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

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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

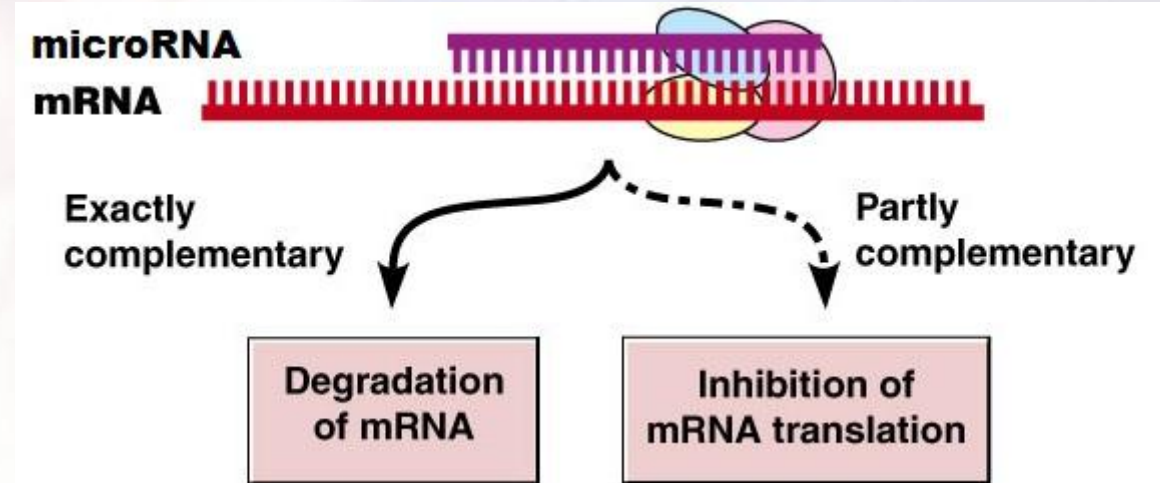
Advantages for forensic science

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

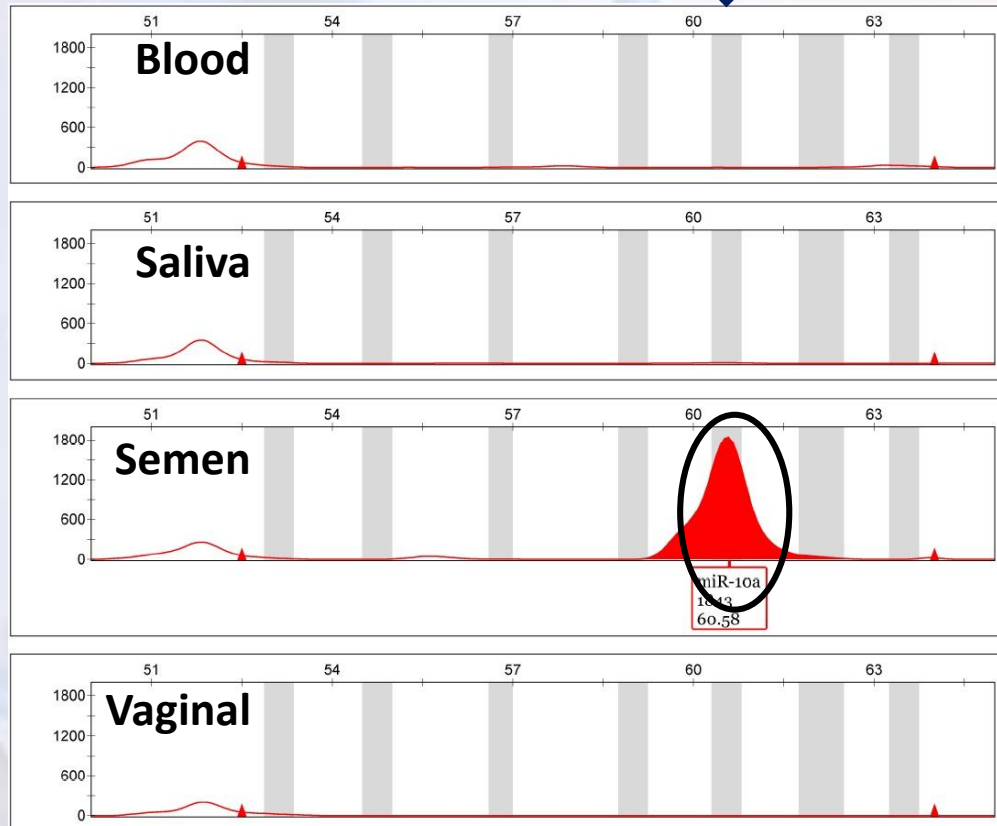
- Analyse microRNAs with capillary electrophoresis (CE)
 - Multiplex microRNAs in single test
 - Possibility to combine microRNA analysis with DNA profiling

Methods and materials

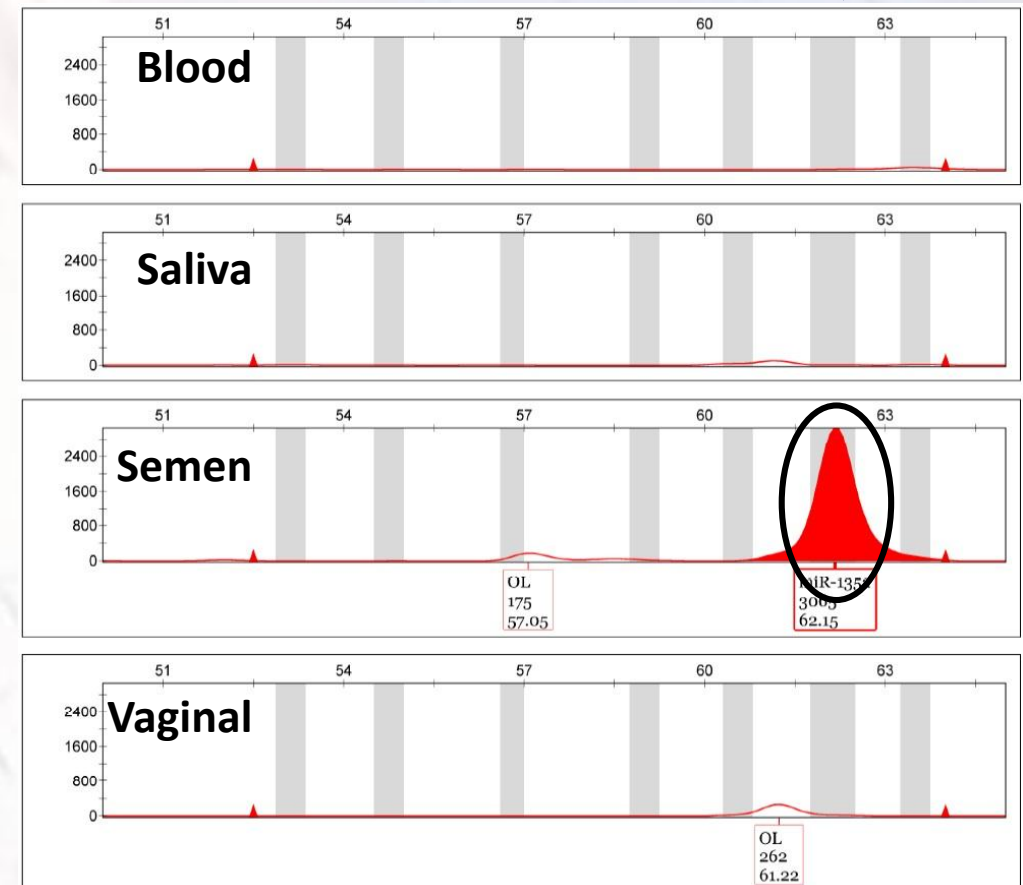
- 5 samples of 4 tissue types
 - Blood, saliva, semen and vaginal material
- DNA extraction
- Normalised to 0.5ng/μl human DNA
- Tested for 9 markers
- Multiplex stem-loop reverse transcription
- ROX-labelled primers

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

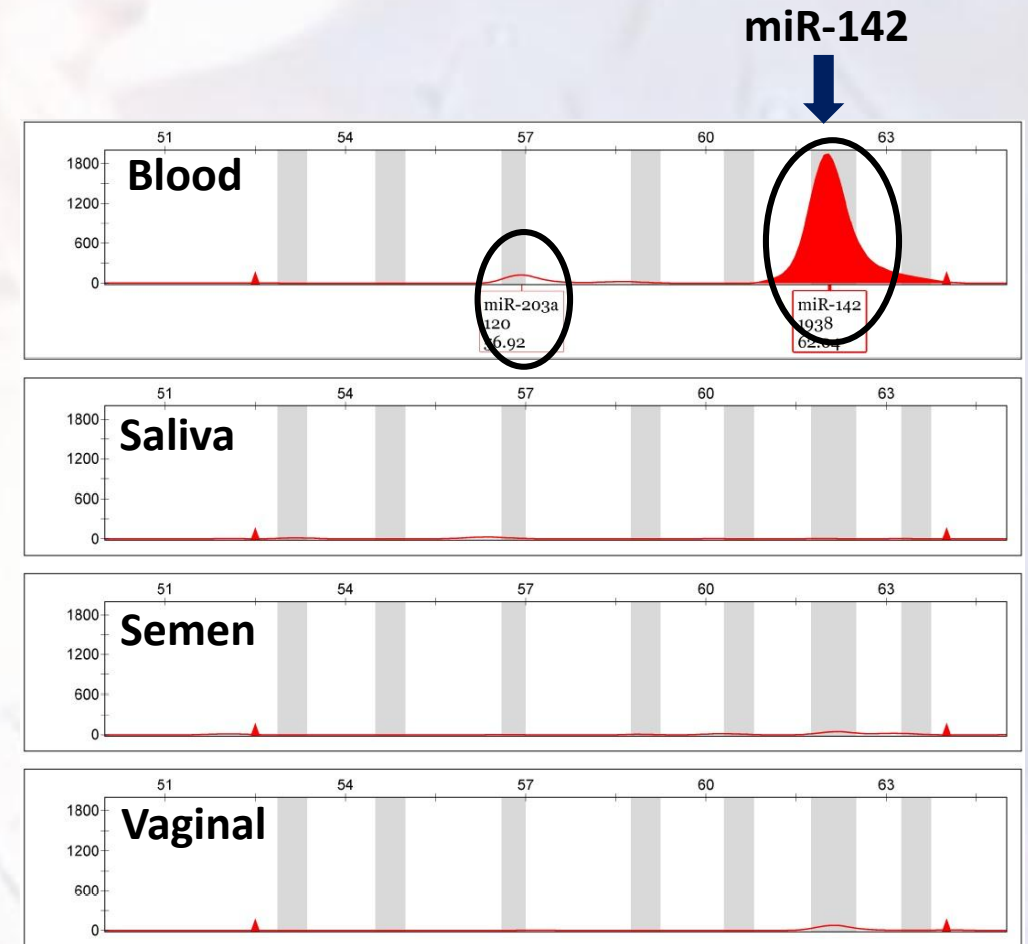
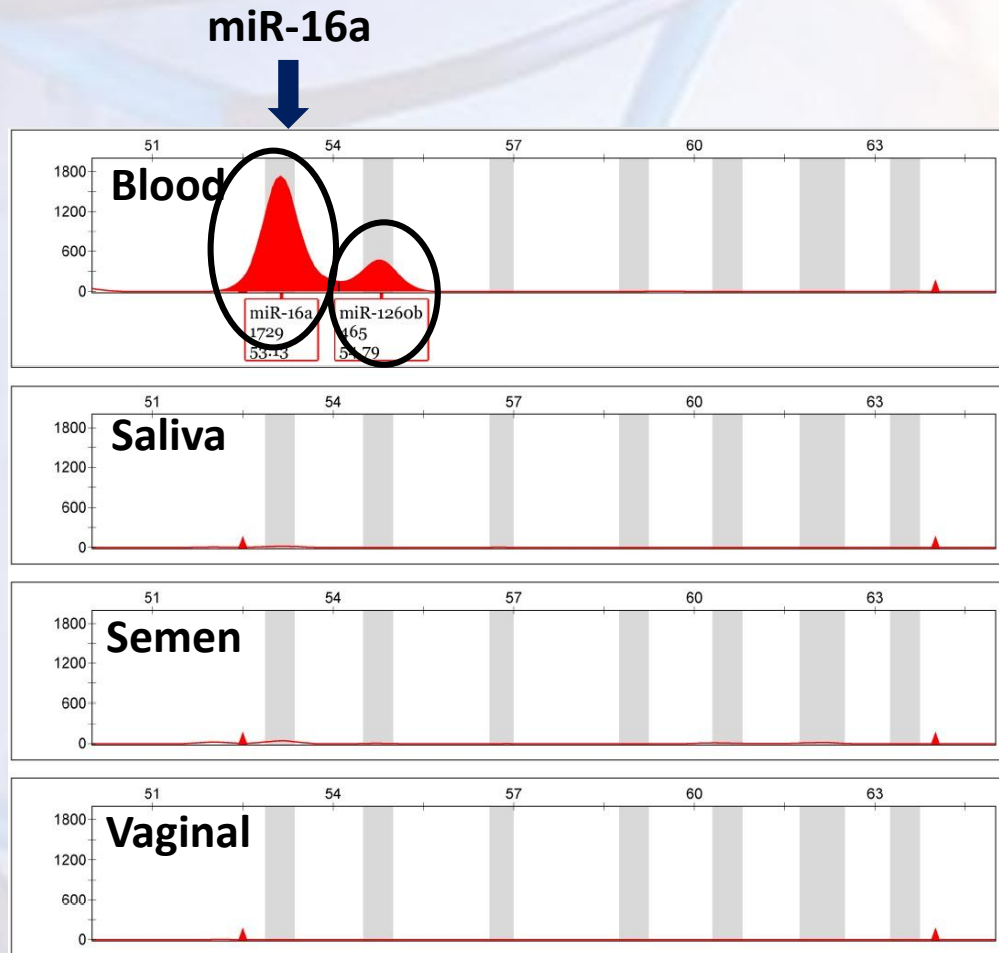
miR-10a



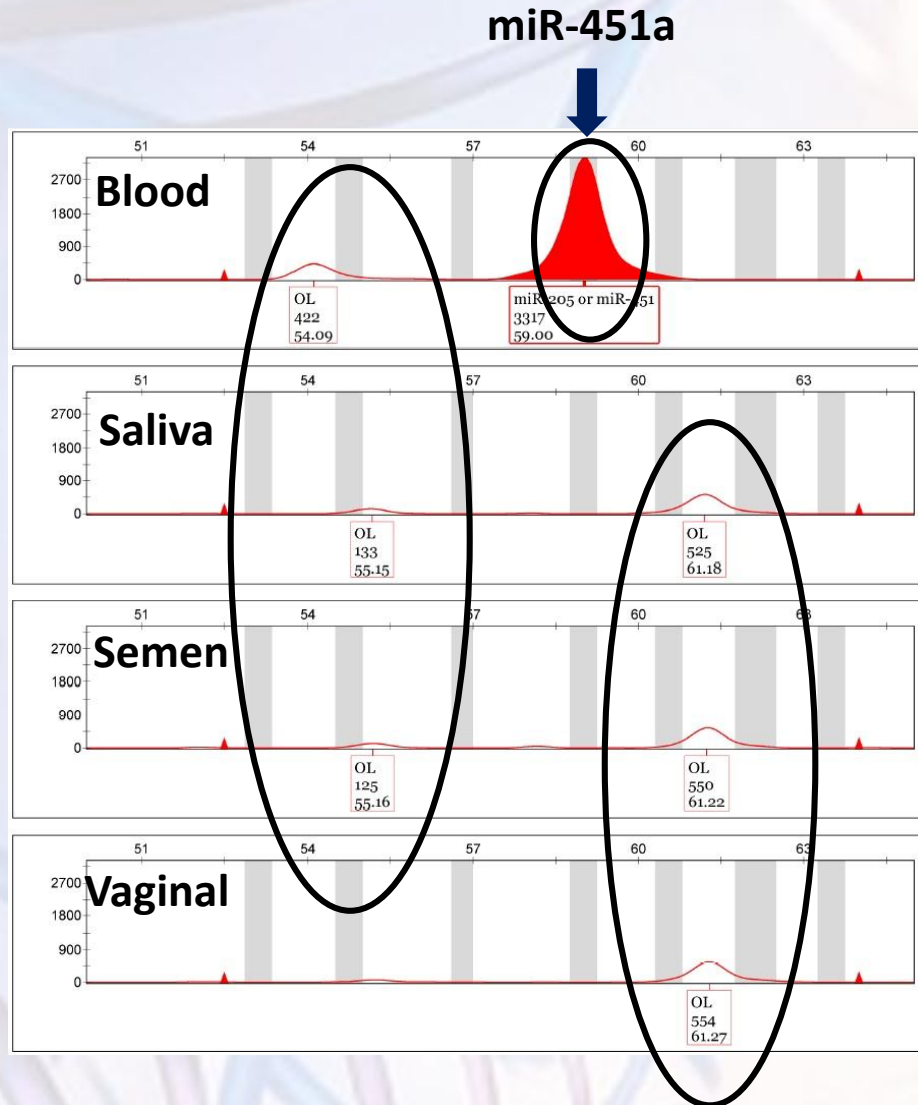
miR-135a



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



miR-451a is exclusively detected in blood



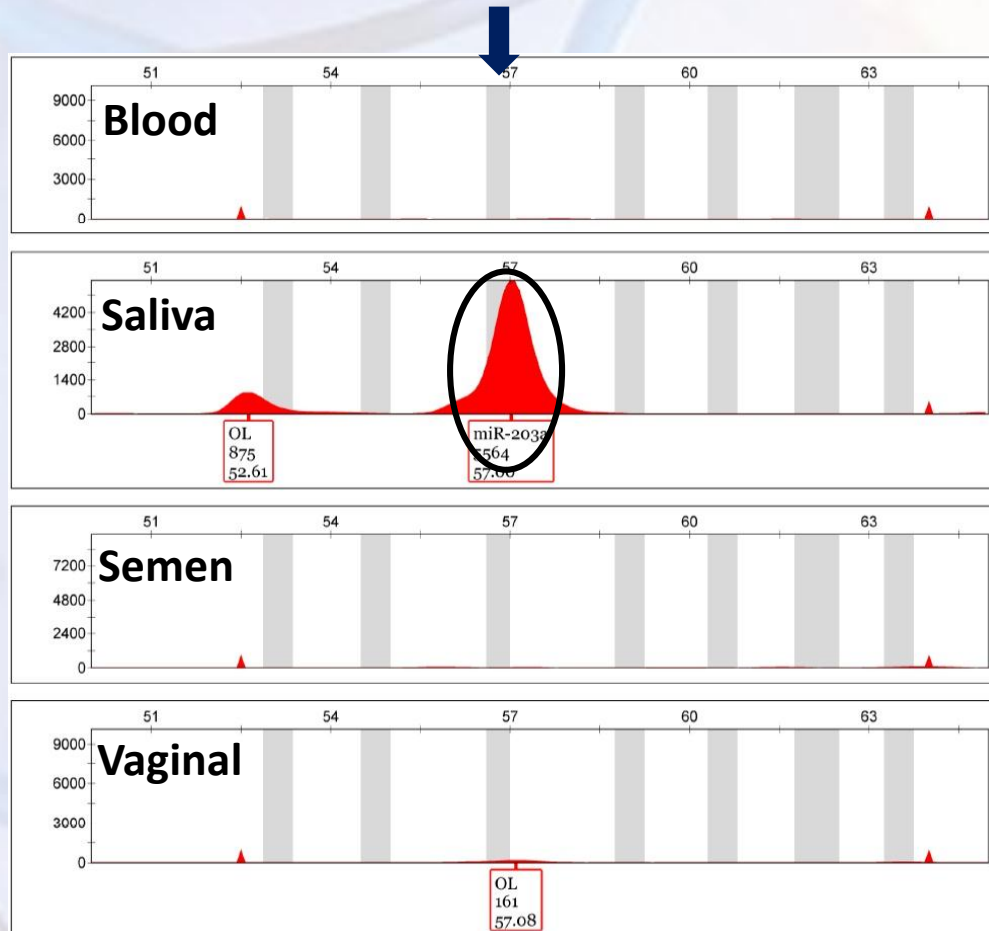
Much lower peaks of
by-products found in all
tissues

miR-203a is mainly detected in saliva

Expressed in epithelial cells

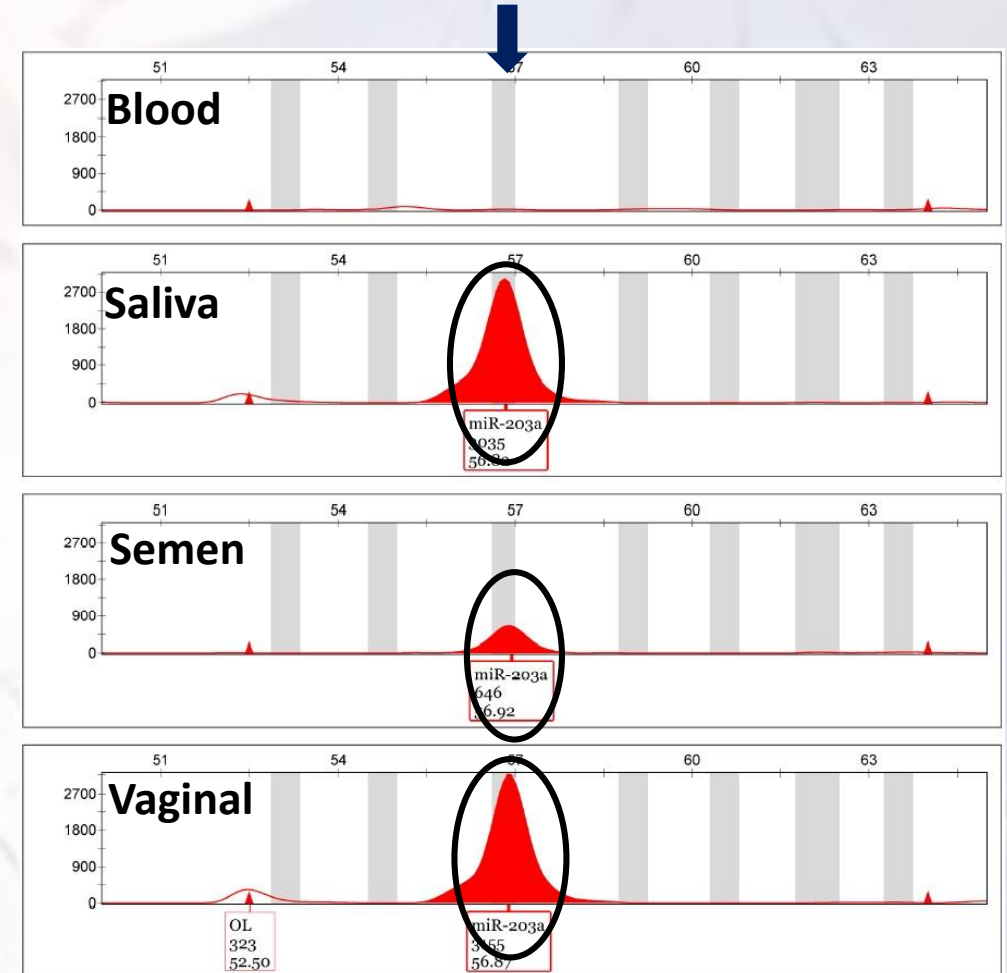
Sample set 1

miR-203a



Sample set 2

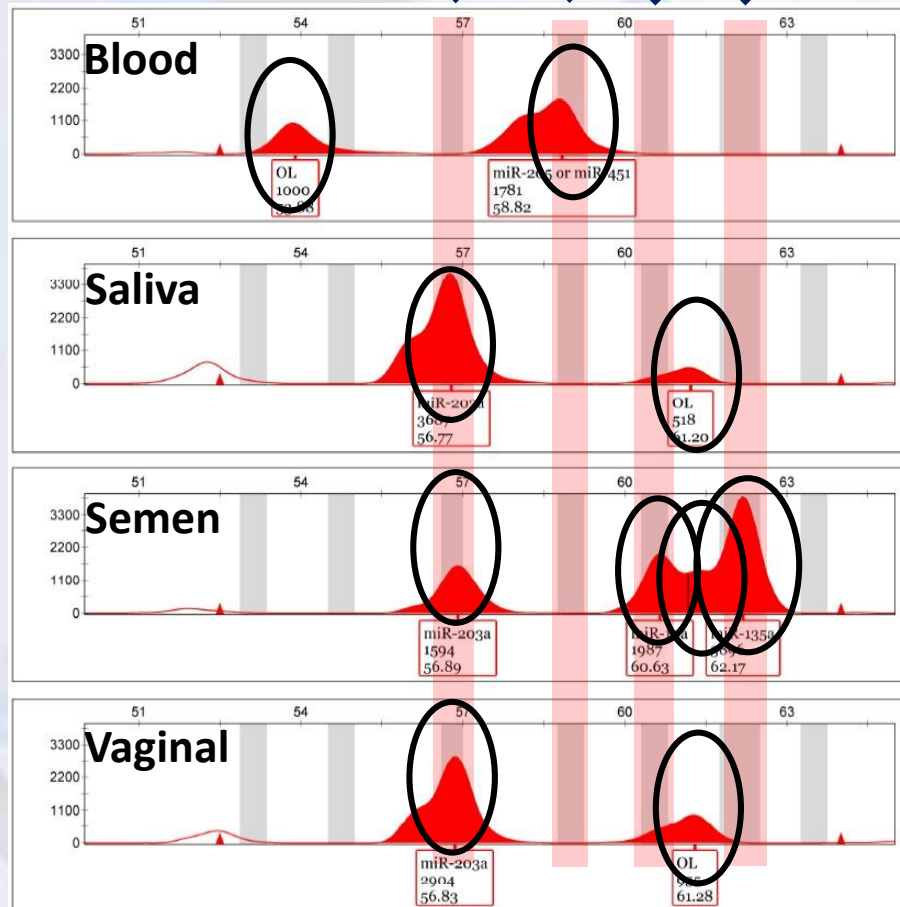
miR-203a



Multiplexing multiple markers yields expected results

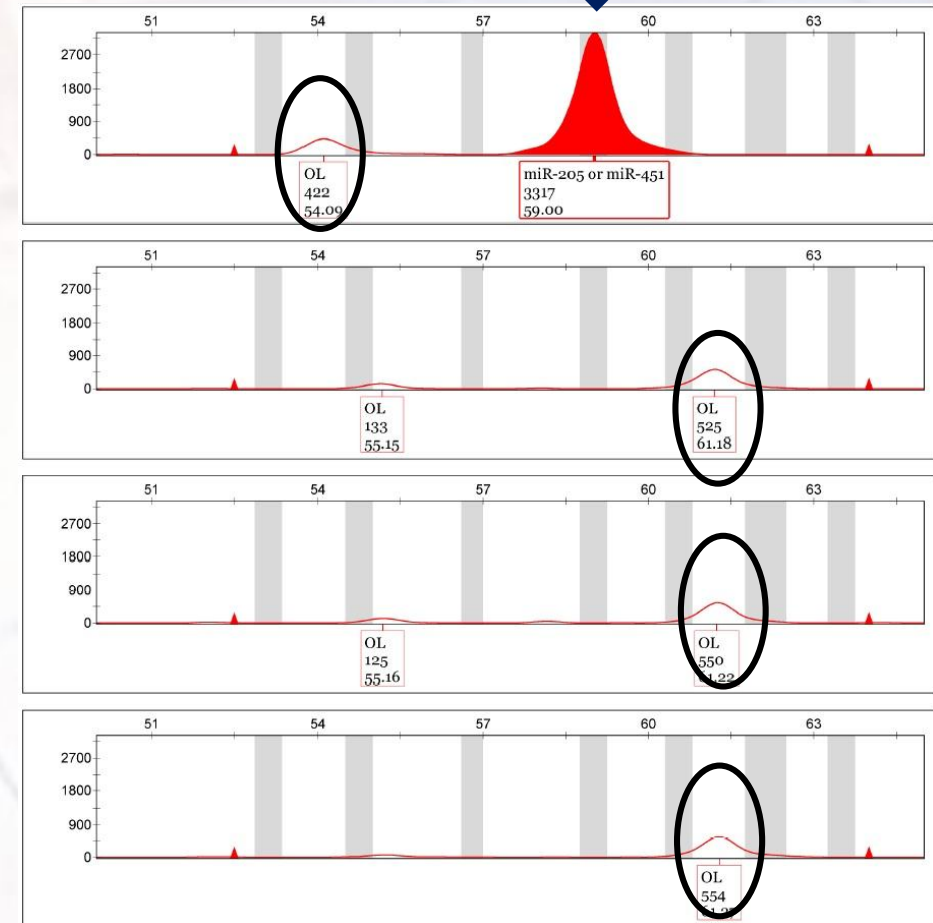
Multiplex

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



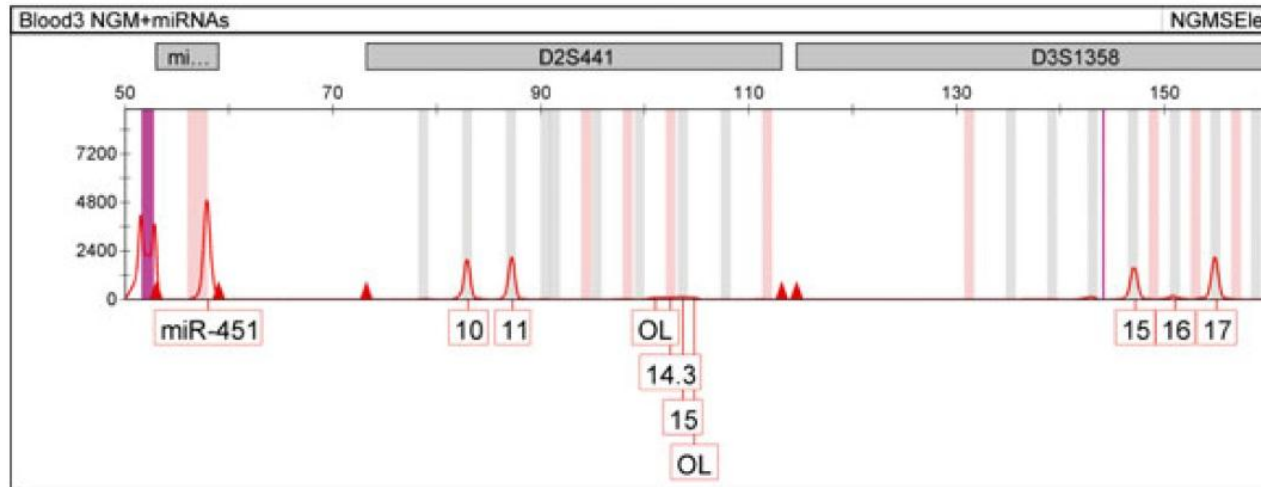
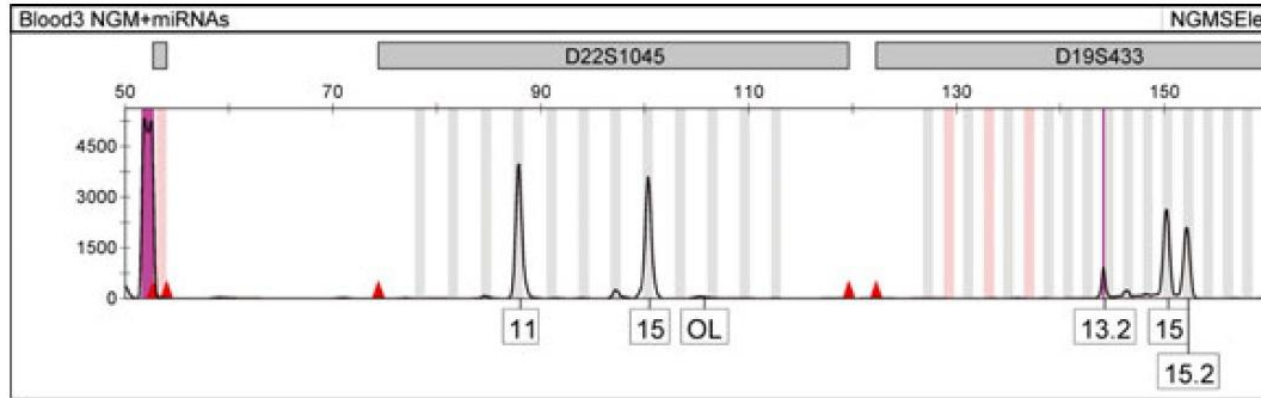
Singleplex

miR-451a

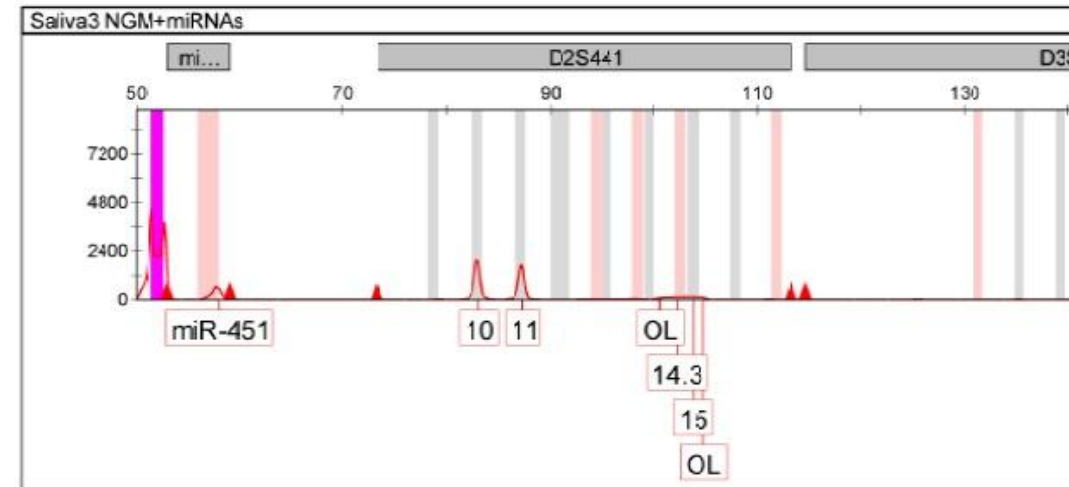
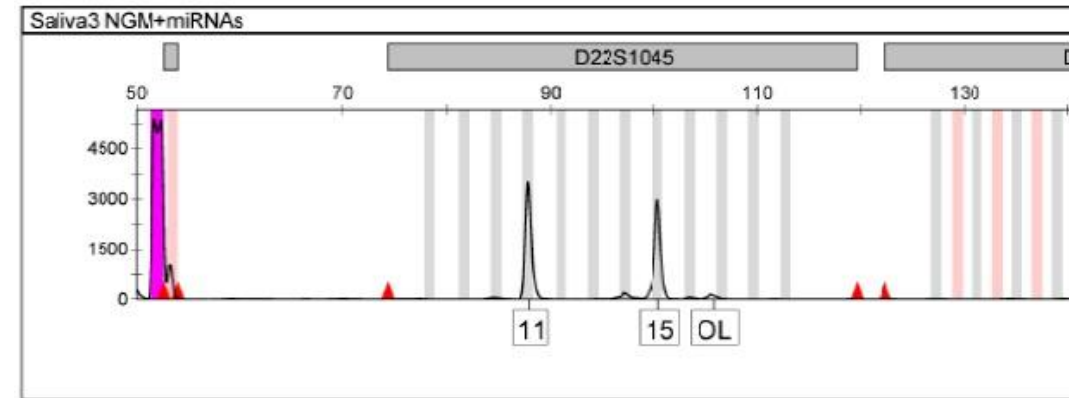


Multiplex with STR markers

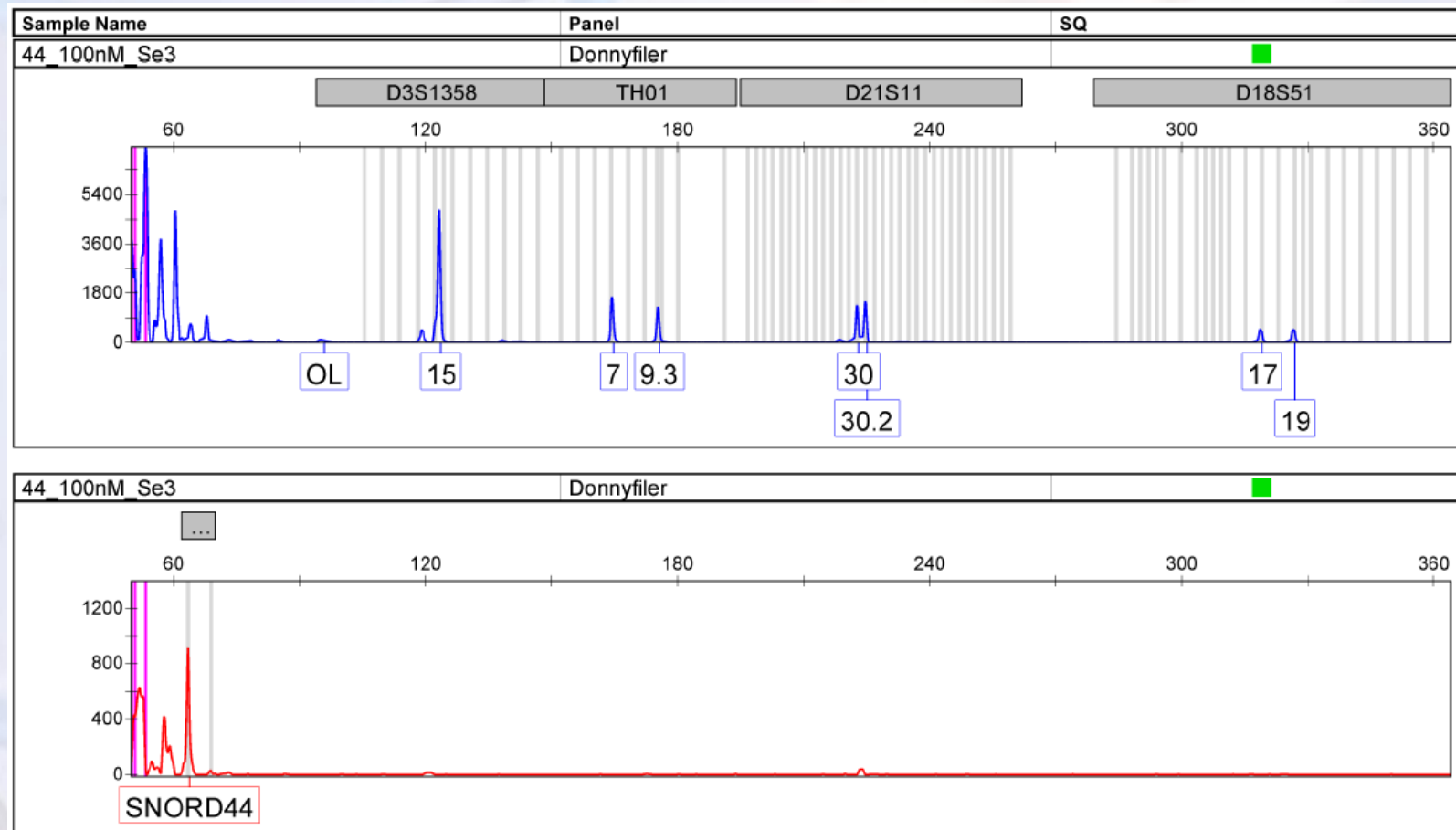
Blood



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 soft, artistic illustration of DNA double helices in shades of blue and purple, intertwined with a glowing, golden molecular structure that resembles a protein or a complex enzyme. The overall aesthetic is scientific and ethereal.

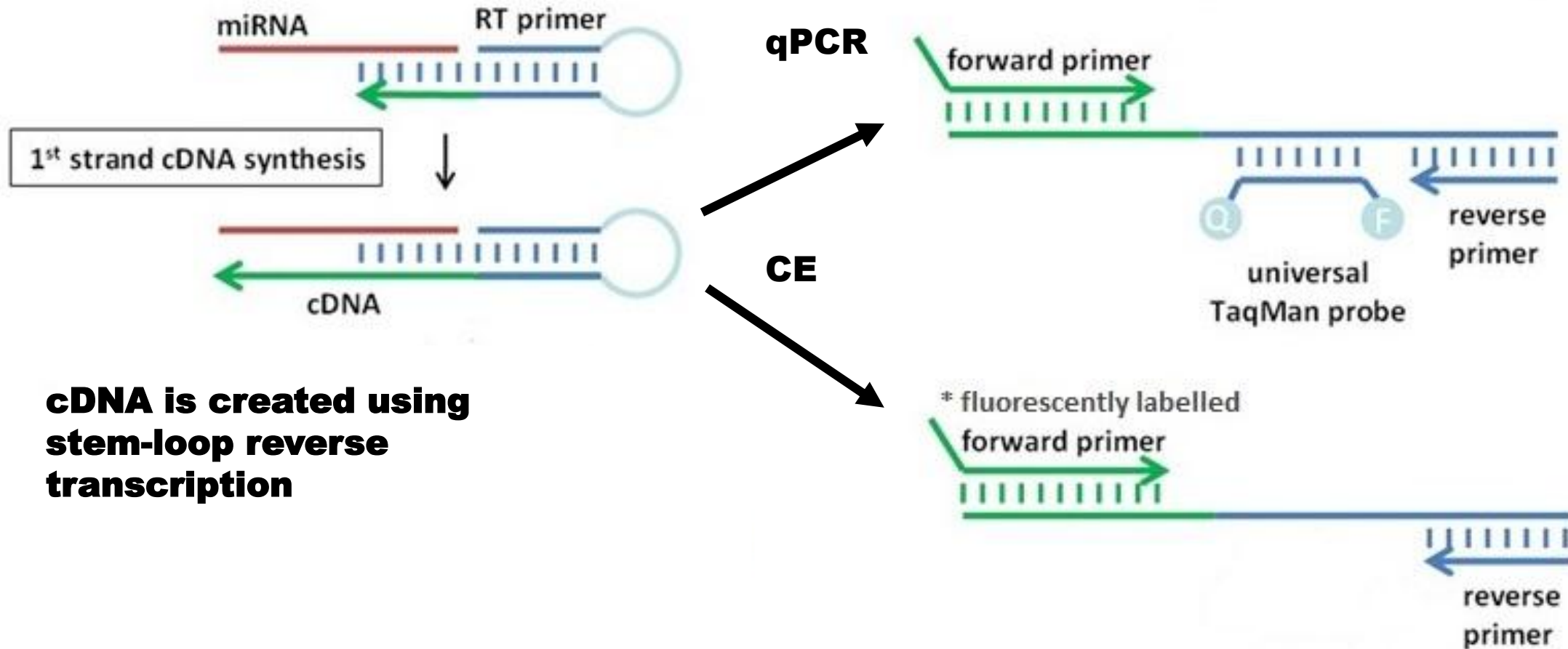
Thank you

d.vandermeer@hud.ac.uk



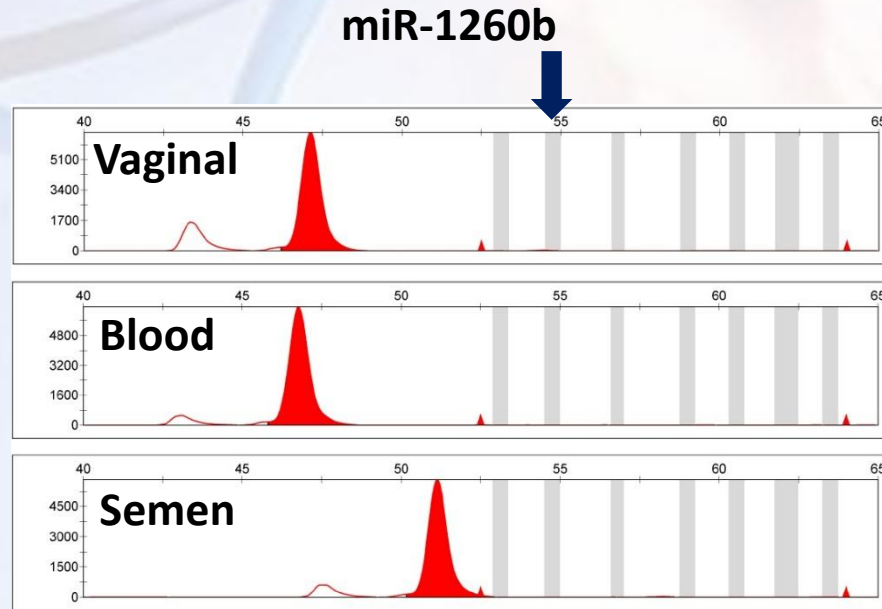
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Our workflow

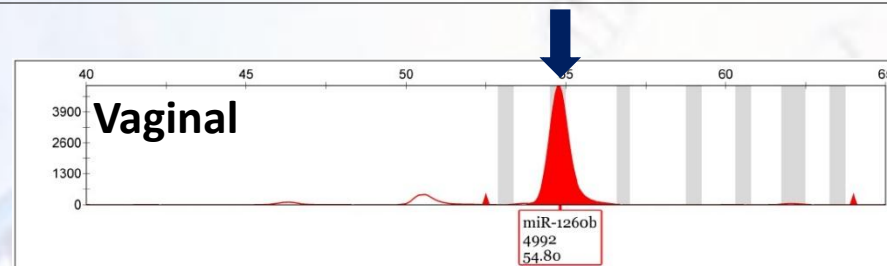


miR-1260b and miR-205 fail due to multiplexing reverse transcription

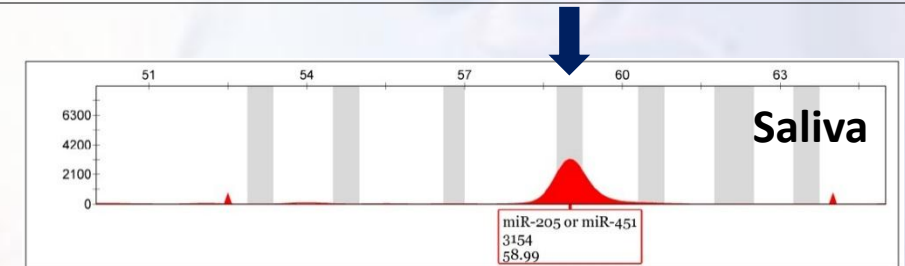
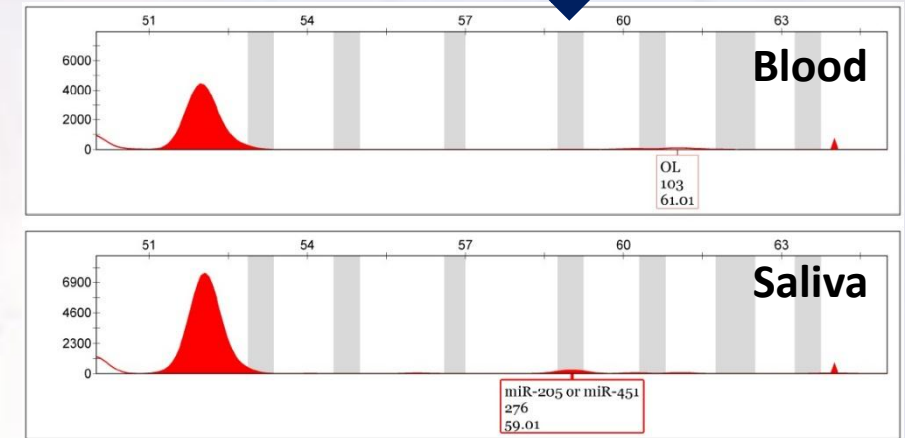
Multiplex RT



Singleplex RT



miR-205



Multiplexing multiple markers yields expected results

