Development and Initial Evaluation of a miRNA System for Forensically Relevant Body Fluids using Capillary Electrophoresis

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INTRODUCTION

Determining the body fluid origin of a stain may provide probative information about the events that transpired during the commission of a crime. miRNAs have shown tissue and cell-type specific expression and the stability necessary for a robust BFID system. A common strategy for miRNA profiling systems is to analyze relative expression (ΔCT) values of various miRNAs compared to an endogenous reference gene using RT-qPCR. However, most instrumentation for qPCR can detect up to five different fluorescent dyes, which would limit the qPCR. However, most instrumentation for qPCR can detect up to five different fluorescent dyes, which would limit the use of stem-loop primers during the commission of a crime. Li et al. [1] addressed these potential problems by designing a linear primer set composed of three primers working in conjunction to amplify specific miRNAs. While the linear primer system does address the technical challenges of the stem-loop primers, only one universal primer for one dye channel was designed and tested in that study [1]. As novel markers are continually being discovered for BFID and normalization, it would be advantageous to expand the linear primer system to additional dye channels and incorporate more markers and reference markers.

RESULTS & DISCUSSION

Marker Selection and Primer Design

Two miRNA markers were chosen for each of menstrual blood, venous blood, semen, and saliva based on a consensus of candidate markers for body fluid identification in various published studies [1, 4-6]. To minimize non-specific binding, sequences previously designed by Lindblad-Toh et al. [7] and M13 sequences were used for the reverse transcription/universal paired binding sequence.

<table>
<thead>
<tr>
<th>Body Fluid</th>
<th>Venous Blood</th>
<th>Menstrual Blood</th>
<th>Semen</th>
<th>Saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marker</td>
<td>miR-451</td>
<td>miR-142-3</td>
<td>miR-891</td>
<td>miR-205</td>
</tr>
</tbody>
</table>

Table 1. miRNA markers included in the multiplex reaction.

The multiplex is able to distinguish between venous blood, menstrual blood, and semen.

- Marker miR-451 appeared to be specific to venous blood and markers miR-891 and miR-10 were specific for semen.
- Saliva primers amplified genomic DNA and were removed from the multiplex system.
- The cross-reactivity observed for miR-141-3 (menstrual blood marker) with semen has not been previously reported.
- Marker balance of miR-142-3 between venous blood and menstrual blood has been difficult to achieve due to different expression levels between fluids.
- Low level transcription of miR-141-3 and miR-412 was observed in semen samples. Logistic regression analysis may be necessary.
- A standardized method of quantification in necessary to regulate the amount of input cDNA in the PCR reaction.

REFERENCES


MATERIALS AND METHODS

Sample Collection

- Samples of venous blood, menstrual blood, semen, and saliva (n=5) were collected with Sam Houston State University IRB approval (#2015-09-26124).

DNA/RNA co-extraction

- Samples were extracted with the AllPrep™ DNA/RNA Micro Kit (Qiagen) according to manufacturer Small RNA Purification protocol with the addition of 5 µL 14.3 M β-mercaptoethanol and an incubation of 2 hrs at 56°C.

DNA Quantification and STR Amplification

- The Quantifier™ Human DNA Quantification Kit was used to quantify DNA extracts on a 7500 Real Time PCR System.
- DNA was amplified using the Globalfiler™ PCR Amplification Kit (ThermoFisher Scientific).

Reverse Transcription

- Reverse transcription was performed using the SuperScript® III First Strand Synthesis System (ThermoFisher Scientific) with PAGE purified custom reverse transcription primers (IDT).

miRNA Amplification

- PCR amplification was performed with the Type-It® Microsatellite PCR Kit (Qiagen) in a reaction volume of 20 µL composed of 10 µL 2X PCR Mix, 1 µL cDNA, and 9 µL of specific primers (IDT) and fluorescently tagged universal primers (ThermoFisher Scientific).

Capillary Electrophoresis

- PCR products were separated and detected with an ABI 3500 Genetic Analyzer. Data was analyzed using Genemapper® Software v. 5.3 (ThermoFisher Scientific). An analytical threshold of 175 RFU was applied.

CONCLUSIONS

The authors thank Sam Houston State University for providing funding for this research as well as the volunteers that donated samples.