



University of HUDDERSFIELD

University of Huddersfield Repository

van der Meer, Dieudonne J. and Williams, Graham

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

Original Citation

van der Meer, Dieudonne J. and Williams, Graham (2015) On combining microRNA analysis with DNA profiling in a single stream process. In: American Academy of Forensic Sciences 67th Annual Meeting, Orlando, FL, USA, 16th - 21st February 2015, Orlando, FL, USA.

This version is available at <http://eprints.hud.ac.uk/24006/>

The University Repository is a digital collection of the research output of the University, available on Open Access. Copyright and Moral Rights for the items on this site are retained by the individual author and/or other copyright owners. Users may access full items free of charge; copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational or not-for-profit purposes without prior permission or charge, provided:

- The authors, title and full bibliographic details is credited in any copy;
- A hyperlink and/or URL is included for the original metadata page; and
- The content is not changed in any way.

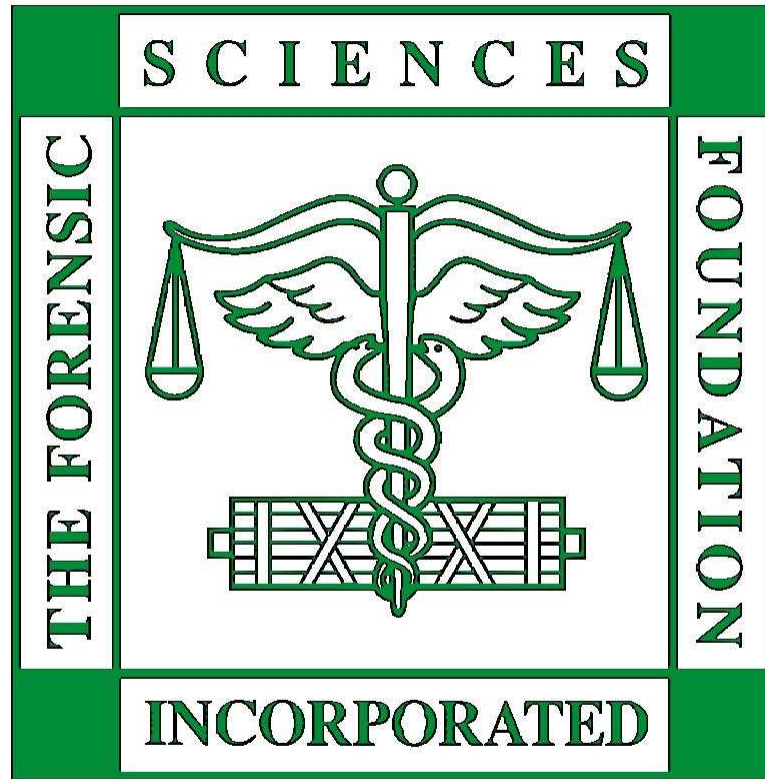
For more information, including our policy and submission procedure, please contact the Repository Team at: E.mailbox@hud.ac.uk.

<http://eprints.hud.ac.uk/>

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

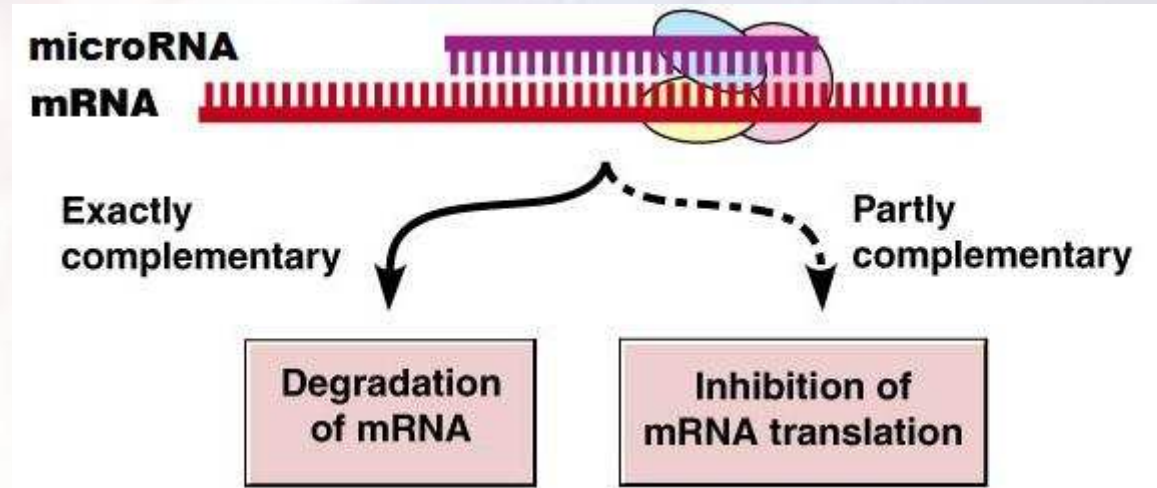
Advantages for forensic science

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

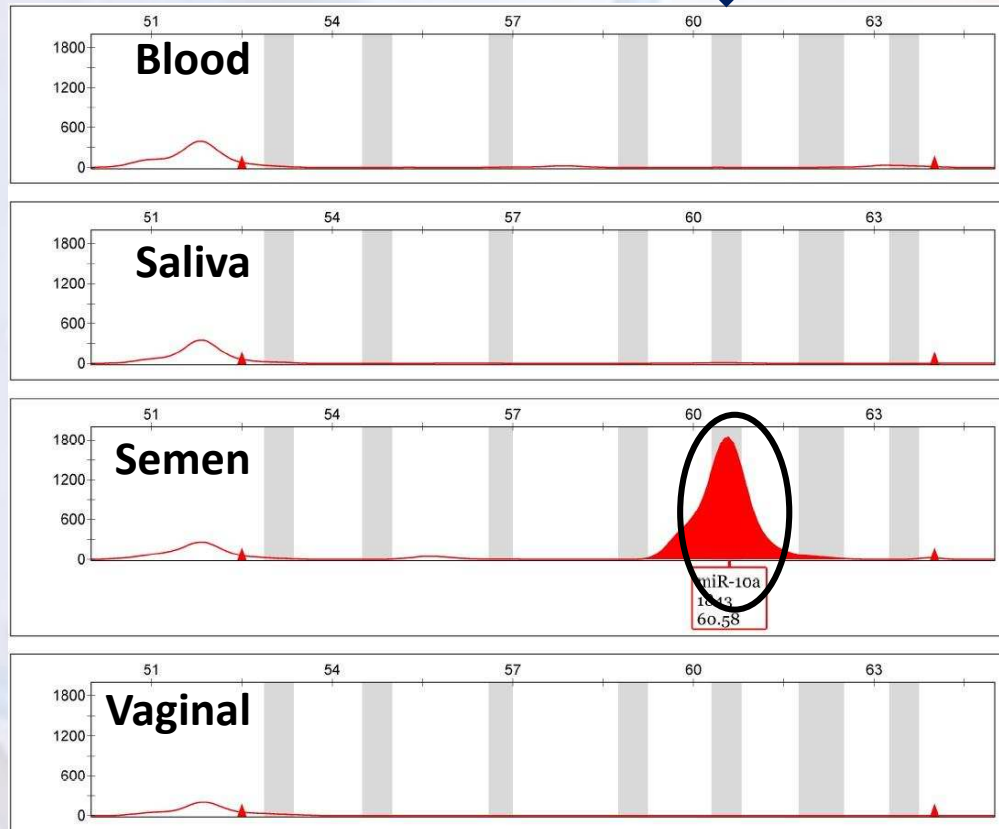
- Analyse microRNAs with capillary electrophoresis (CE)
 - Multiplex microRNAs in single test
 - Possibility to combine microRNA analysis with DNA profiling

Methods and materials

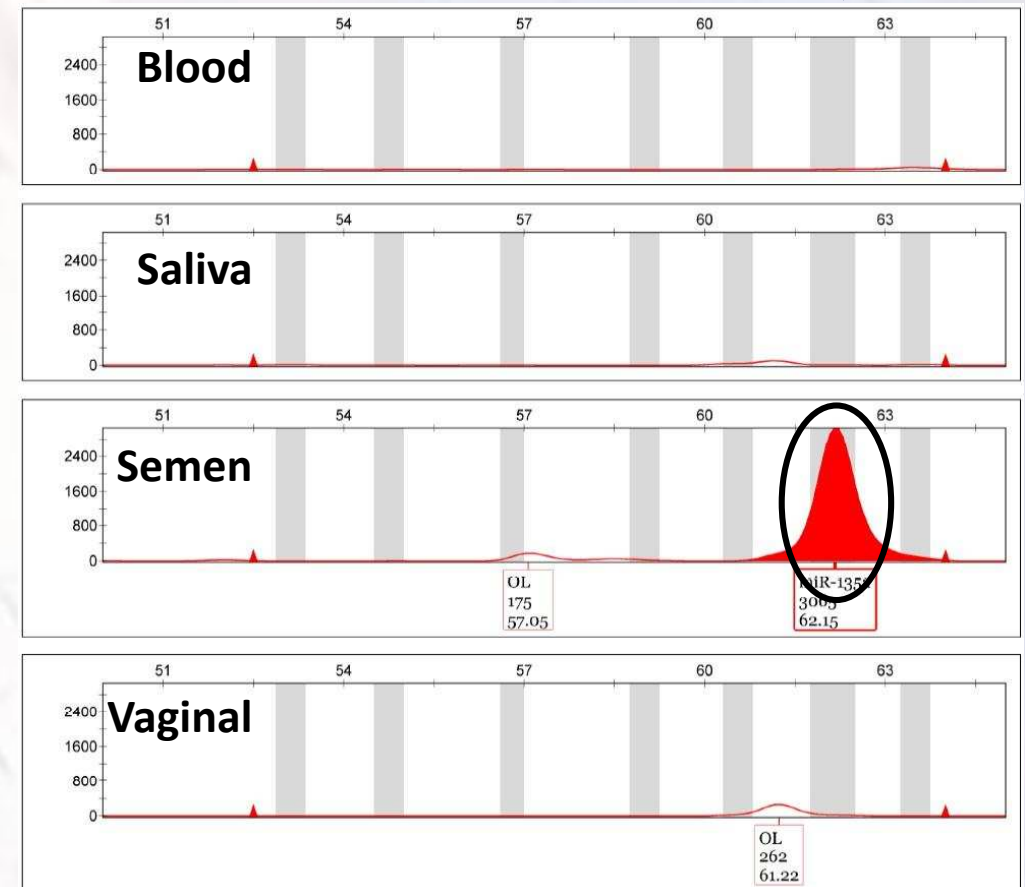
- 5 samples of 4 tissue types
 - Blood, saliva, semen and vaginal material
- DNA extraction
- Normalised to 0.5ng/ μ l human DNA
- Tested for 9 markers
- Multiplex stem-loop reverse transcription
- ROX-labelled primers

miR-10a and miR-135a are exclusively detected in semen

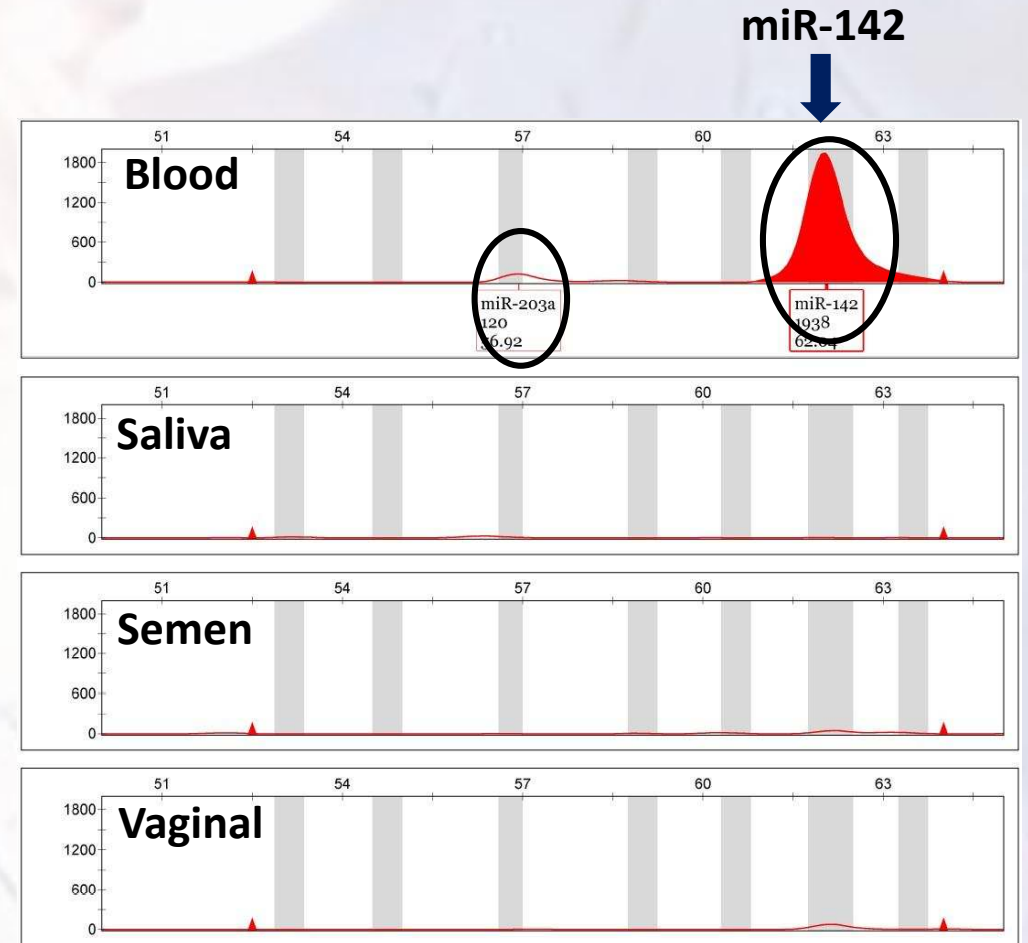
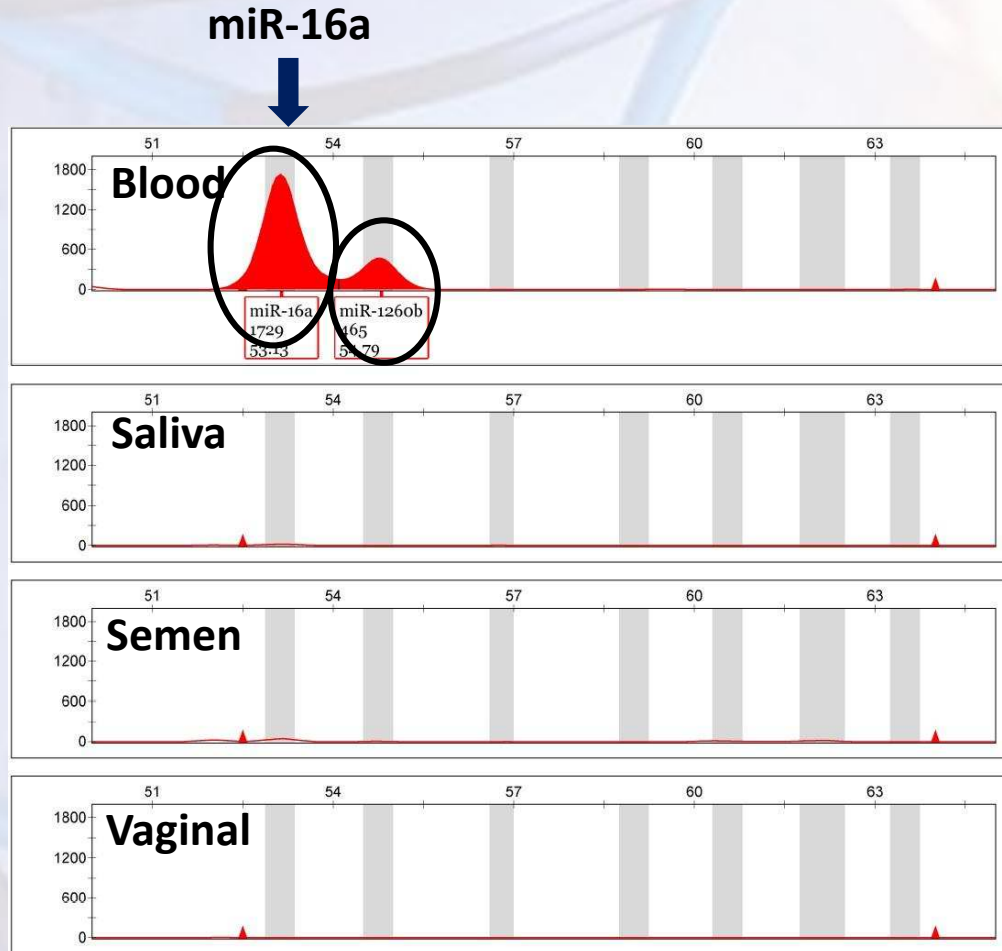
miR-10a



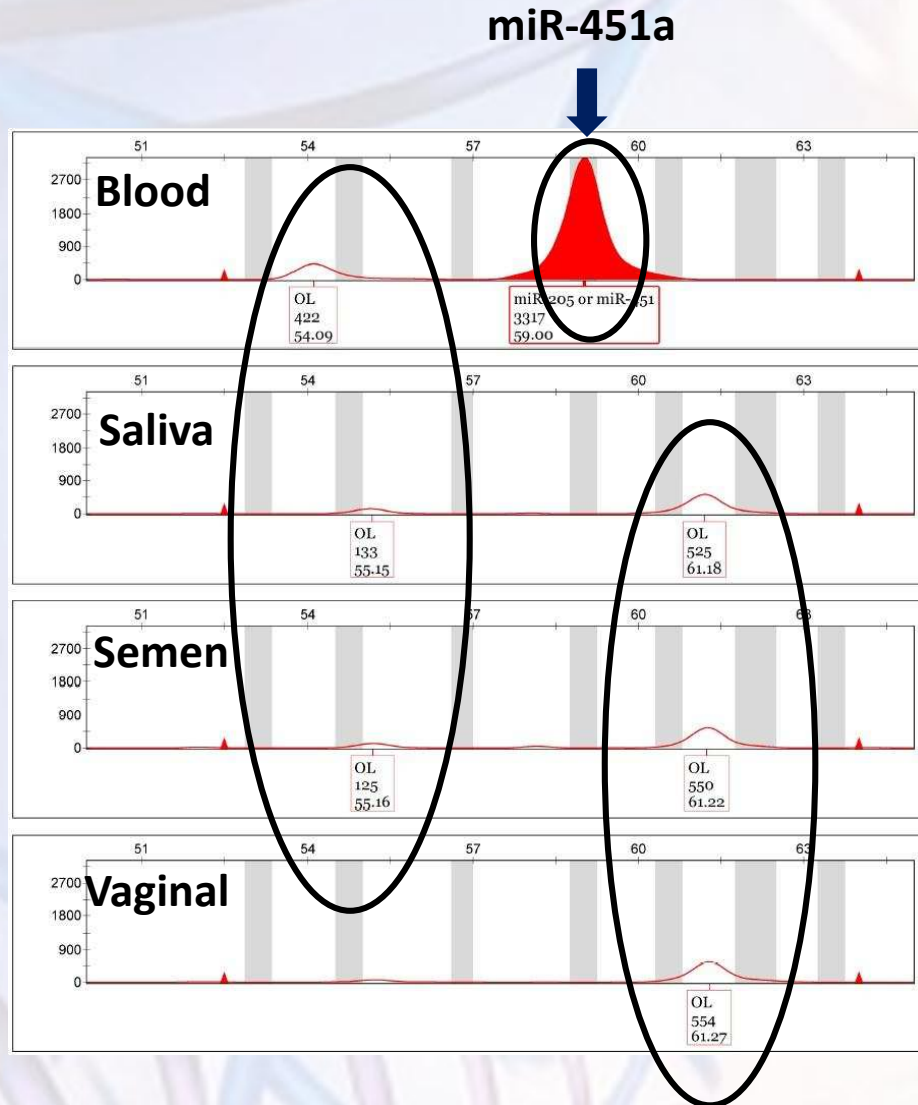
miR-135a



miR-16a and miR-142 are exclusively detected in blood



miR-451a is exclusively detected in blood



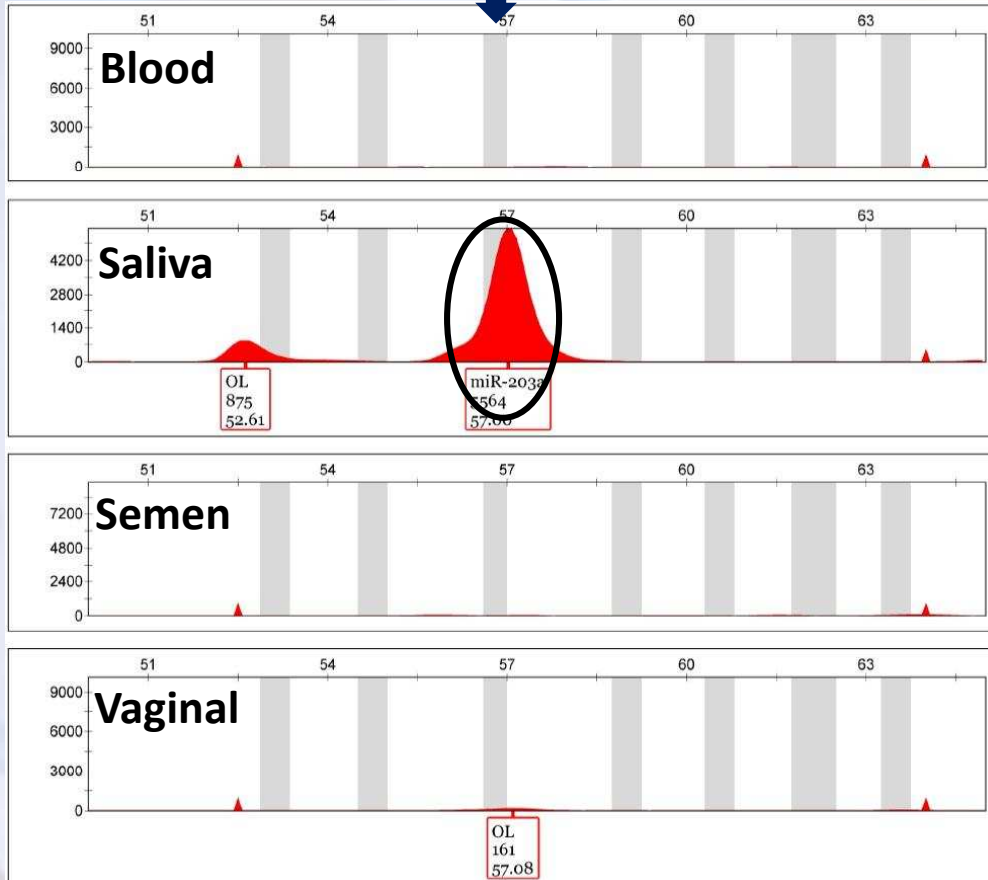
Much lower peaks of by-products found in all tissues

miR-203a is mainly detected in saliva

Expressed in epithelial cells

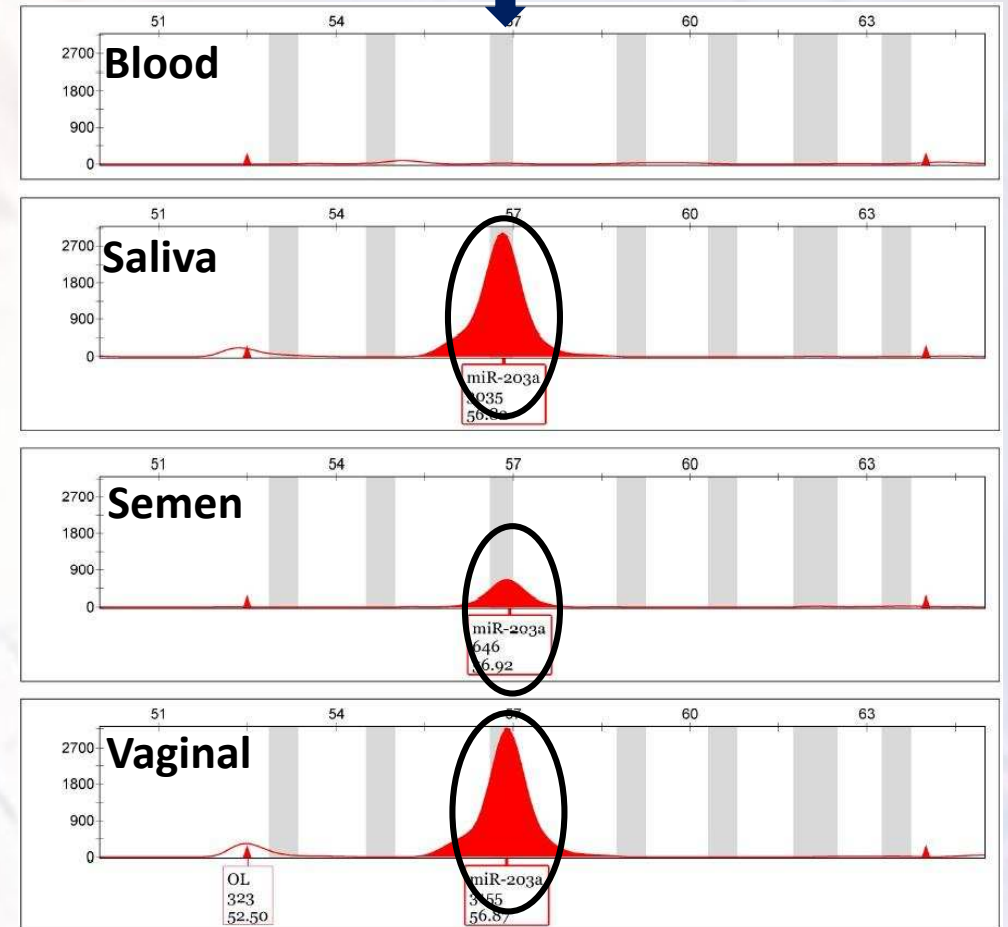
Sample set 1

miR-203a



Sample set 2

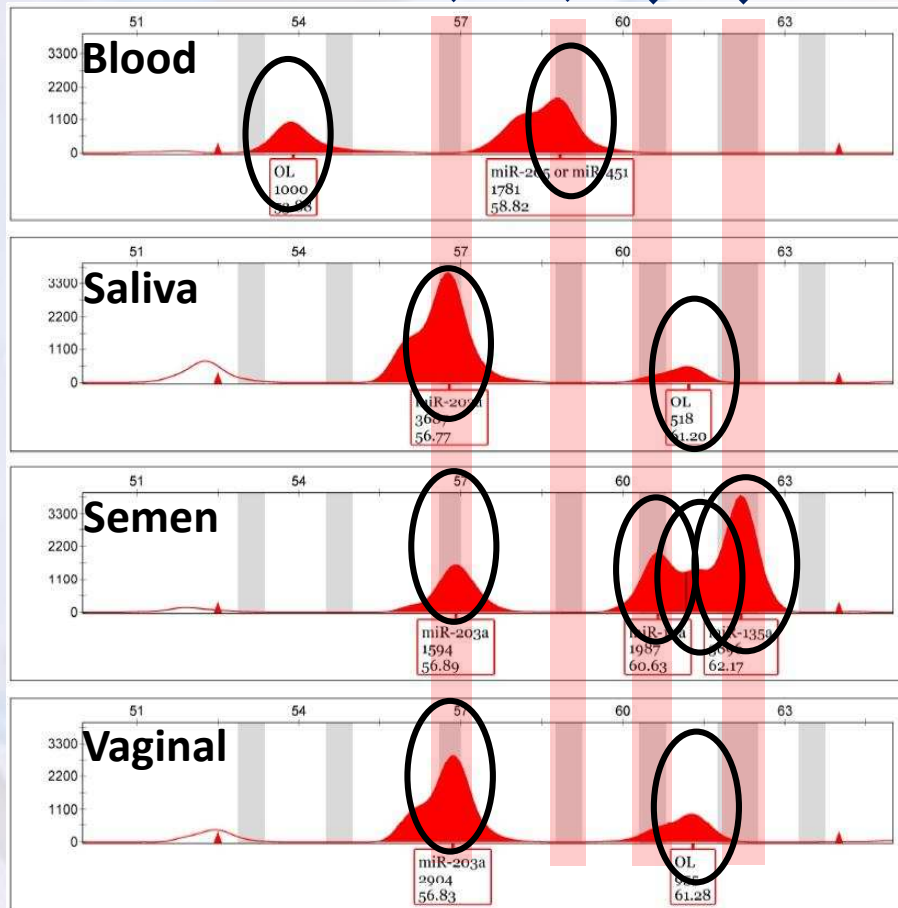
miR-203a



Multiplexing multiple markers yields expected results

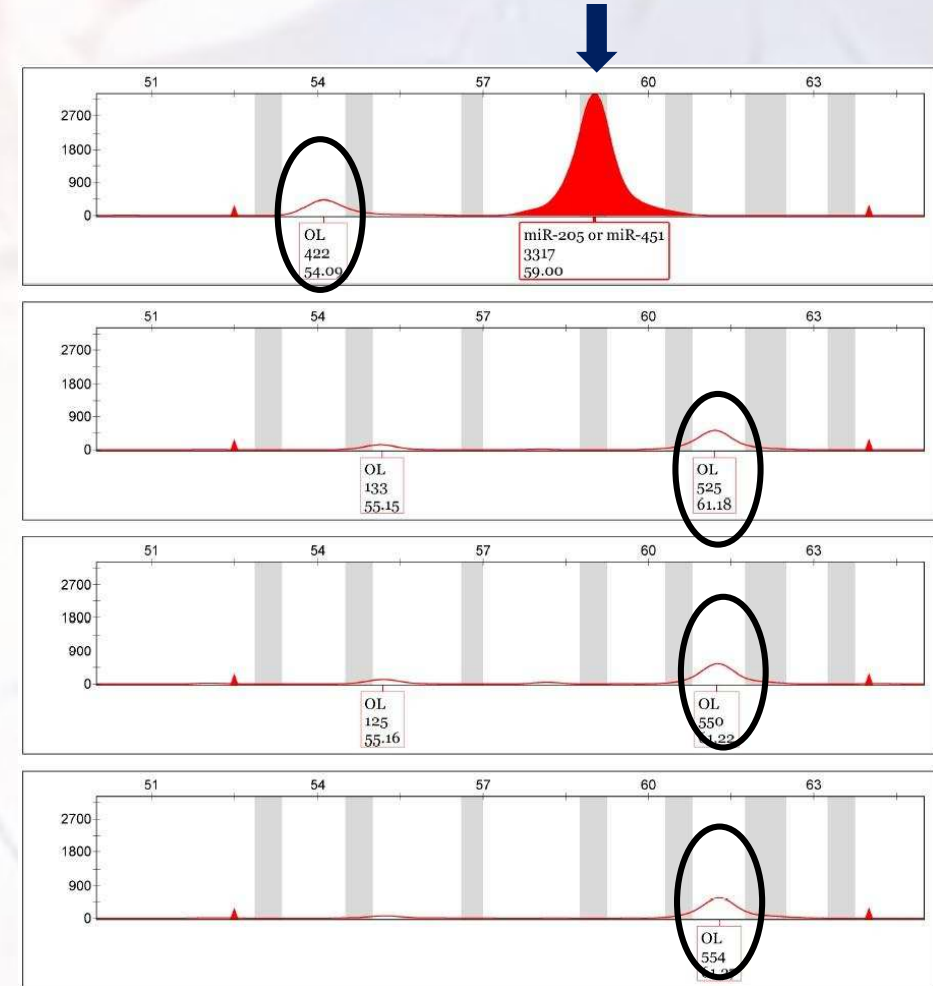
Multiplex

Saliva 203a
Blood 451a
Semen 10a
Semen 135a



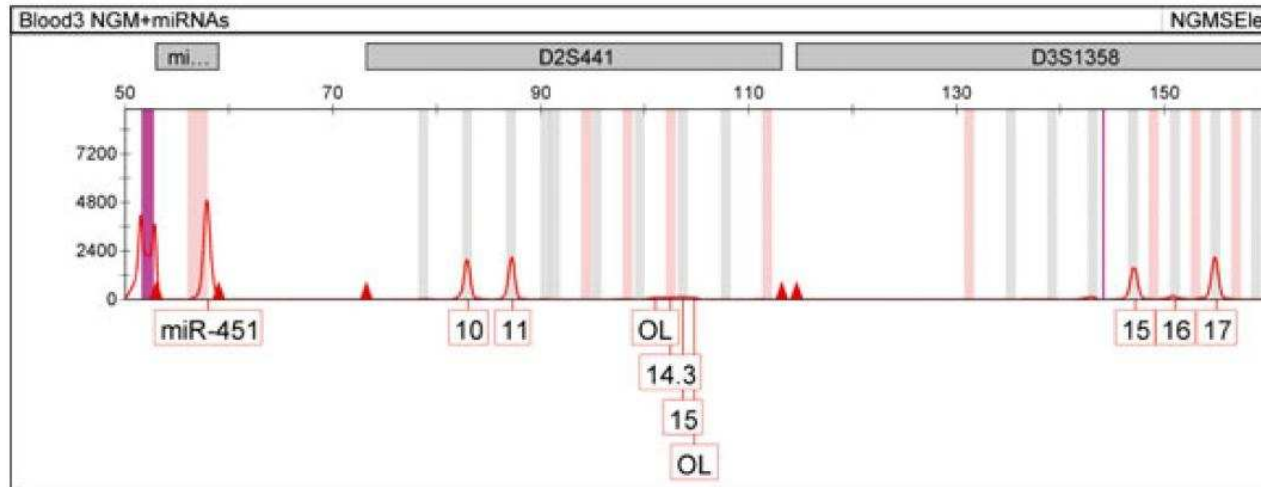
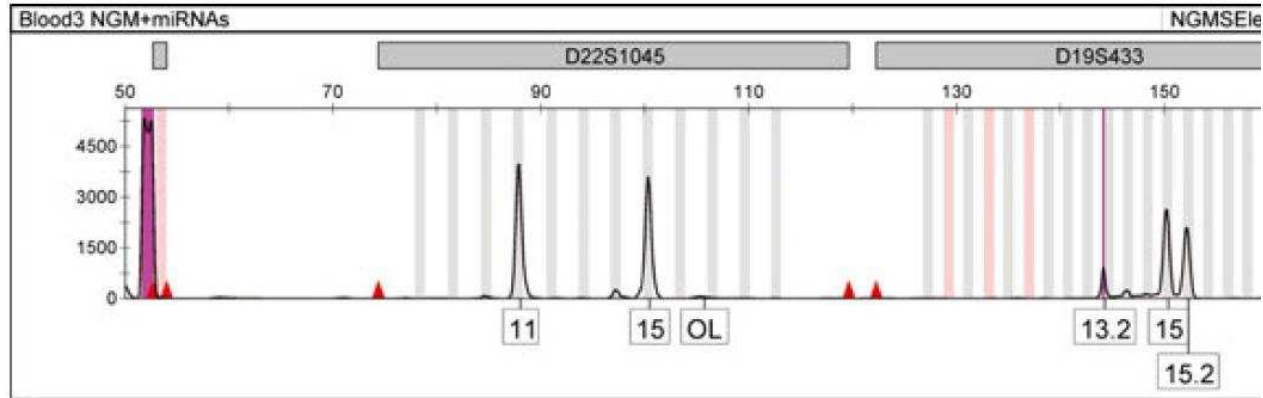
Singleplex

miR-451a

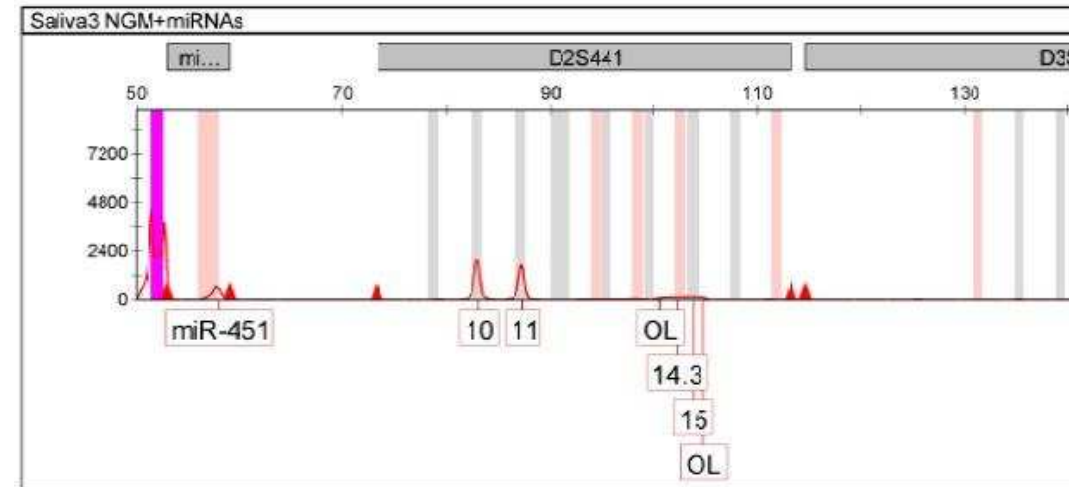
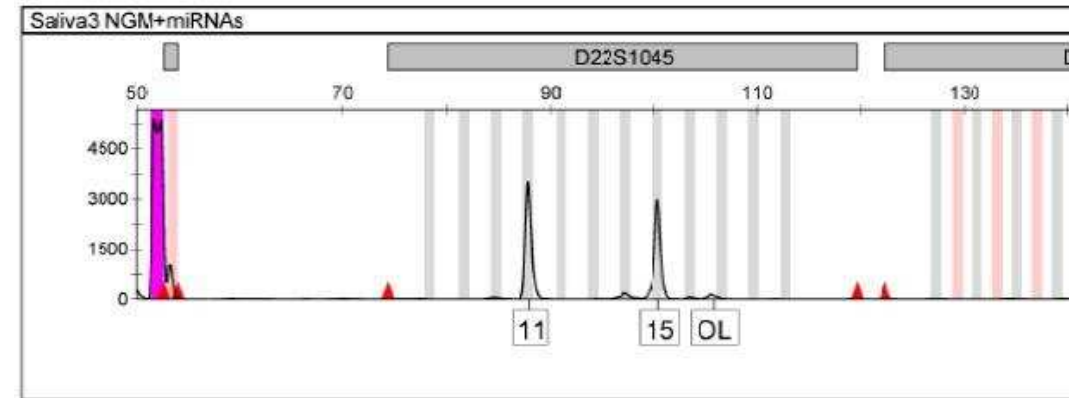


Multiplex with STR markers

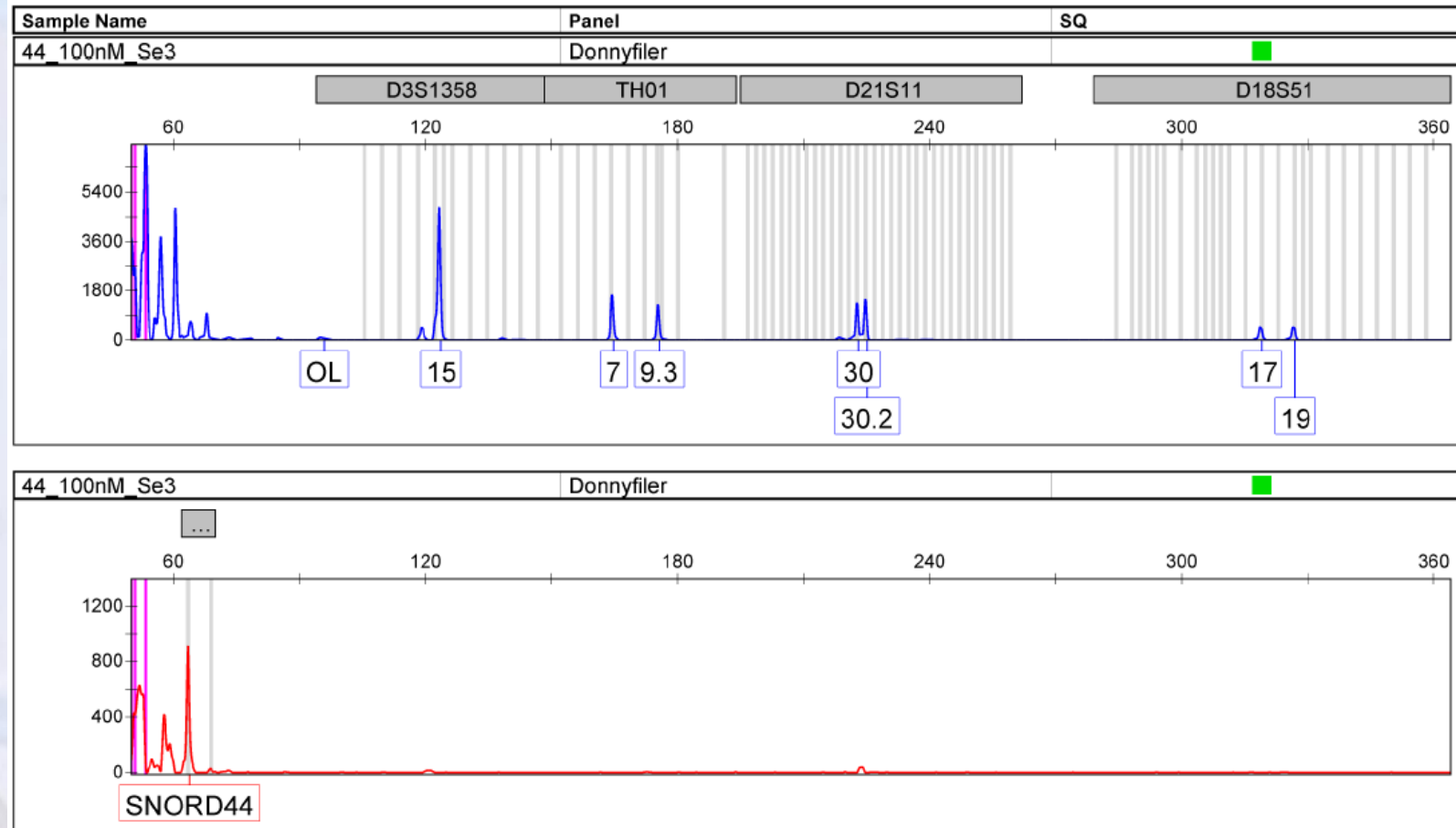
Blood



Saliva



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

The background features a light blue and white color palette with several semi-transparent DNA double helix structures and molecular diagrams scattered across the frame. The DNA strands are rendered in shades of blue and brown, while the molecular structures are depicted with thin lines and small spheres representing atoms.

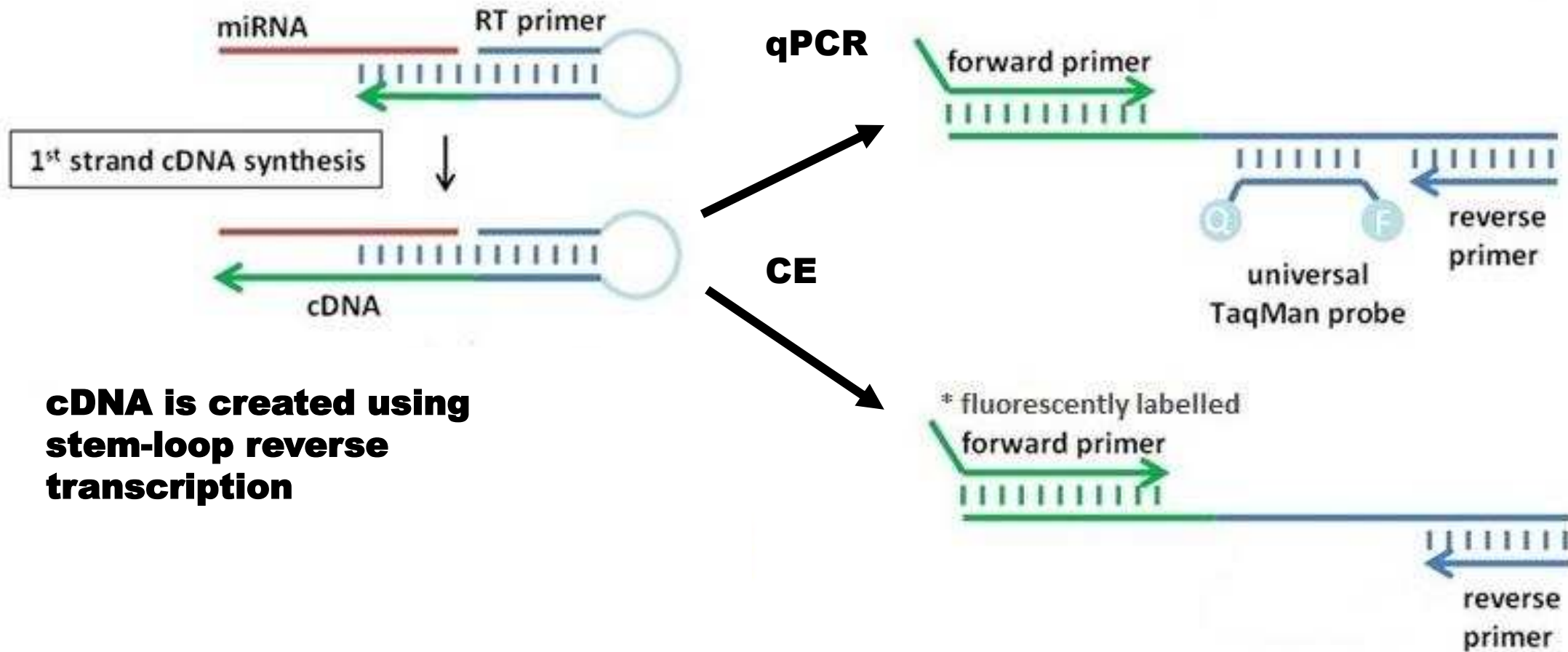
Thank you

d.vandermeer@hud.ac.uk



FSF Emerging Forensic Scientist Award Oral Presentation

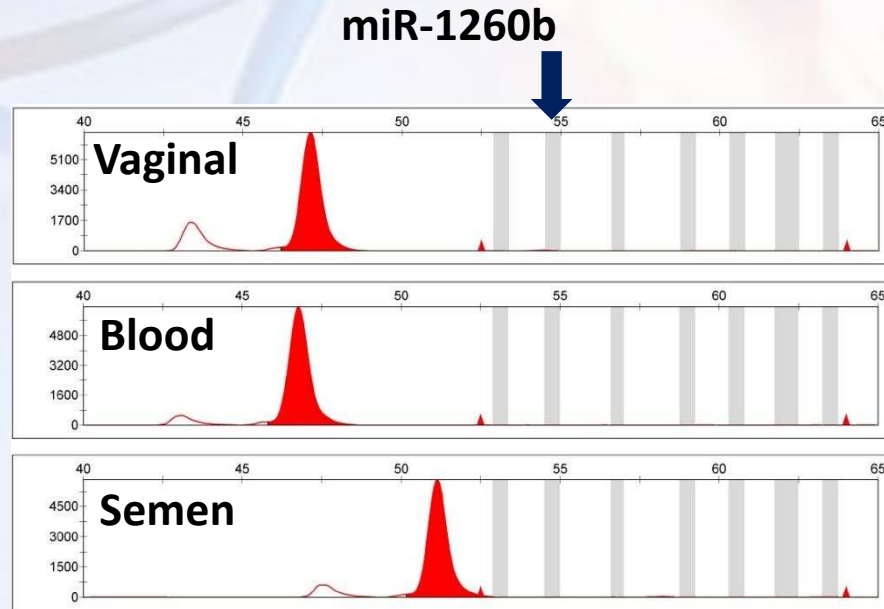
Our workflow



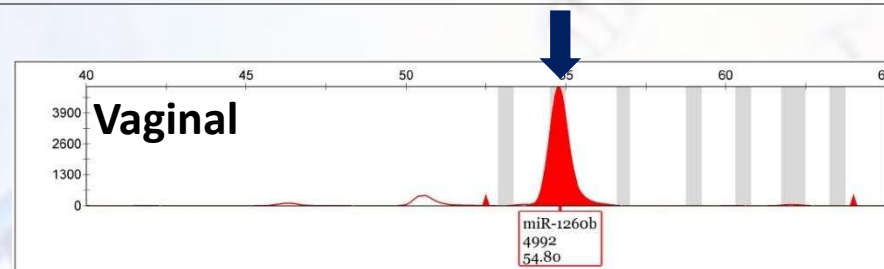
cDNA is created using stem-loop reverse transcription

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

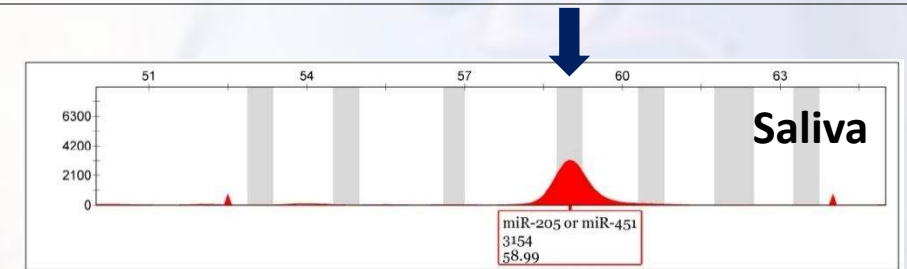
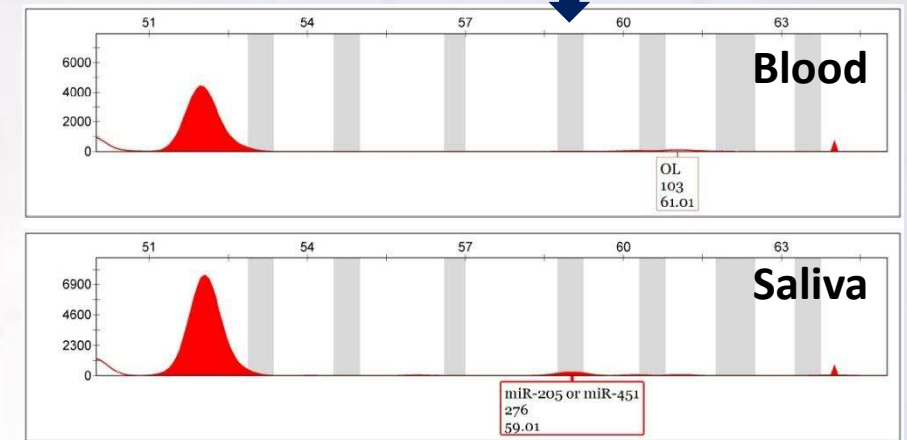
Multiplex RT



Singleplex RT

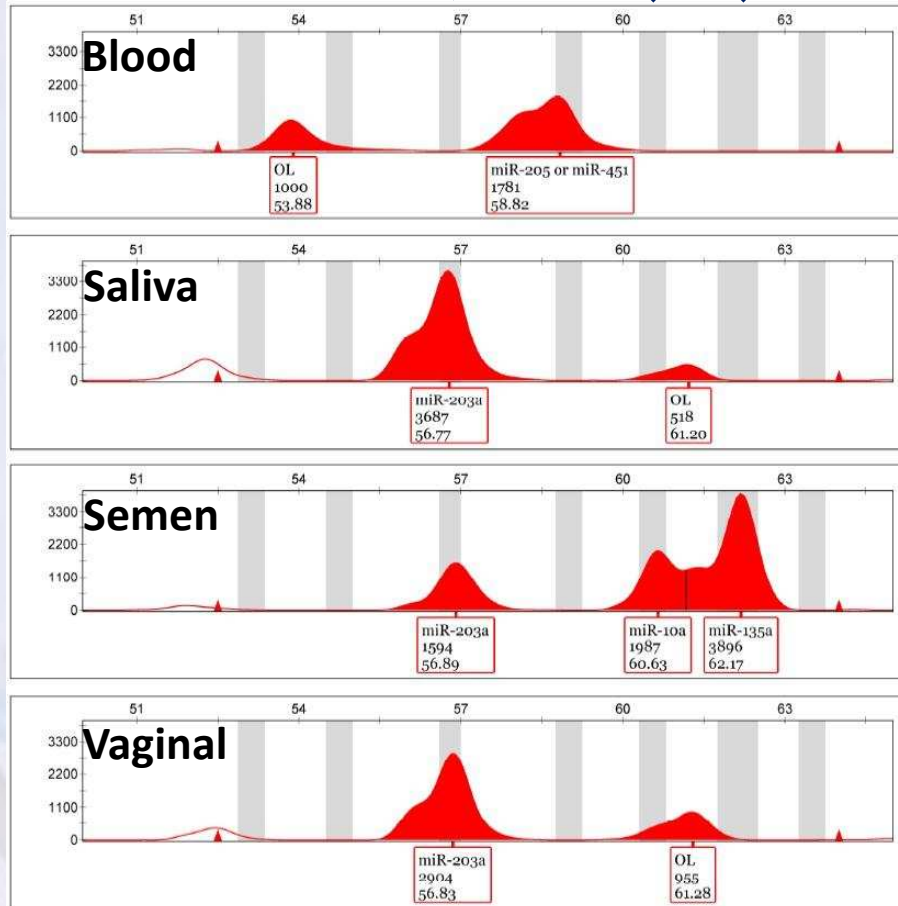


miR-205



Multiplexing multiple markers yields expected results

Vaginal 1260b
Saliva 203a
Blood 451a
Semen 10a
Semen 135a



Blood 16a

Saliva 205

Blood 142

Control SNORD44

