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Capillary electrophoretic analysis of body fluid specific microRNA markers in order to multiplex with STR kits

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1. INTRODUCTION

MicroRNA (miRNA) are small interfering molecules that have a role in the negative regulation of protein expression. These molecules are thought to be more stable than messenger RNA due to their short size and relative abundance within the cell.1,2 In addition, miRNA is thought to be immune from the effect of ribonuclease activity. These characteristics, along with its tissue specific expression, make the molecules a superior candidate for body fluid identification. Current efforts in body fluid identification focus on developing a process where RNA and DNA are separately analysed, requiring two sets of resources.3,4 This project seeks to enhance STR analysis by incorporating body fluid specific microRNA primers within the STR PCR mix.5 Proof of principle has already been demonstrated by van der Meer et al.6 The aim of this study is to expand the number of body fluid specific markers and to evaluate them in context of STR analysis

2. METHODS AND MATERIALS

3. CAPILLARY ELECTROPHORETIC ANALYSIS OF MICRONRNA

4. CE OF DNA AND MiR

The EPG above was produced following 29 PCR cycles amplification of hsa-miR-205 in a saliva sample whilst multiplexed with the NGS SE Kit (Life Technologies). This result demonstrated that miRNA can still be characterised following 29 PCR cycles. This result also demonstrated that microRNA probes can be incorporated into the STR kit to simultaneously produce both a readable DNA profile and a body fluid specific miRNA profile. Optimisation of stem-loop RT primers and PCR primers is yet to be completed. The inclusion of the DNA profile highlights one of the major advantages of targeting miRNA markers for body fluid identification in that the amplicons do not appear to interfere with any of the STR markers.

7. DISCUSSION

The aim of this study was to assess whether or not CE could be used to characterise miRNA markers with a view to differentiating between body fluids. This was explored by extracting samples from a range of body fluids. These then underwent cDNA synthesis before undergoing qPCR. Fourteen body fluid specific miRNA markers were targeted and five markers were subsequently selected for CE and presented above. The results presented in Section 3 indicated that miRNA markers could be detected and that individual markers can be expressed differently in various body fluids. Thus CE has the potential for characterising miRNA markers sufficiently for RFID. There are indications of over-amplification; which is to be expected given that qPCR was carried out using 40 PCR cycles. Section 4 demonstrates that 29 PCR cycles is still sufficient for analysis. This section also demonstrates that such miRNA markers can be characterised alongside STR markers thus enhancing the information that can be gathered from a DNA profile

8. REFERENCES