Characterisation of body fluid specific microRNA markers by capillary electrophoresis

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Abstract. The characterisation of RNA molecules for the purpose of body fluid identification is currently a major field in forensic genetics; with a great deal of effort going towards the analysis of messenger RNA (mRNA). There is also some effort with targeting microRNA (miRNA) which is a more stable RNA molecule than mRNA; due to its short size and role in RNA interference. Most research into forensic miRNA analysis is based around quantitative PCR (qPCR). No substantial research has yet been carried out on capillary electrophoretic (CE) analysis of miRNA. Thus the aim of this study was to explore the viability of CE of miRNA. Samples of blood, saliva, semen, and vaginal material were obtained from a number of volunteers with their informed consent. All samples then underwent standard DNA extraction using QIAAmp DNA mini kit. cDNA synthesis was carried out using stem-loop reverse transcription and commercially available stem-loop primers. qPCR was performed using a 7500 Fast Real-Time PCR Machine and commercially available miRNA assays. The amplified product then underwent fragment analysis using an ABI 3130 genetic analyser. The findings have demonstrated that CE analysis of miRNA markers could be viable for the purpose of forensic genetics. The fragment sizes (between 30 and 70 bp) suggest that such CE based miRNA assays could be multiplexed with STR kits with minimal modification; thus enhancing the capability of DNA profiling.

Keywords: MicroRNA; body fluid identification; capillary electrophoresis; blood; saliva

1. Introduction

RNA-based body fluid identification has developed to the stage where it is almost ready for implementation in case work. Messenger RNA work has successfully developed a system where it can be characterised by capillary electrophoresis [1]. Work is also being carried out on microRNA, but currently the analyses are being carried out by quantitative PCR. This study is looking at the validity of conducting CE upon amplified miRNA products. This builds upon the work of van der Meer *et al* [2] and explores further body fluid specific markers. The main benefit of CE analysis is the multiplexing with STR kits to create a much more useful electropherogram for the reporting practitioner.

2. Methods and materials

2.1 Sample acquisition and extraction

Blood, saliva, seminal fluid and vaginal material samples were collected from 5 volunteers with informed consent and ethical approval. Blood was recovered by the finger prick method and spotted on to filter paper. All other body fluid samples were collected by swabbing/using sterile swabs. Sections of the stained filter paper and the swabs then underwent extraction using the QIAAmp DNA mini kit (Qiagen, UK) as per the Omelia *et al* study [3].

2.2 slRT-qPCR

Each sample extract then underwent stem loop reverse transcription [4] and quantitative PCR targeting miR-451 (blood), miR-205 (saliva), miR-891a (seminal fluid) and miR-617 (vaginal material). slRT-qPCR was carried out using the miRNA assay kits and Taqman master mix on a Veriti thermocycler and Fast 7500 Real-Time PCR Machine (Life Technologies) as per manufacturer's instructions. 40 PCR cycles were utilised during each experiment.

2.3. Capillary electrophoresis

All samples, following slRT-qPCR, then underwent CE on the AB3130 Genetic Analyser (Life Technologies, UK), using the same run conditions as the NGM SElect STR kit. The bins and panels were left in, for reference purposes.

2.4. Presentation of data

Although multiplexing of the probes have yet to take place, a 'virtual multiplex' was created by overlaying the EPGs for each marker on the same body fluid.

3. Results

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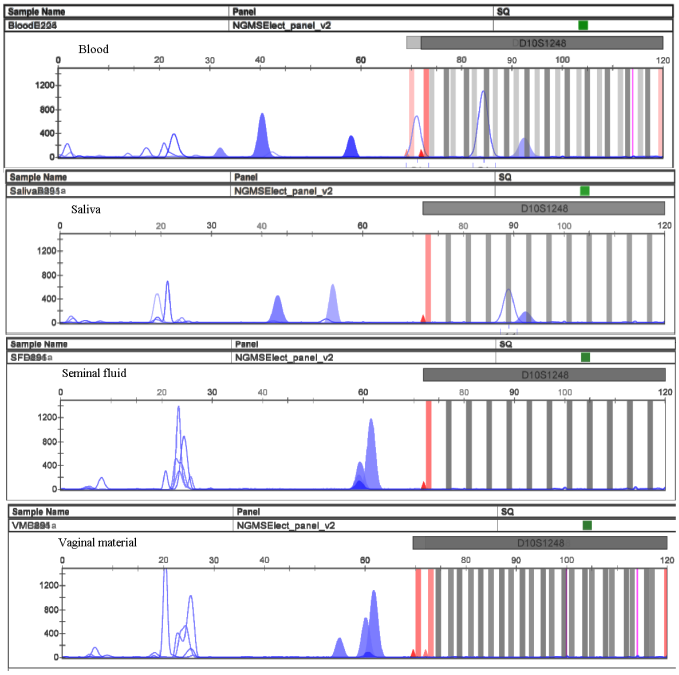

Figure 1: Composite electropherogram derived from an overlapping of 4 different markers (miR-451, miR-205, miR-224 and miR-891a) in four different body fluids (blood, saliva, seminal fluid and vaginal material). Peaks below 30bp are low molecular weight artefacts. The peak variation between individuals is ~±0.7bp. The data presented is from one individual, but is representative of five different individuals.

As indicated in Figure 1, most of the four markers are being detected in the four different body fluids. However, the amplicon sizes of each of the markers vary (as well as peak height). For example, a peak at 62bp is only present in seminal fluid and vaginal material. Each different body fluid has a different arrangement of peaks (or different 'profile'); thus indicating that CE analysis of microRNA for body fluid identification is possible. Concerns relating to its proximity to the low molecular weight artefacts and the bins and panels of the NGM SElect kit are real. However, it can be observed that there is a range of peaks between 40bp and 65bp which can be utilised.

Further work will include the CE analysis of additional body fluid specific markers and PCR optimisation studies. Initial work relating to the optimisation of PCR cycle number have indicated that miRNA markers can be detected by CE after 30 PCR cycles; in line with the majority of STR kits PCR conditions.

5. Role of funding

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7. Conflict of interest

Conflict of interest: none.

8. References

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