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On combining microRNA analysis with DNA profiling in a single stream process

Donny van der Meer MSc
Supervisor: Dr Graham Williams
FSF Emerging Forensic Scientist Award
Oral Presentation
What are microRNAs and why are we interested in them?

Small (~22nt) non-coding RNAs
Regulate mRNA expression

Advantages for forensic science
Stable
High expression levels
Sensitive and specific detection
Co-extracted with DNA
MicroRNAs can be used for body fluid identification

More than 2500 microRNAs in humans
Tissue specific expression patterns

Previously identified markers
**Blood:** miR-16a, miR-142 and miR-451a
**Saliva:** miR-203a and miR-205
**Semen:** miR-10a and miR-135a
**Vaginal material:** miR-1260b
**Control:** SNORD44

Improve current methodology with our novel method

Current
• microRNAs: RT-qPCR
  • Separate reaction per microRNA

Our novel method
• Analyse microRNAs with capillary electrophoresis (CE)
  • Multiplex microRNAs in single test
  • Possibility to combine microRNA analysis with DNA profiling
Methods and materials

• 5 samples of 4 tissue types
  • Blood, saliva, semen and vaginal material
• DNA extraction
• Normalised to 0.5ng/µl human DNA
• Tested for 9 markers
• Multiplex stem-loop reverse transcription
• ROX-labelled primers
miR-10a and miR-135a are exclusively detected in semen.
miR-16a and miR-142 are exclusively detected in blood.
miR-451a is exclusively detected in blood

Much lower peaks of by-products found in all tissues
miR-203a is mainly detected in saliva

Expressed in epithelial cells

Sample set 1

miR-203a

Sample set 2

miR-203a
Multiplexing multiple markers yields expected results

**Multiplex**

- Blood
- Saliva
- Semen 10a
- Semen 135a

**Singleplex**

- miR-451a

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**Blood**

**Saliva**

**Semen**

**Vaginal**
Multiplex with STR markers
Multiplex with STR markers
Conclusion

• Analysing microRNAs with CE is viable

• Potential for future single confirmatory test

• Combining microRNA analysis with DNA profiling is technically feasible
Future work

• Reduce non-specific amplification

• Physically separate markers
  • Increase product length

• Optimise multiplex reaction

• Combination with DNA profiling
Thank you

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Our workflow

cDNA is created using stem-loop reverse transcription

miR-1260b and miR-205 fail due to multiplexing reverse transcription

Multiplex RT

miR-1260b

Vaginal

Blood

Semen

miR-205

Blood

Saliva

Singleplex RT

Vaginal

miR-1260b

Saliva

miR-205
Multiplexing multiple markers yields expected results

- Blood
- Saliva
- Semen
- Vaginal

![Graphs showing expected results for Blood, Saliva, Semen, and Vaginal samples]