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

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Supervisor: Dr Graham Williams



*University of*  
**HUDDERSFIELD**  
Inspiring tomorrow's professionals



## 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

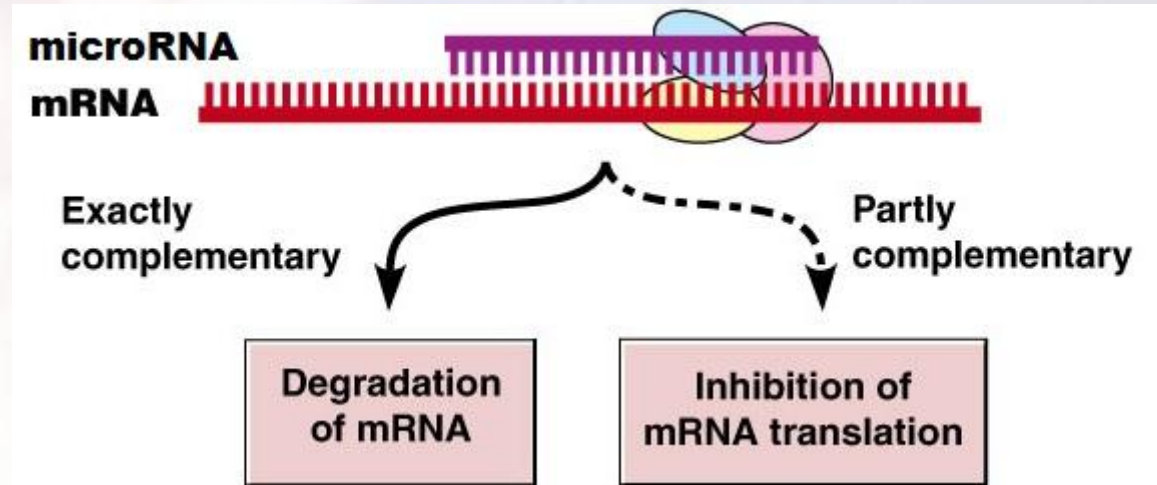
## Advantages for forensic science

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

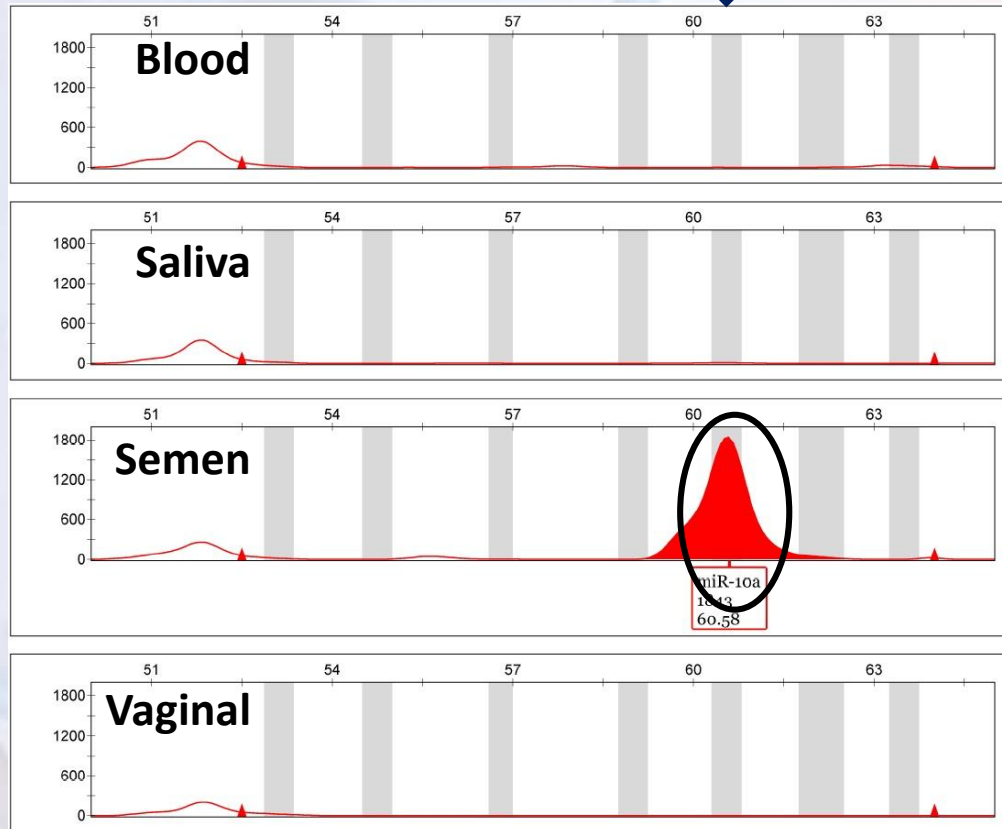
- Analyse microRNAs with capillary electrophoresis (CE)
  - Multiplex microRNAs in single test
  - Possibility to combine microRNA analysis with DNA profiling

# Methods and materials

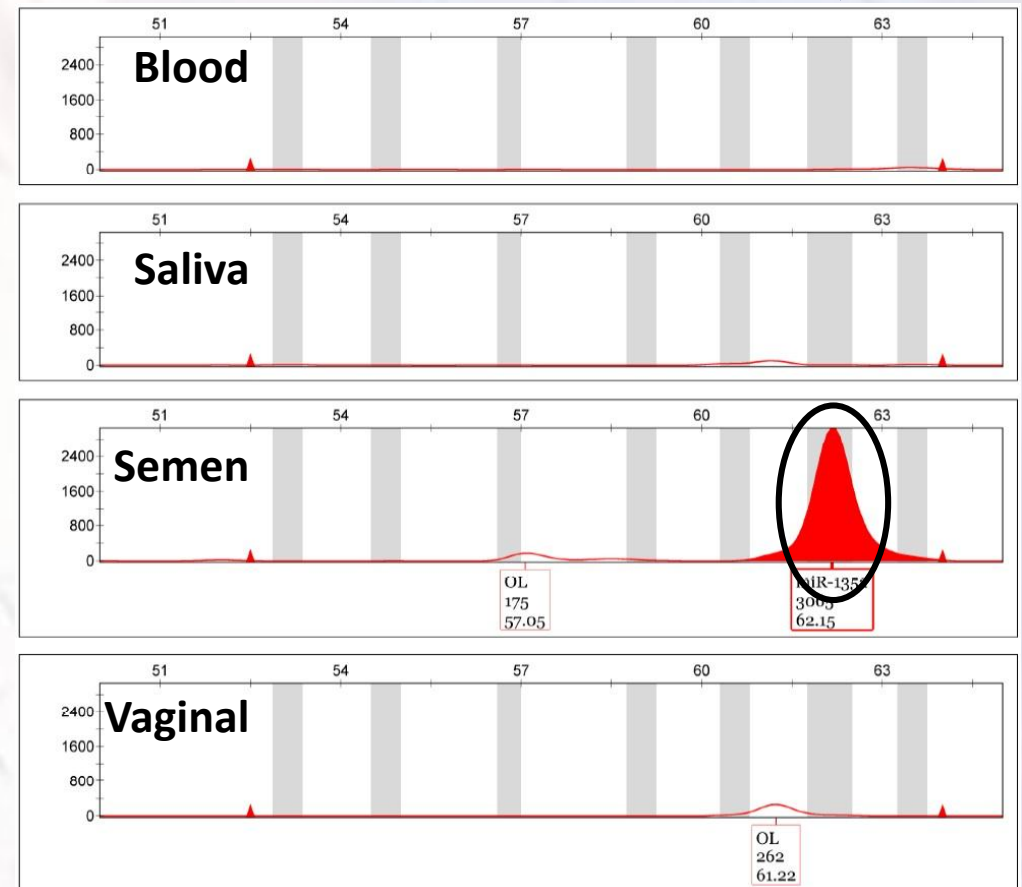
- 5 samples of 4 tissue types
  - Blood, saliva, semen and vaginal material
- DNA extraction
- Normalised to 0.5ng/ $\mu$ l human DNA
- Tested for 9 markers
- Multiplex stem-loop reverse transcription
- ROX-labelled primers

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

miR-10a

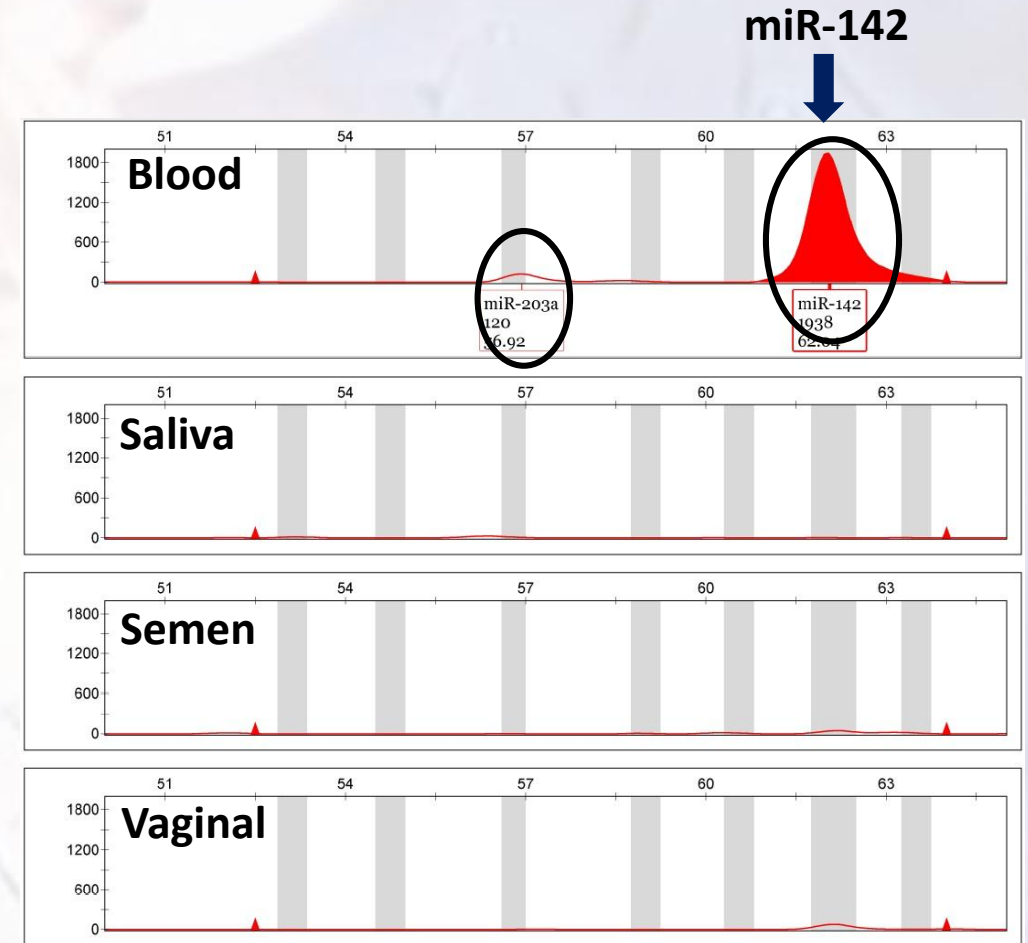
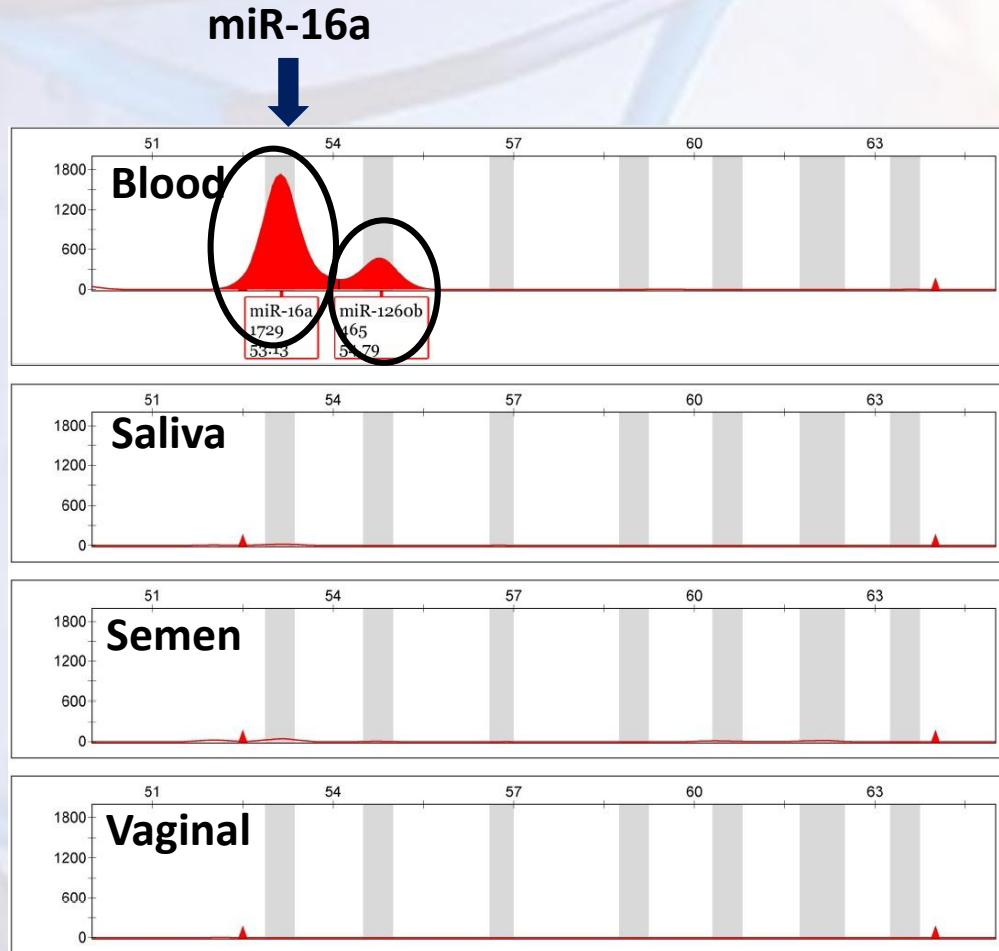


miR-135a

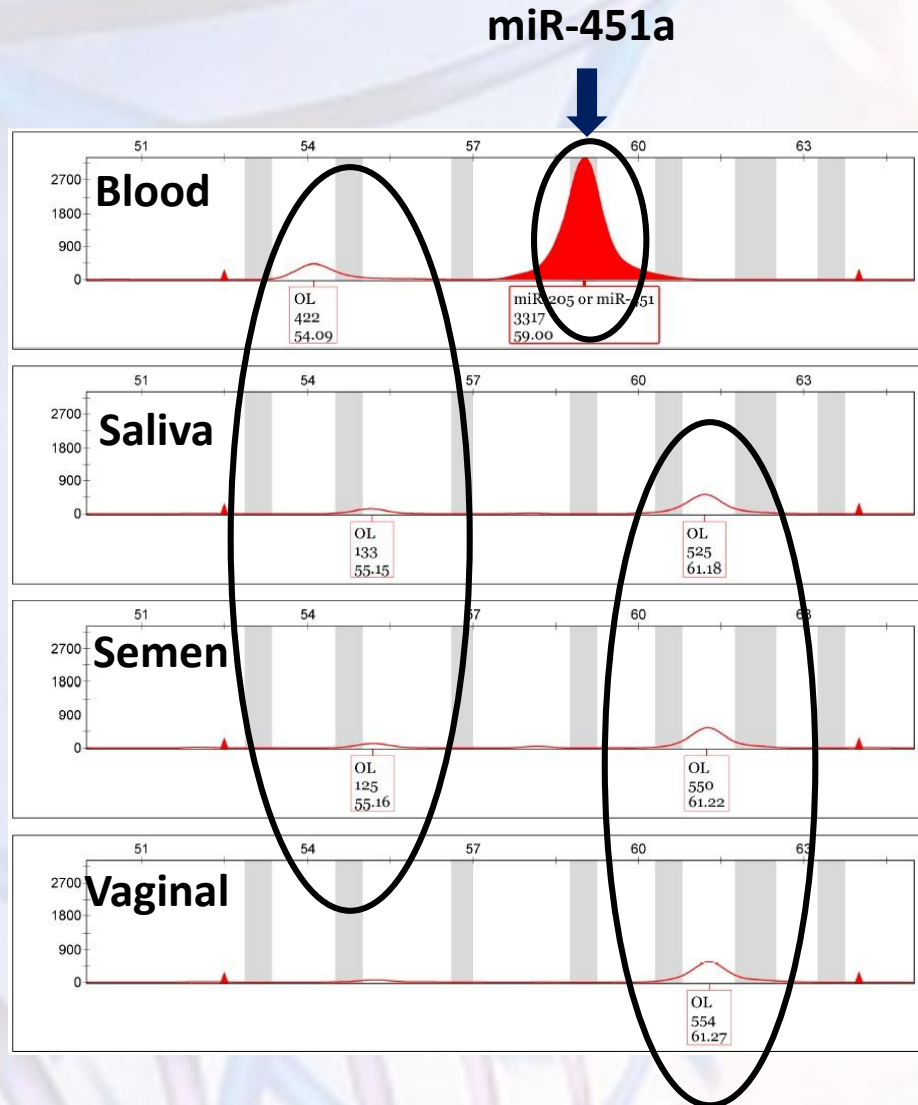




# miR-16a and miR-142 are exclusively detected in blood



# miR-451a is exclusively detected in blood



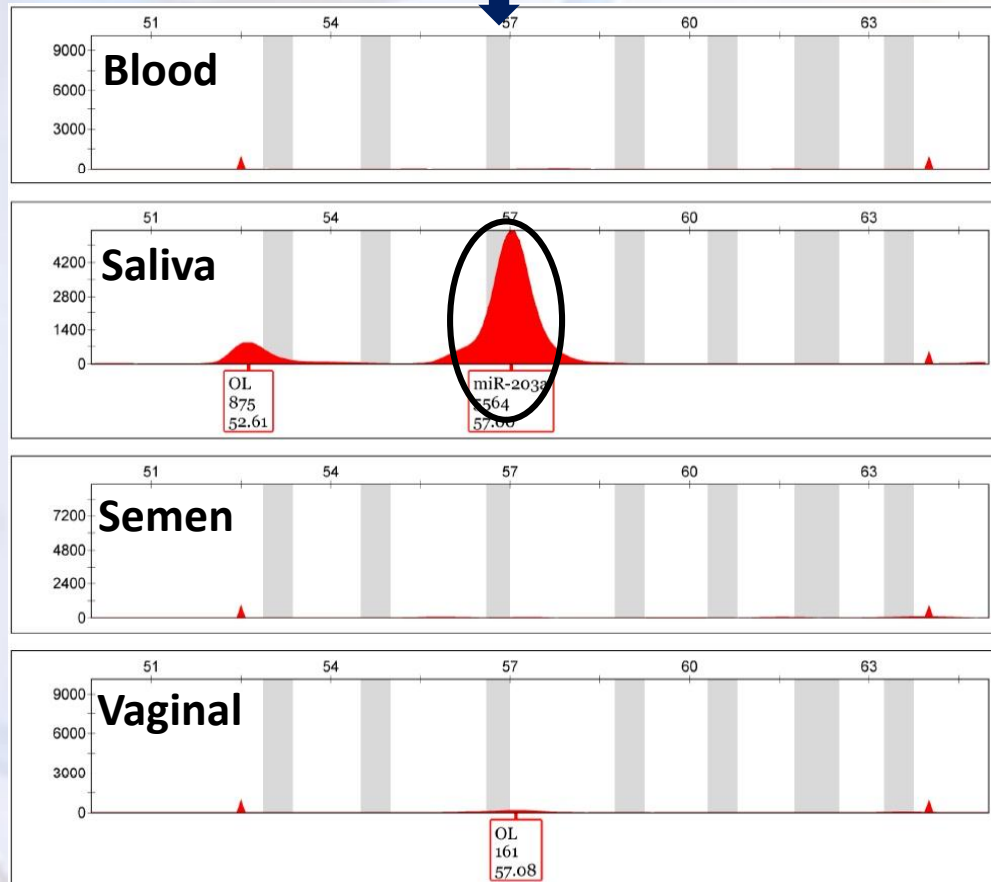
Much lower peaks of by-products found in all tissues

# miR-203a is mainly detected in saliva

**Expressed in epithelial cells**

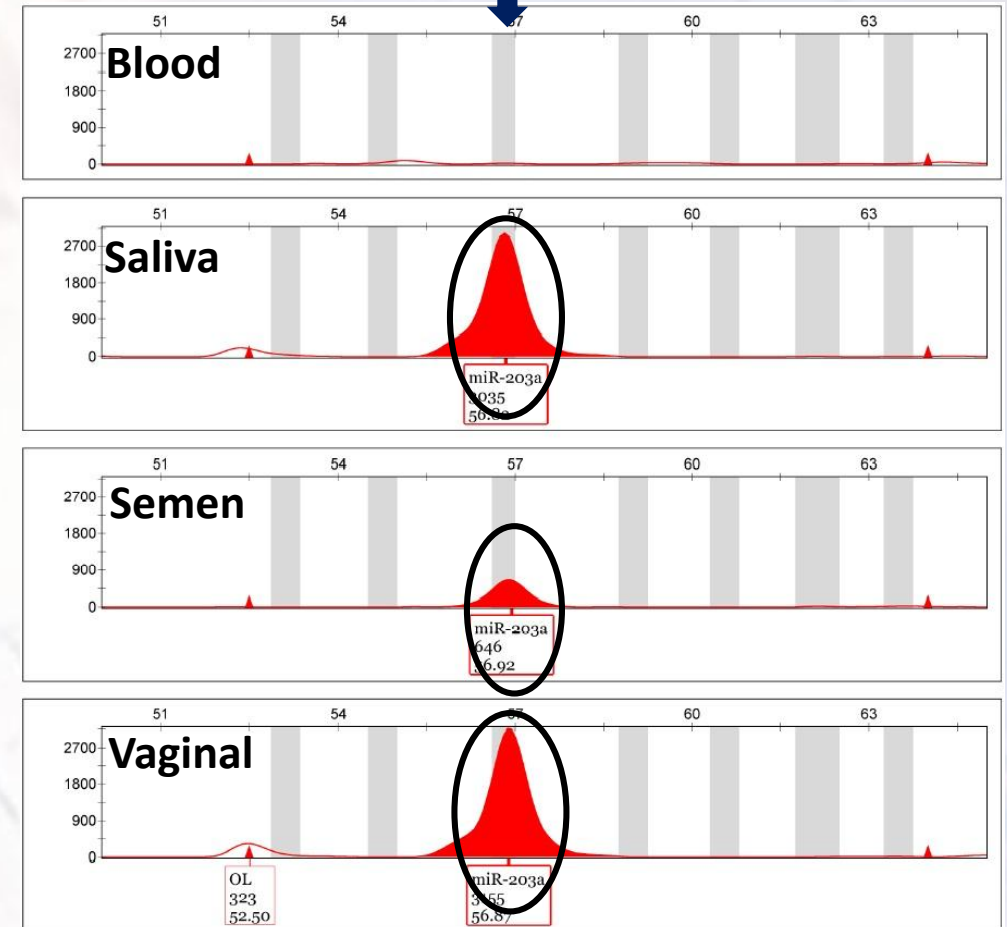
**Sample set 1**

miR-203a



**Sample set 2**

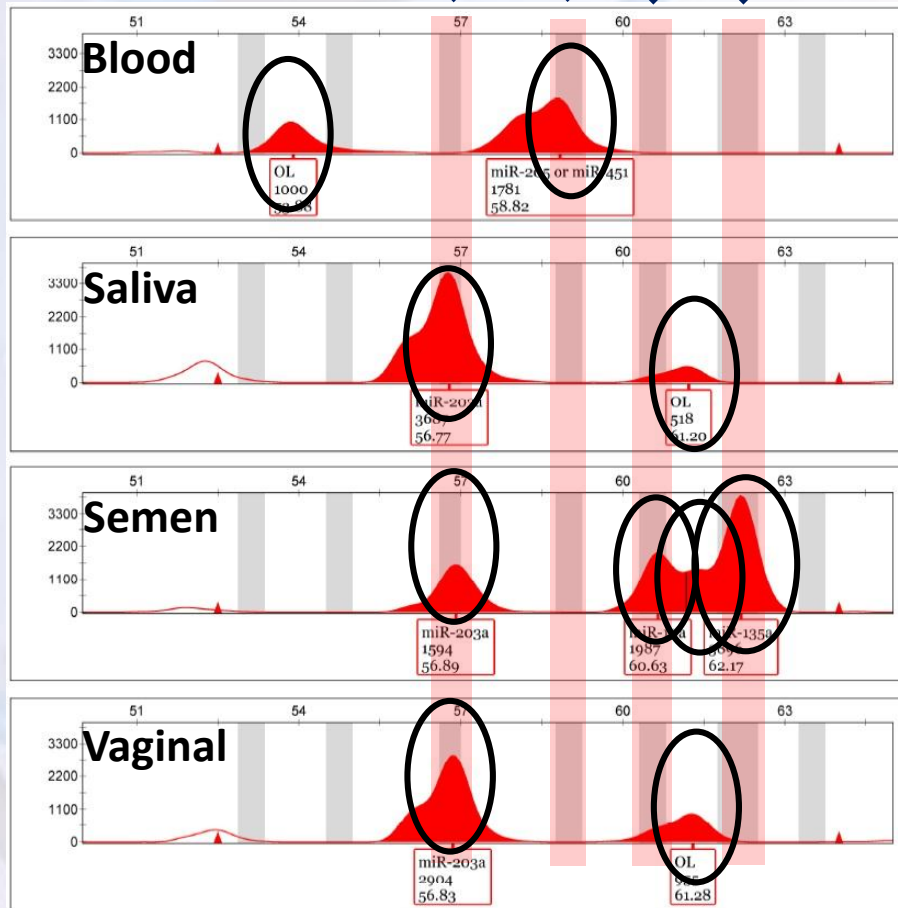
miR-203a



# Multiplexing multiple markers yields expected results

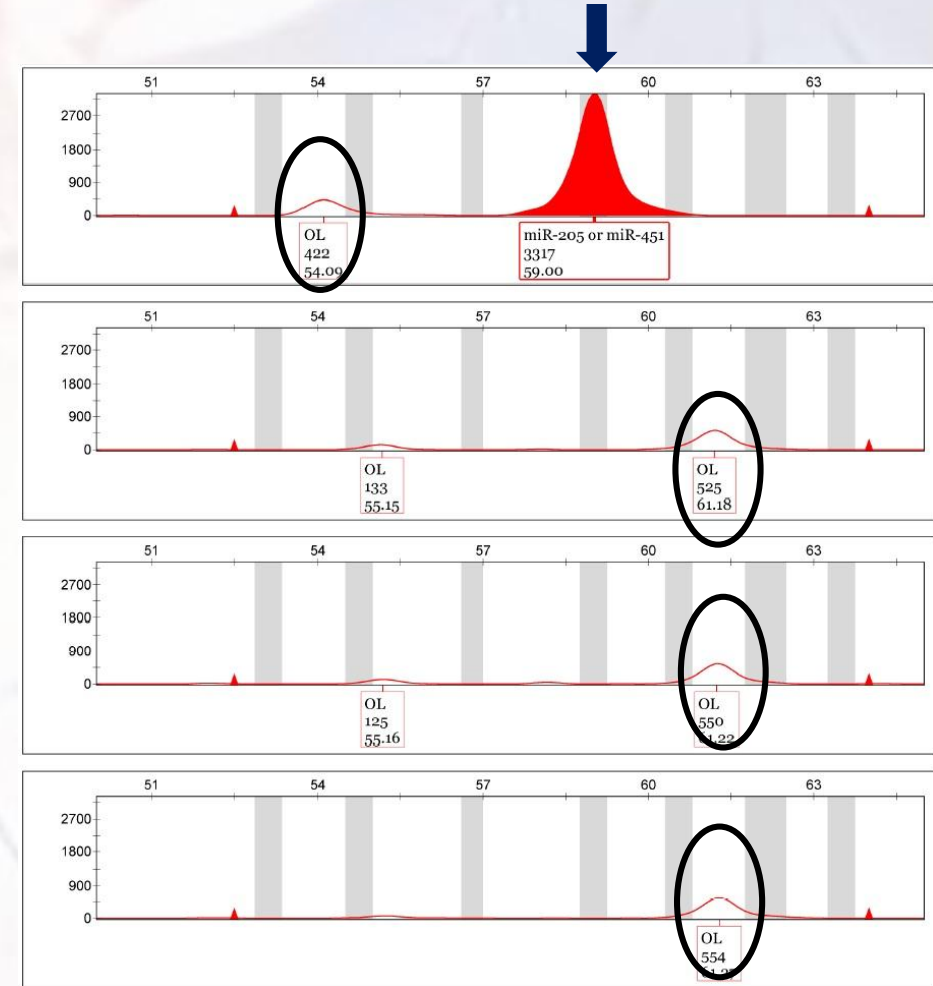
Multiplex

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



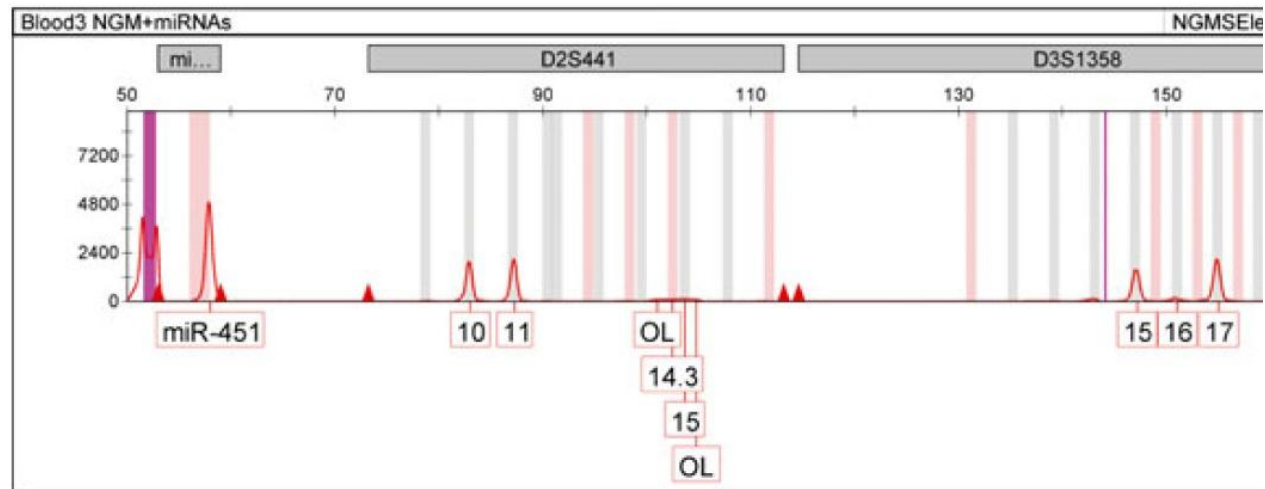
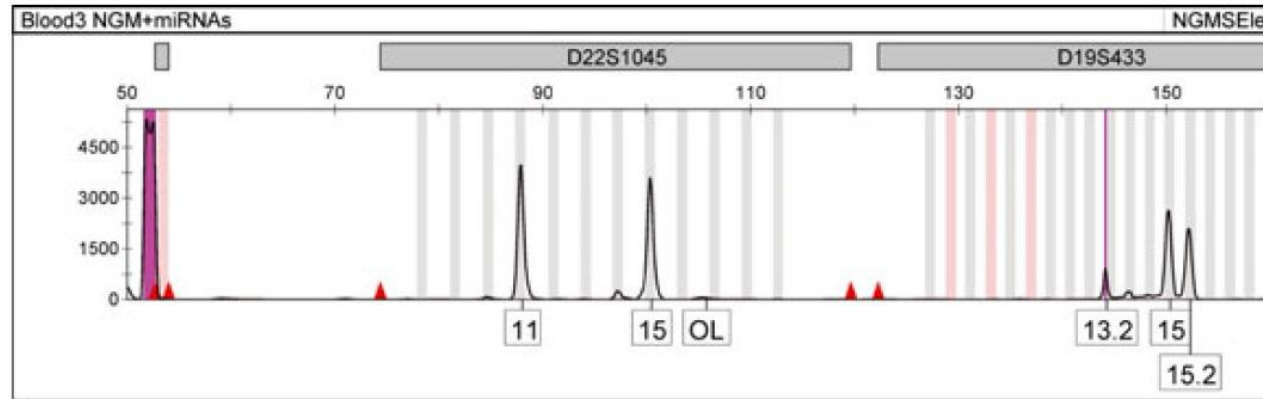
Singleplex

miR-451a

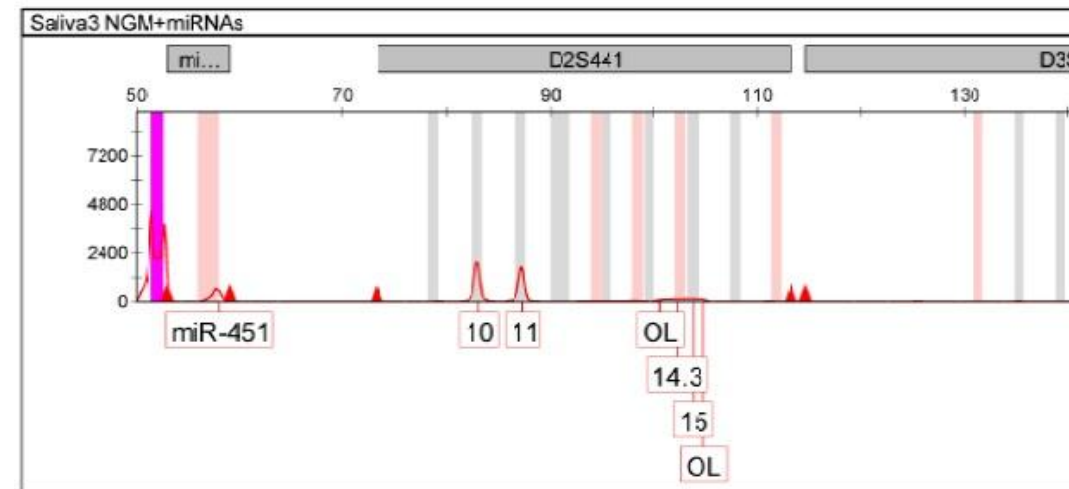
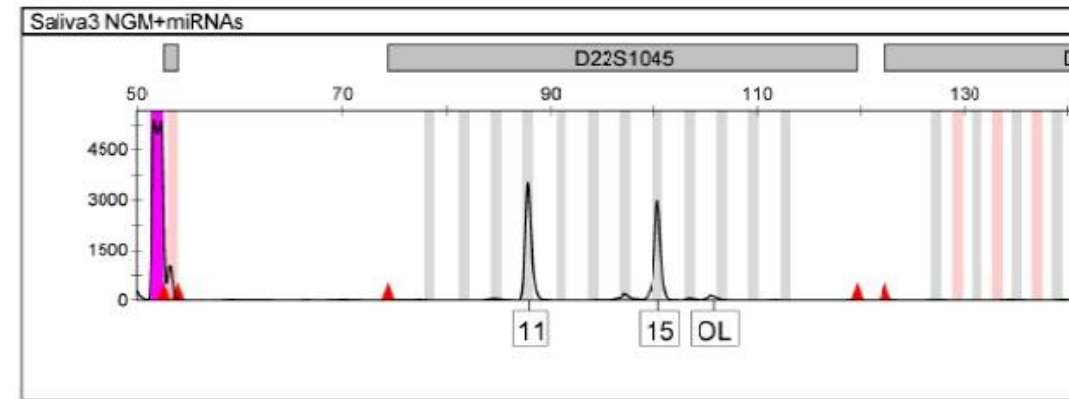


# Multiplex with STR markers

## Blood

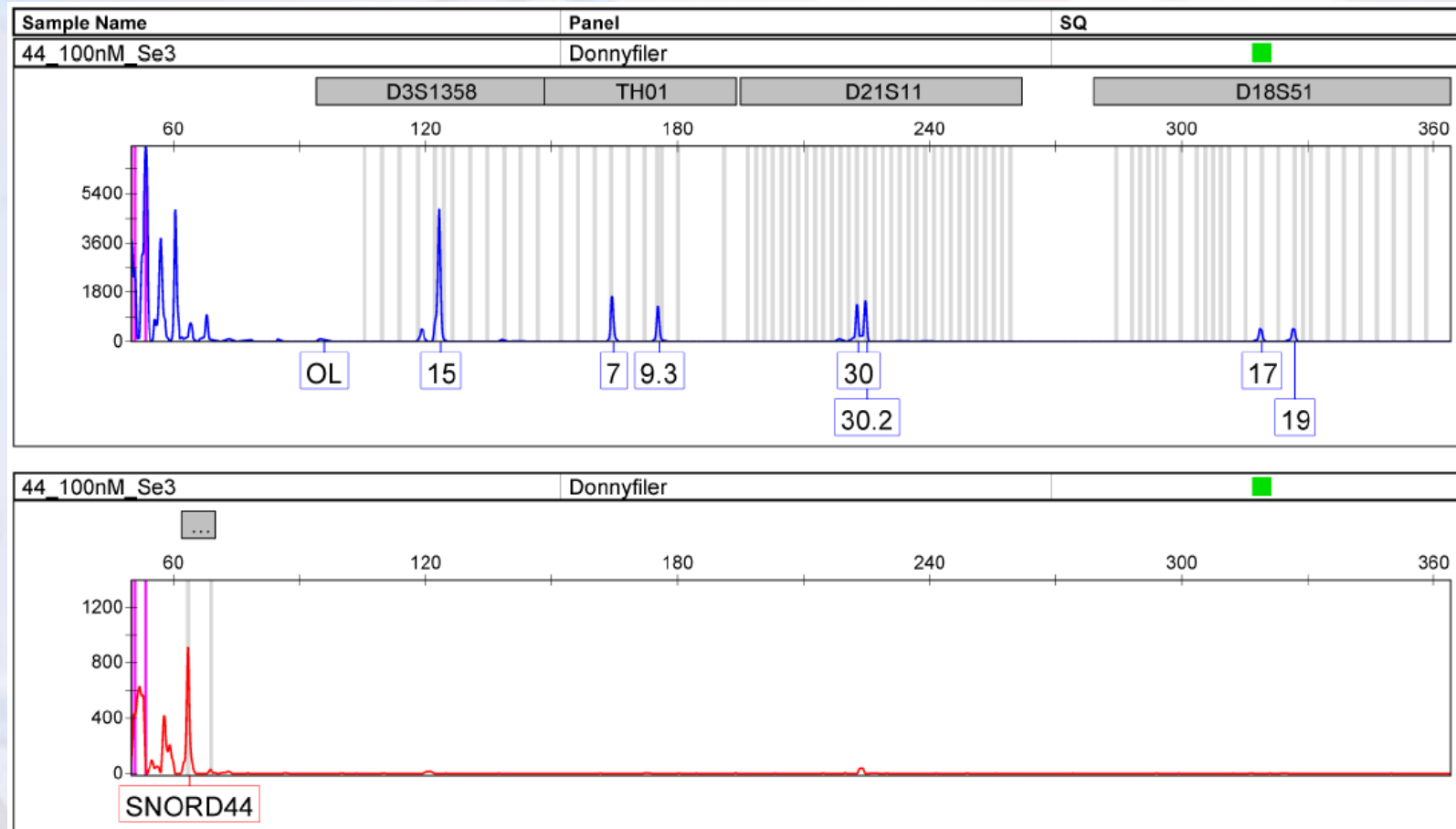


## Saliva





# 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

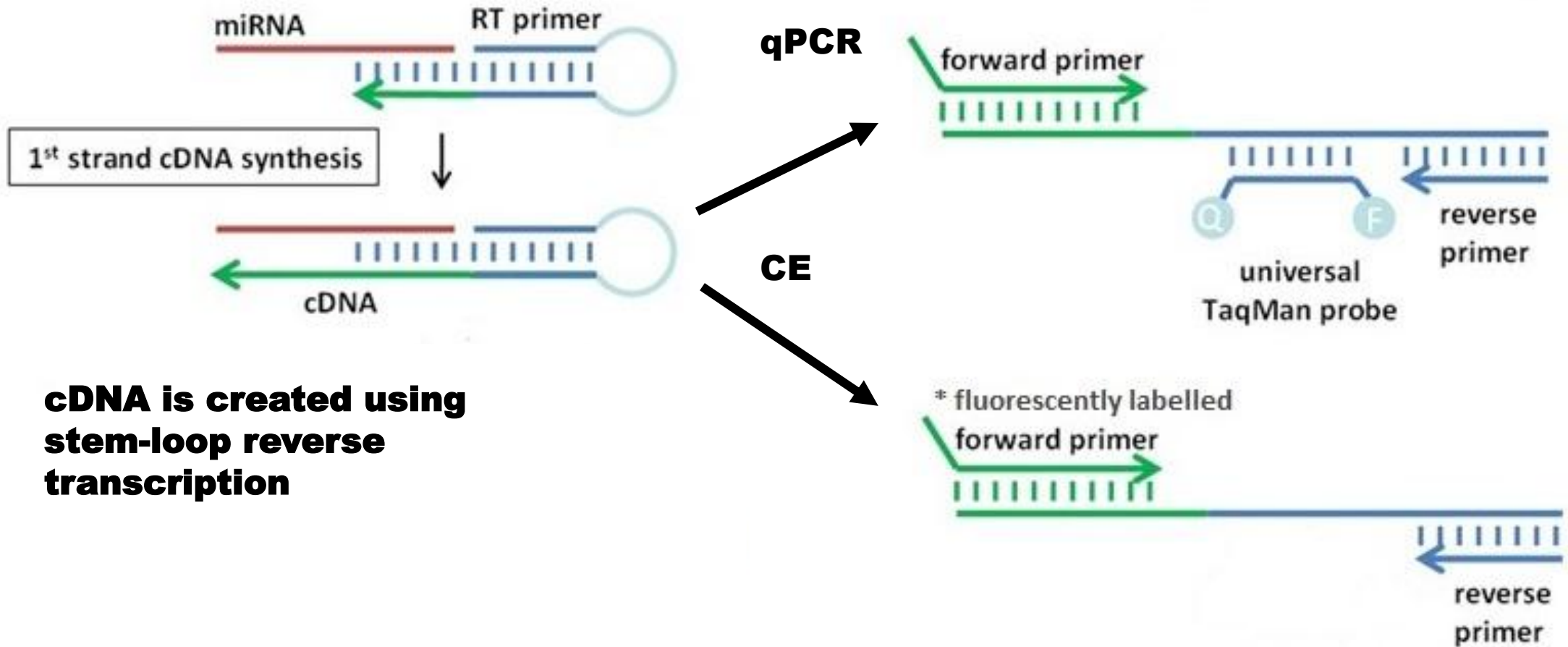
[d.vandermeer@hud.ac.uk](mailto:d.vandermeer@hud.ac.uk)



# FSF Emerging Forensic Scientist Award Oral Presentation

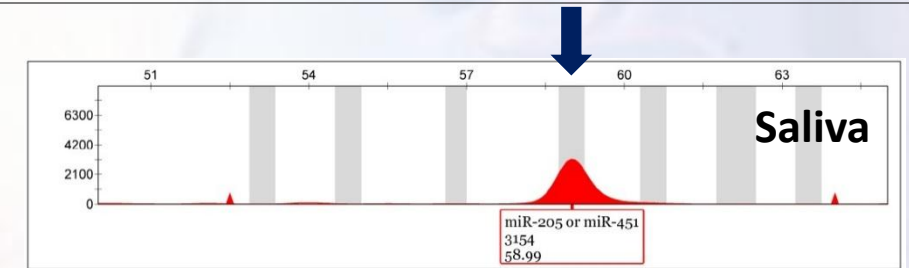
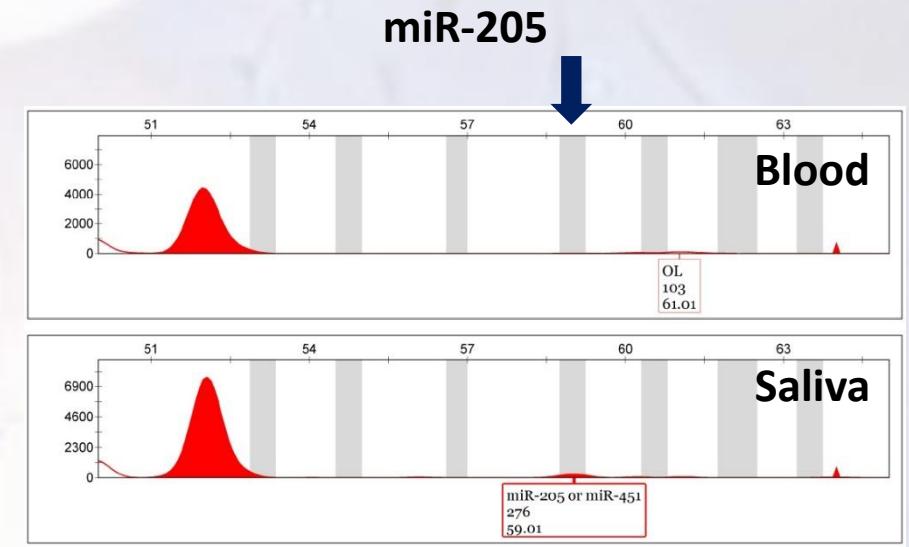
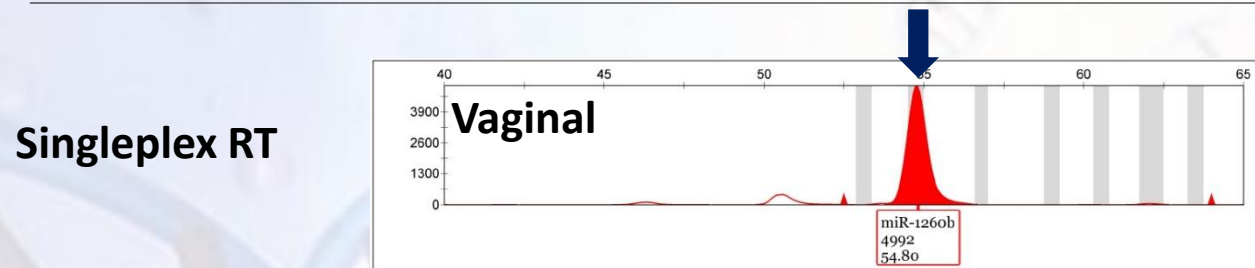
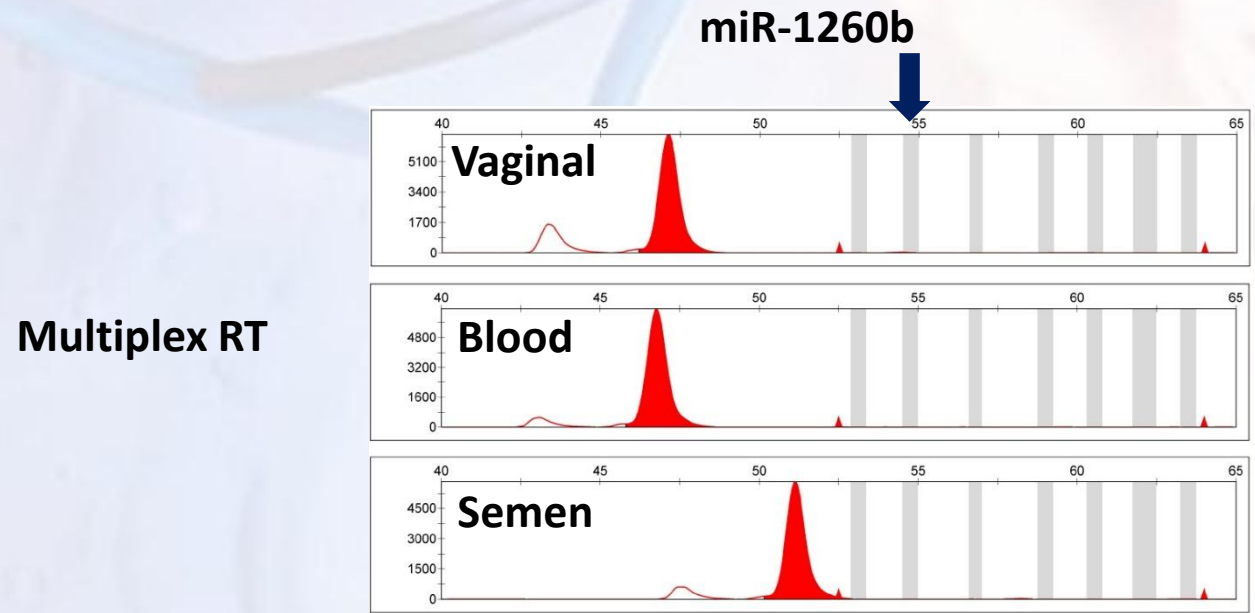


# Our workflow



**cDNA is created using stem-loop reverse transcription**

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



# Multiplexing multiple markers yields expected results

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

Blood 16a  
Saliva 205  
Blood 142  
Control SNORD44

