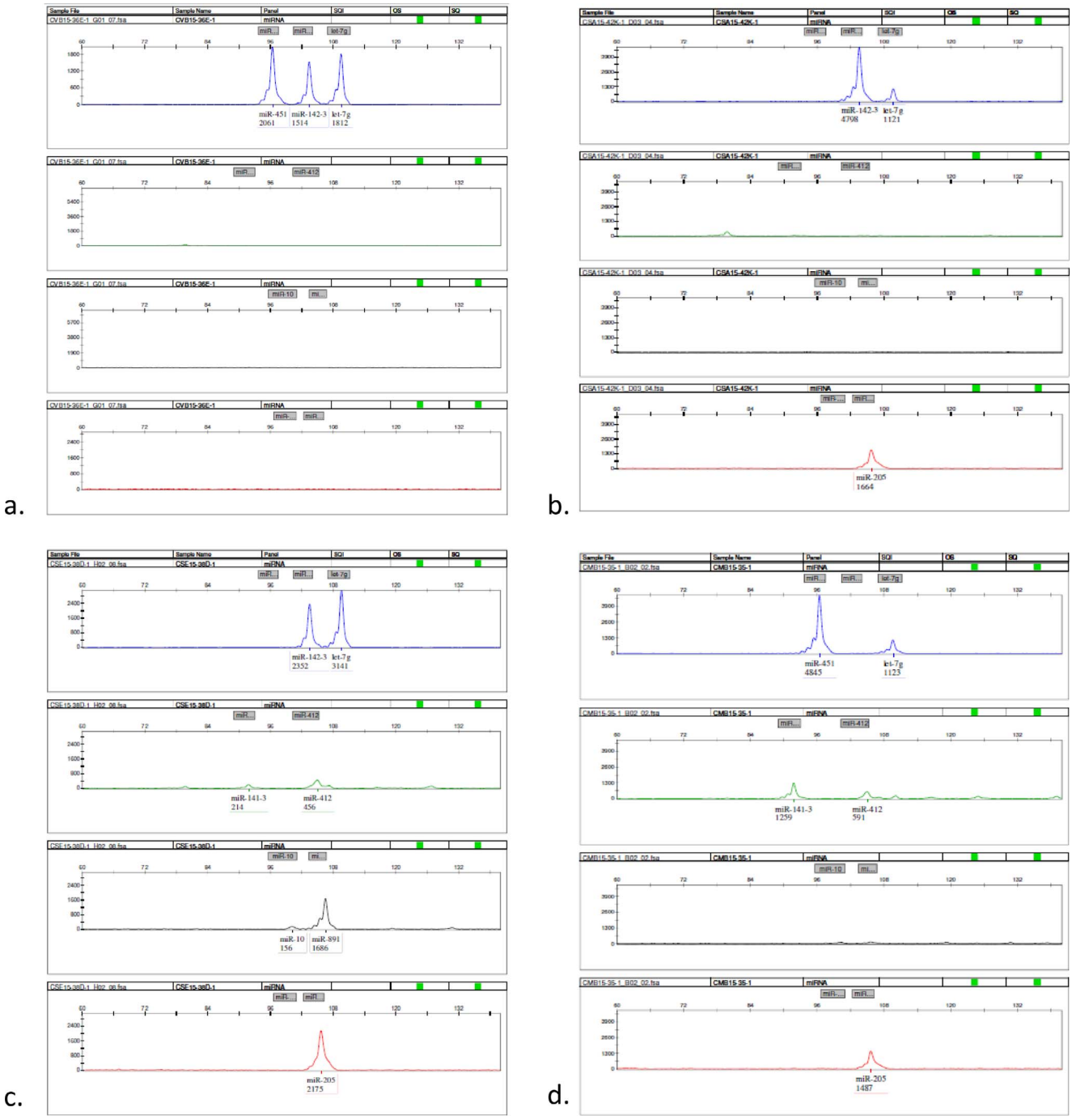

Fig. 1. Representative electropherograms from miRNA multiplex analysis allow for body fluid discrimination. a. Venous blood sample b. Saliva sample c. Semen sample d. Menstrual blood sample.

(50 μL) of venous blood, semen, and saliva were used for extraction, and one swab was used for menstrual blood samples. Samples were extracted using the AllPrep® DNA/RNA Micro Kit (Qiagen®, Valencia, CA) following the Small RNA Purification Protocol [18] with the addition of an initial incubation of 2 h at 56 °C with 900 rpm shaking to improve lysis. The elution volumes for DNA and RNA were 50 μL and 18 μL, respectively.

2.2. DNA quantification and amplification

DNA was quantified with the Quantifiler® Trio DNA Quantification Kit (ThermoFisher Scientific) on a 7500 Real Time PCR System (ThermoFisher Scientific), and amplified using the GlobalFiler™ PCR Amplification Kit (ThermoFisher Scientific) on a Veriti™ Thermal Cycler (ThermoFisher Scientific) as per manufacturer's instructions.

2.3. Marker selection and primer design

Markers were selected based on a consensus of candidate markers in various published studies [6,8–10]. The endogenous reference gene let-7g was included as an internal control, miR-451a and miR-142-3p were chosen for venous blood, miR-141-3p and miR-412-3p for menstrual blood, miR-891a and miR-10b for semen, and miR-205 for saliva. The reverse transcription and specific primers for miR-451a as well as the universal primer designed by Li et al. [17] were also used in this study. Additional primers (reverse transcription and specific primers for all