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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*.[5] 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

SAMPLE RECOVERY
Blood, saliva, seminal fluid and vaginal material were recovered from volunteers with informed consent.

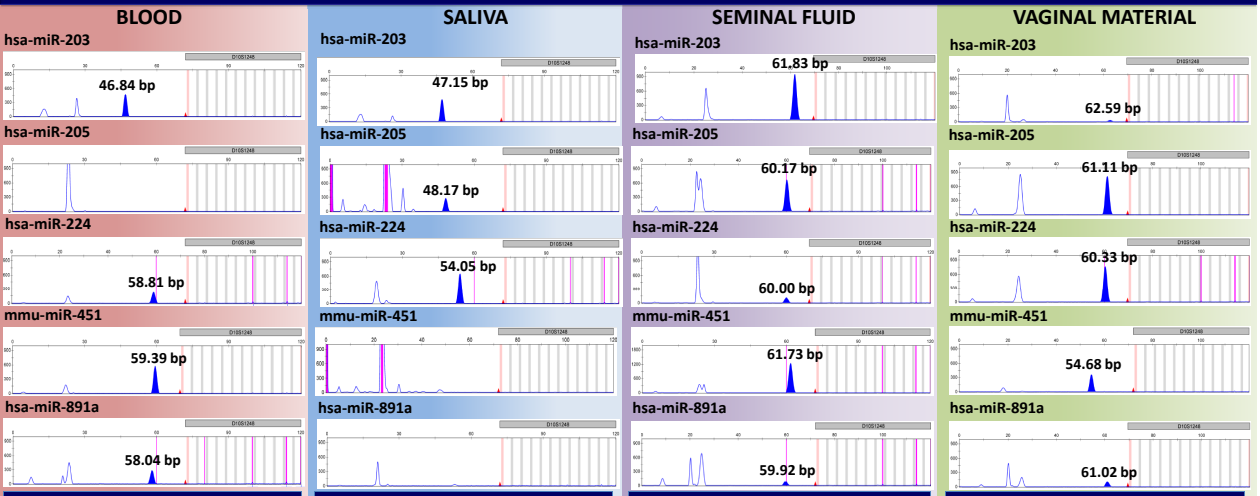
SAMPLE EXTRACTION
Based upon previous research indicating that miRNA is still present in DNA extract all samples under went solid phase DNA extraction.[6]

cDNA SYNTHESIS
All blood, saliva, vaginal material and seminal fluid extracts underwent stem-loop reverse transcription [7]

PCR
cDNA products from all body fluids underwent PCR using labelled microRNA markers. DNA amplification was performed only on saliva.

CE
All PCR products were diluted a 100-fold and STR and miR analysis was carried out using a 3130 genetic analyzer.

3. CAPILLARY ELECTROPHORETIC ANALYSIS OF MICRORNA



BLOOD		
hsa-miR-203	46.78 bp	± 0.09
hsa-miR-205	No Expression	
hsa-miR-224	58.75 bp	± 0.40
mmu-miR-451	59.52 bp	± 0.55
hsa-miR-891a	58.30 bp	± 0.23

SALIVA		
hsa-miR-203	46.99 bp	±0.18
hsa-miR-205	48.13 bp	±0.06
hsa-miR-224	54.03 bp	±0.38
mmu-miR-451	No Expression	
hsa-miR-891a	No Expression	

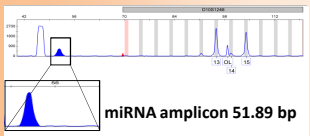
SEMINAL FLUID		
hsa-miR-203	62.35bp	±0.88
hsa-miR-205	59.65 bp	±0.39
hsa-miR-224	60.73 bp	±0.84
mmu-miR-451	60.38 bp	±1.46
hsa-miR-891a	59.44 bp	±0.42

VAGINAL MATERIAL		
hsa-miR-203	64.31 bp	±1.35
hsa-miR-205	61.77 bp	±0.99
hsa-miR-224	60.23 bp	±0.21
mmu-miR-451	54.44 bp	±0.35
hsa-miR-891a	61.78 bp	±1.53

The results in Section 3 are the EPGs associated with the body fluids where the indicated markers were targeted and amplified prior to fragment analysis. The amplicons highlighted in blue demonstrates the base pair sizes, typically between 45 to 65 bp in length. Since the targeted markers amplify to create products of differing base pair lengths for each body fluid and shows little variation between individuals, CE could be used to differentiate between types of body fluid. The peaks visible before the microRNA peak are usually half the size and can be identified as primer dimers. There are some indications of cross-reactivity as demonstrated by similar peaks in different body fluids; however, this is expected given that 40 PCR cycles was used to amplify them along with relatively high primer concentrations.

4. CE OF DNA AND MIR

SALIVA



The EPG above was produced following 29 PCR cycles amplification of hsa-miR-205 in a saliva sample whilst multiplexed with the NGM 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 reportable 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 BFID. 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

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