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

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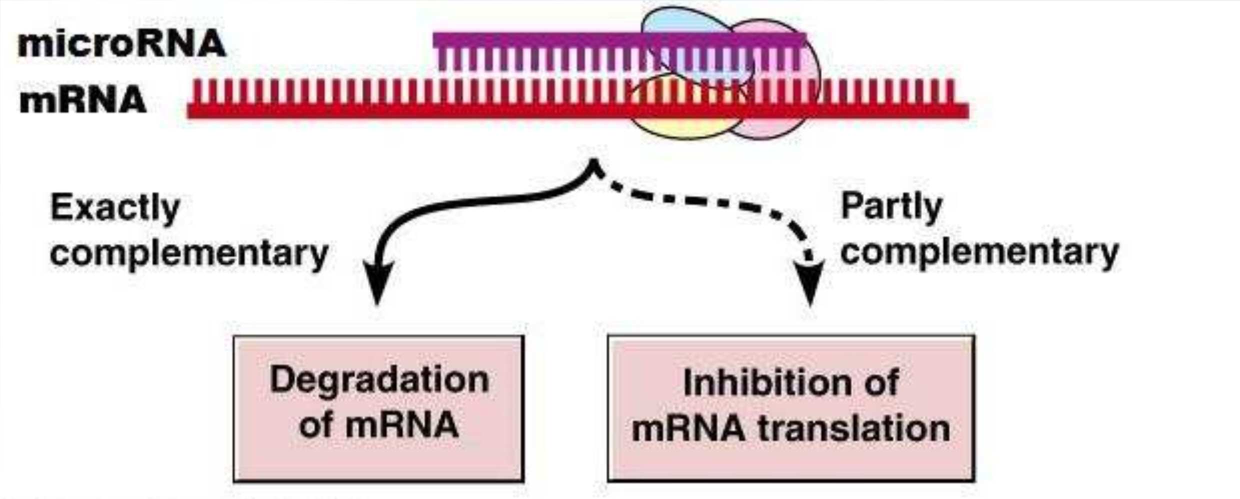
FSF Emerging Forensic Scientist Award
Oral Presentation

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

Hanson, E. K. *et al.* (2009) *Anal Biochem*, *387*(2), 303–14. Zubakov, D. *et al.* (2010) *Int J Leg Med*, *124*(3), 217–26
Park, J.-L. *et al.* (2014) *Electrophoresis* 35(21-22), 3062-8

Improve current methodology with our novel method

Current

- microRNAs: RT-qPCR
    - Separate reaction per microRNA

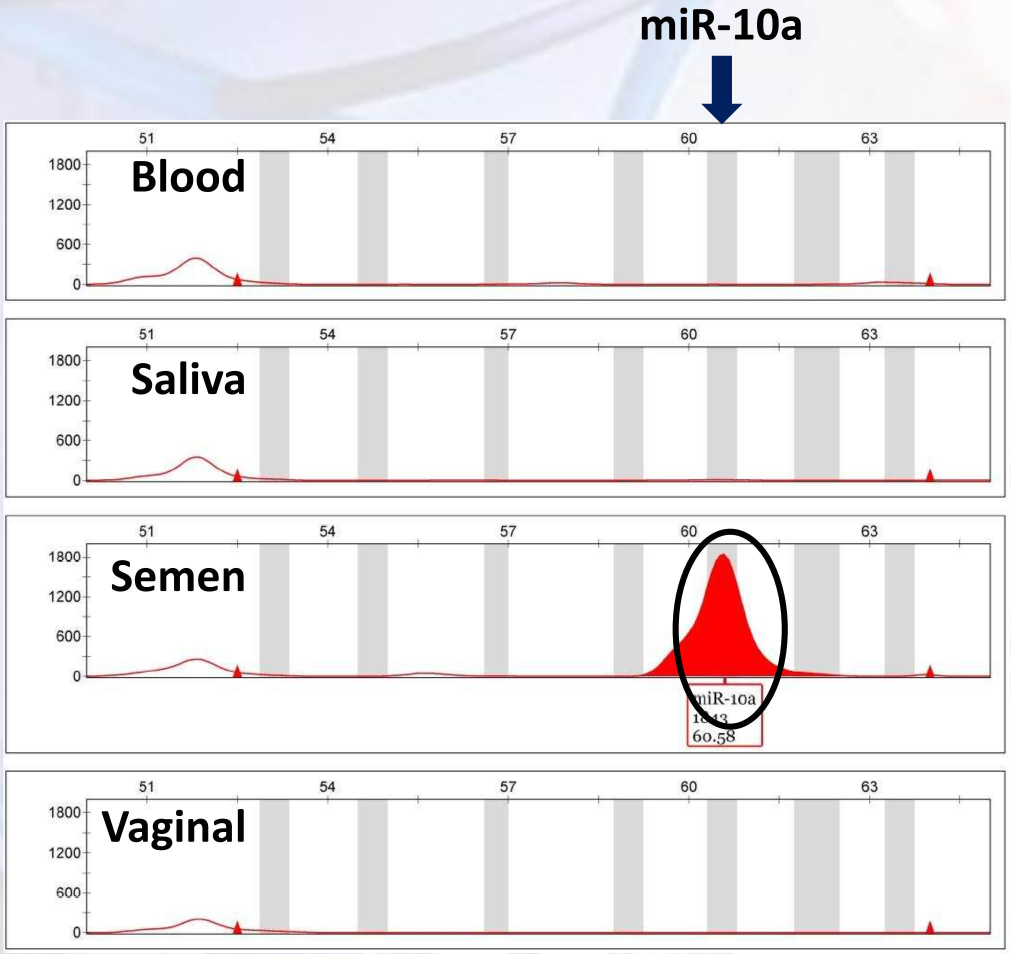
Our novel method

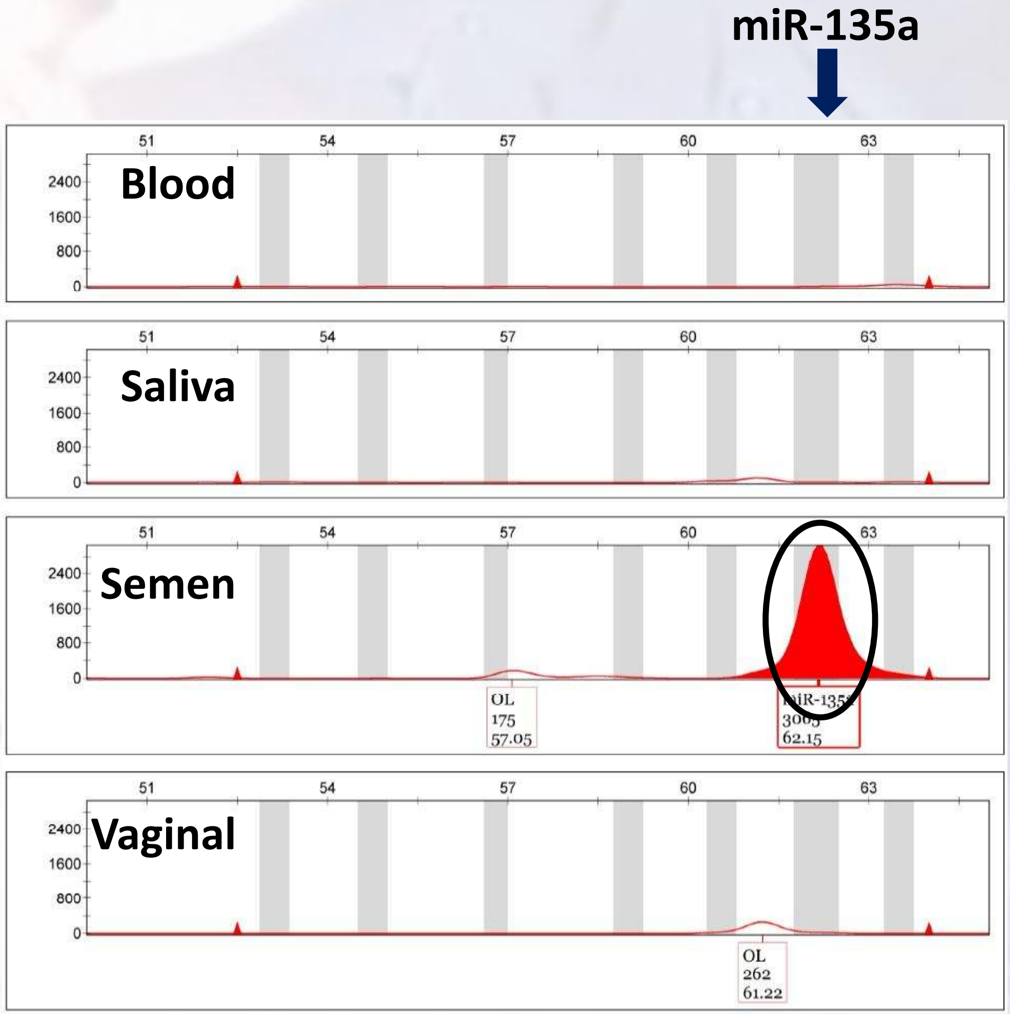
- Analyse microRNAs with capillary electrophoresis (CE)
    - Multiplex microRNAs in single test
    - Possibility to combine microRNA analysis with DNA profiling

Methods and materials

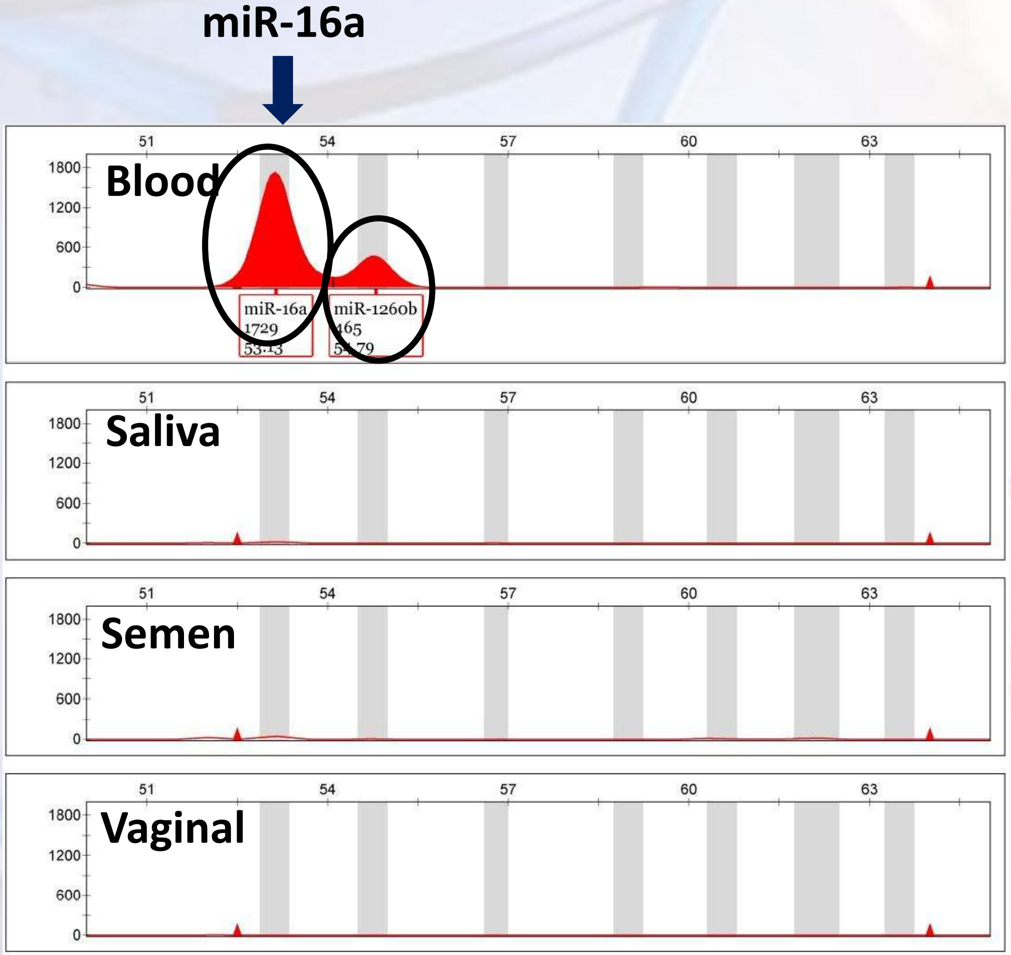
- 5 samples of 4 tissue types
  - Blood, saliva, semen and vaginal material
- DNA extraction
- Normalised to 0.5ng/µl human DNA
- Tested for 9 markers
- Multiplex stem-loop reverse transcription
- ROX-labelled primers

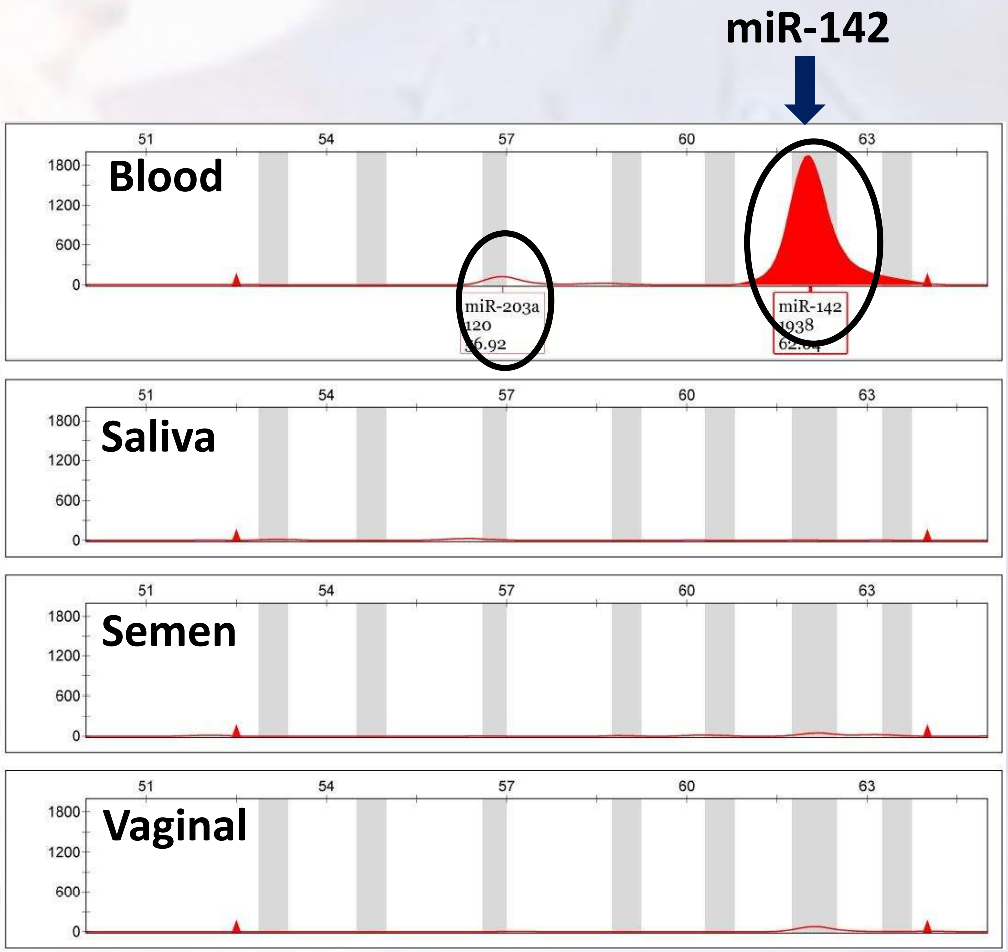
miR-10a and miR-135a are exclusively detected in semen



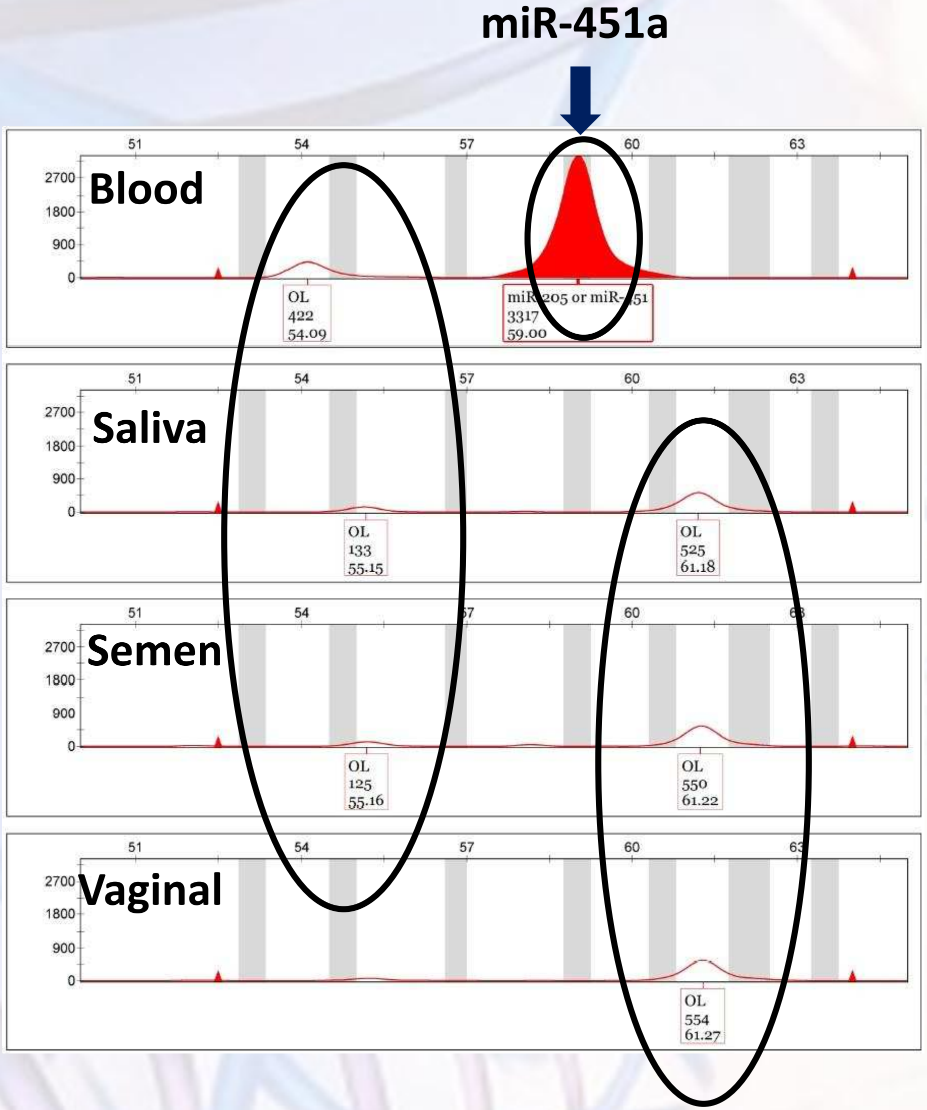


miR-16a and miR-142 are exclusively detected in blood





miR-451a is exclusively detected in blood

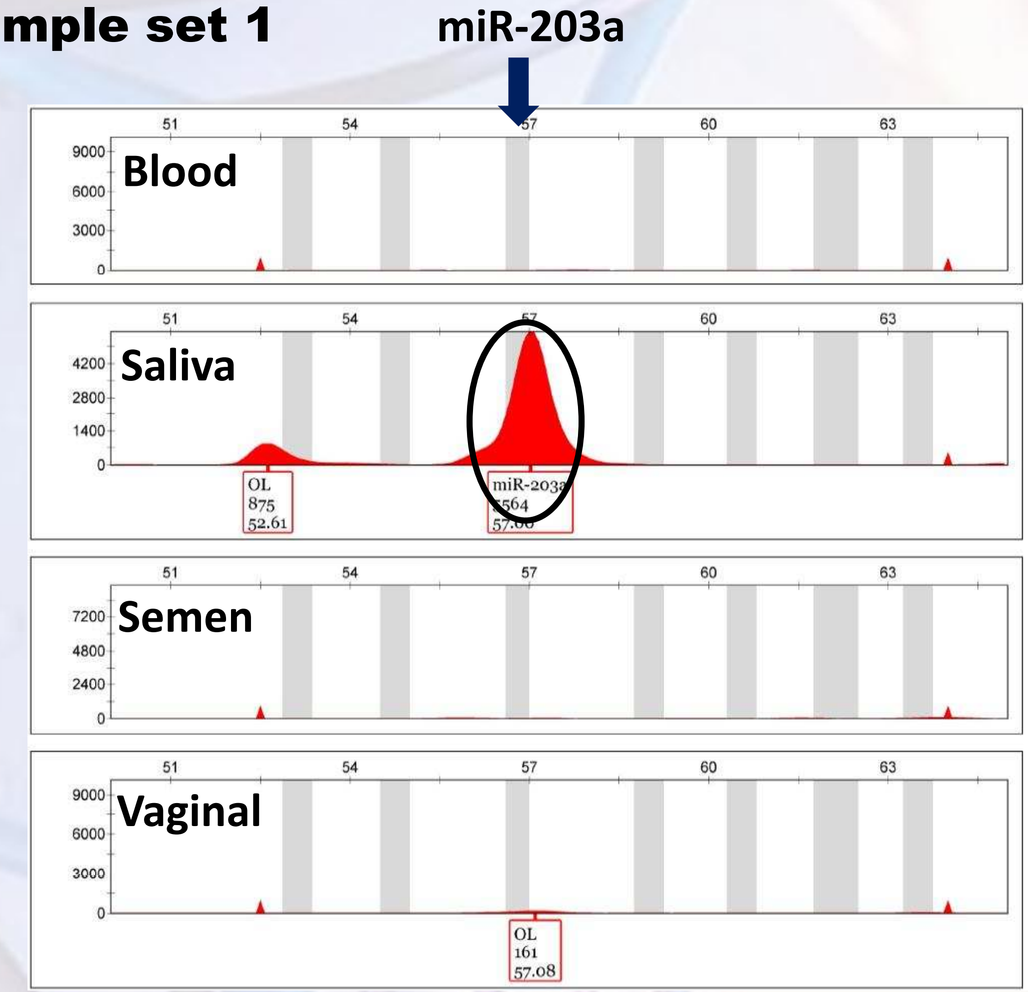


Much lower peaks of by-products found in all tissues

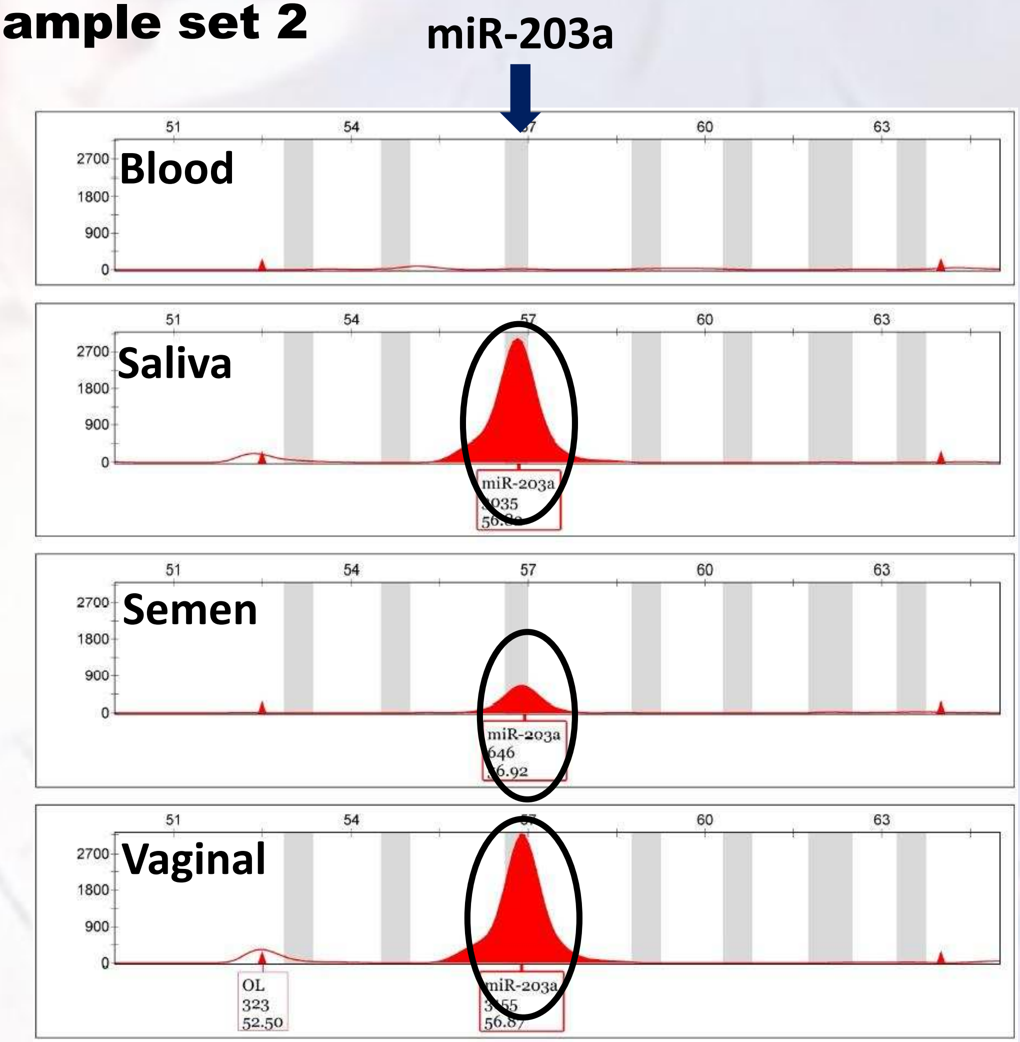
miR-203a is mainly detected in saliva

Expressed in epithelial cells

Sample set 1

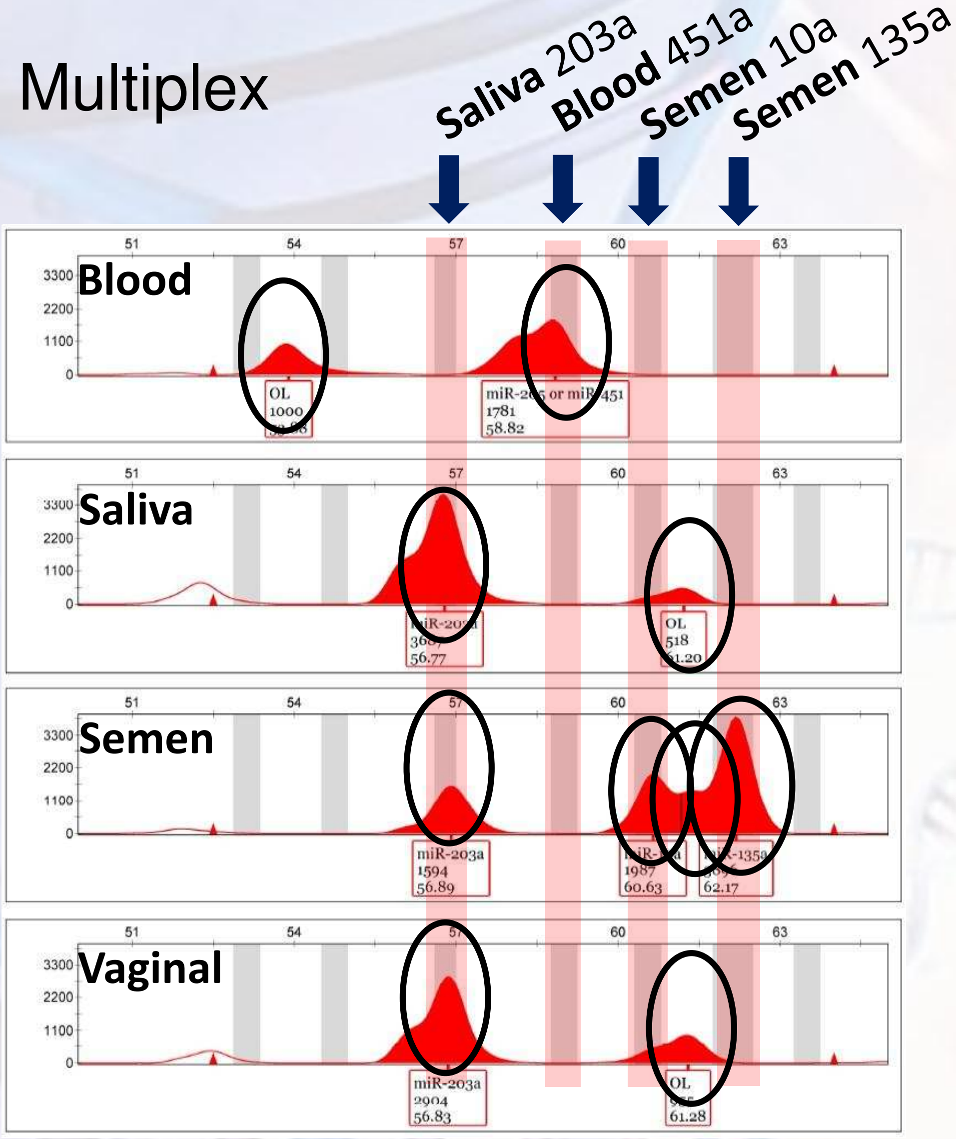


Sample set 2

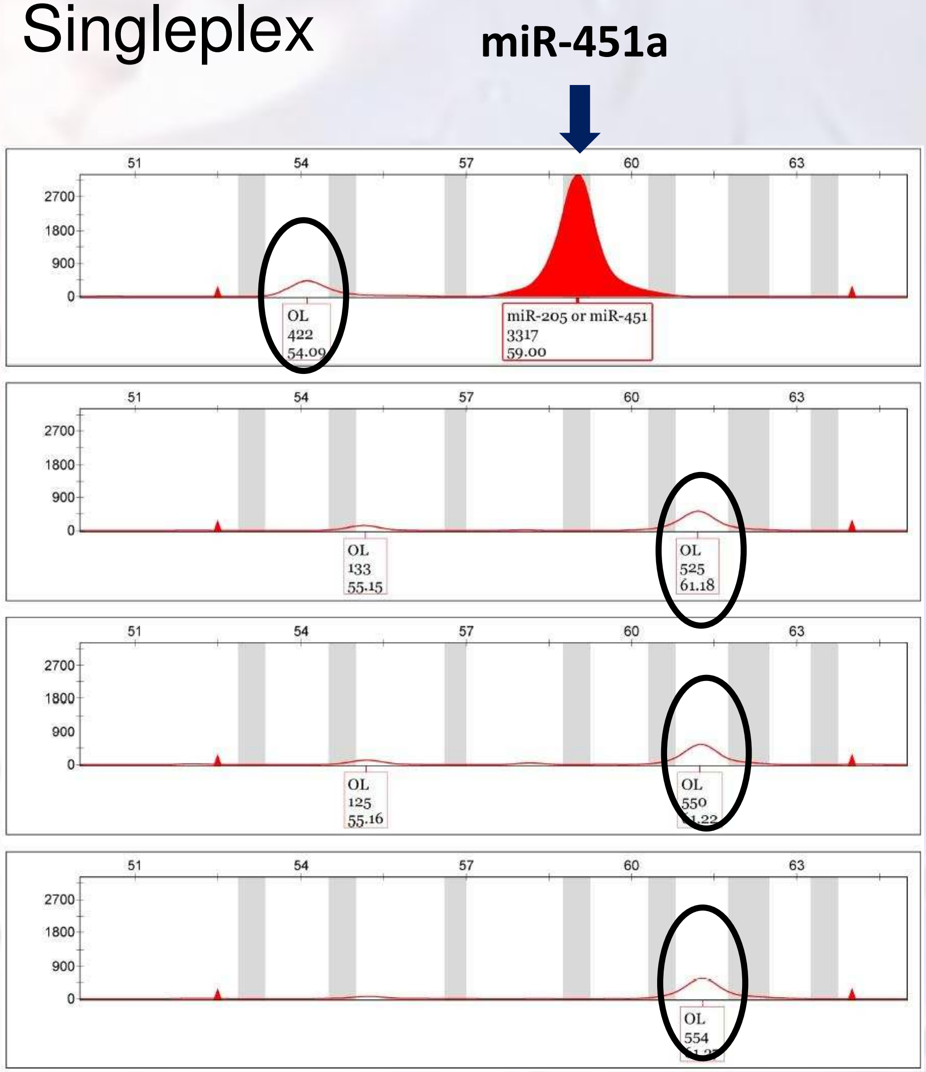


Multiplexing multiple markers yields expected results

Multiplex

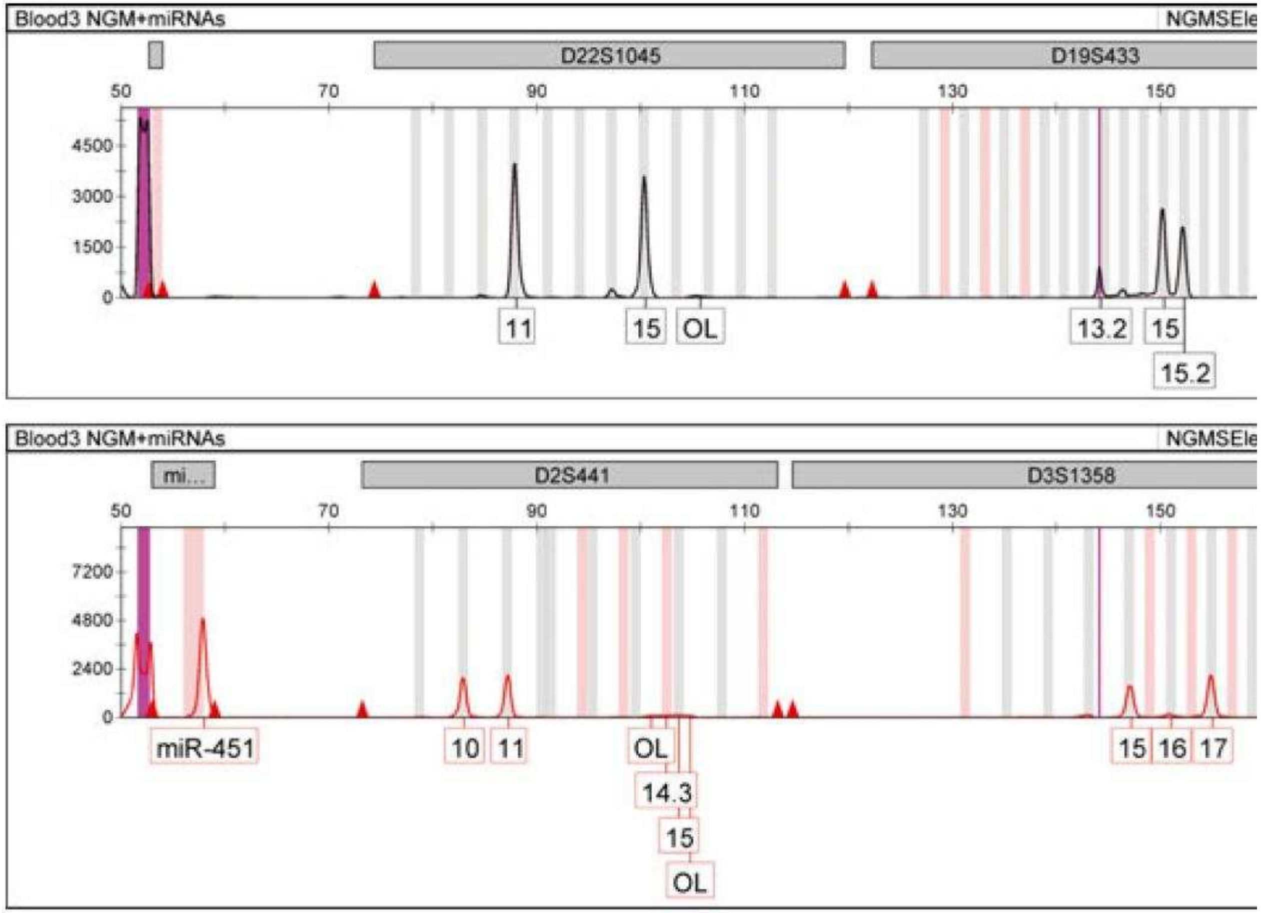


Singleplex

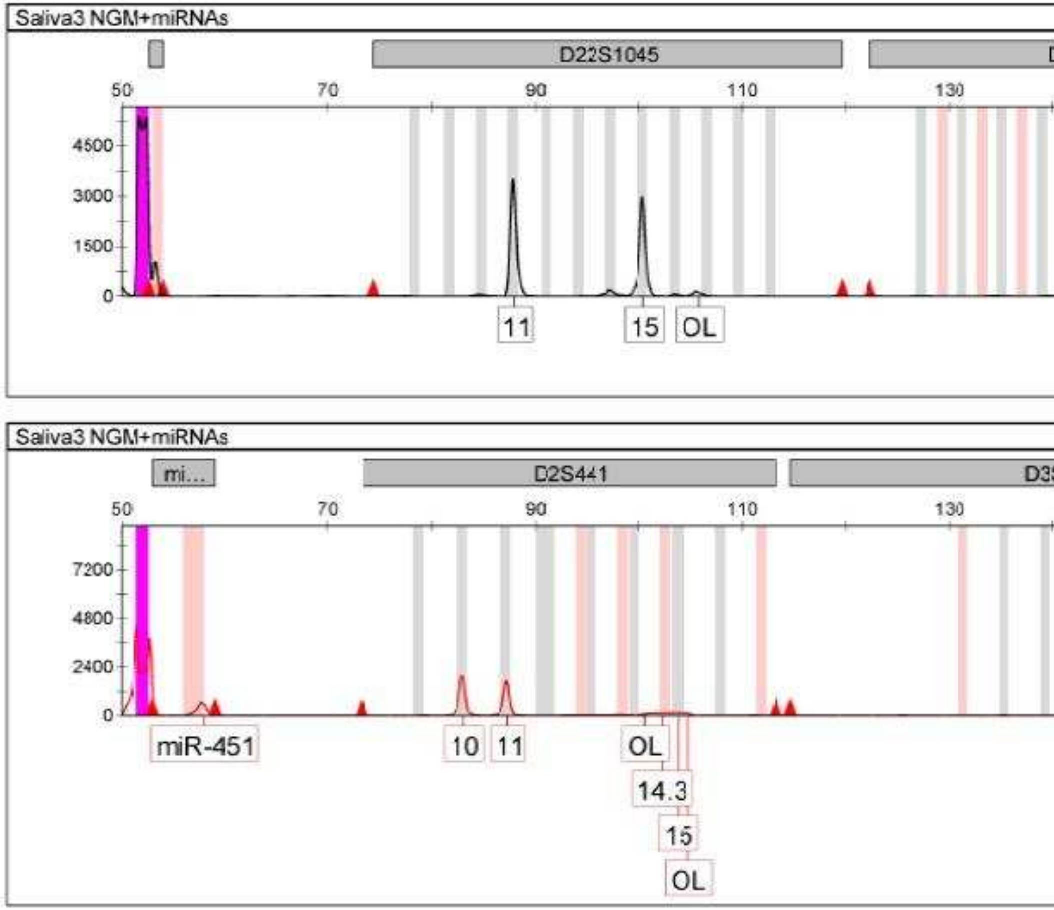


Multiplex with STR markers

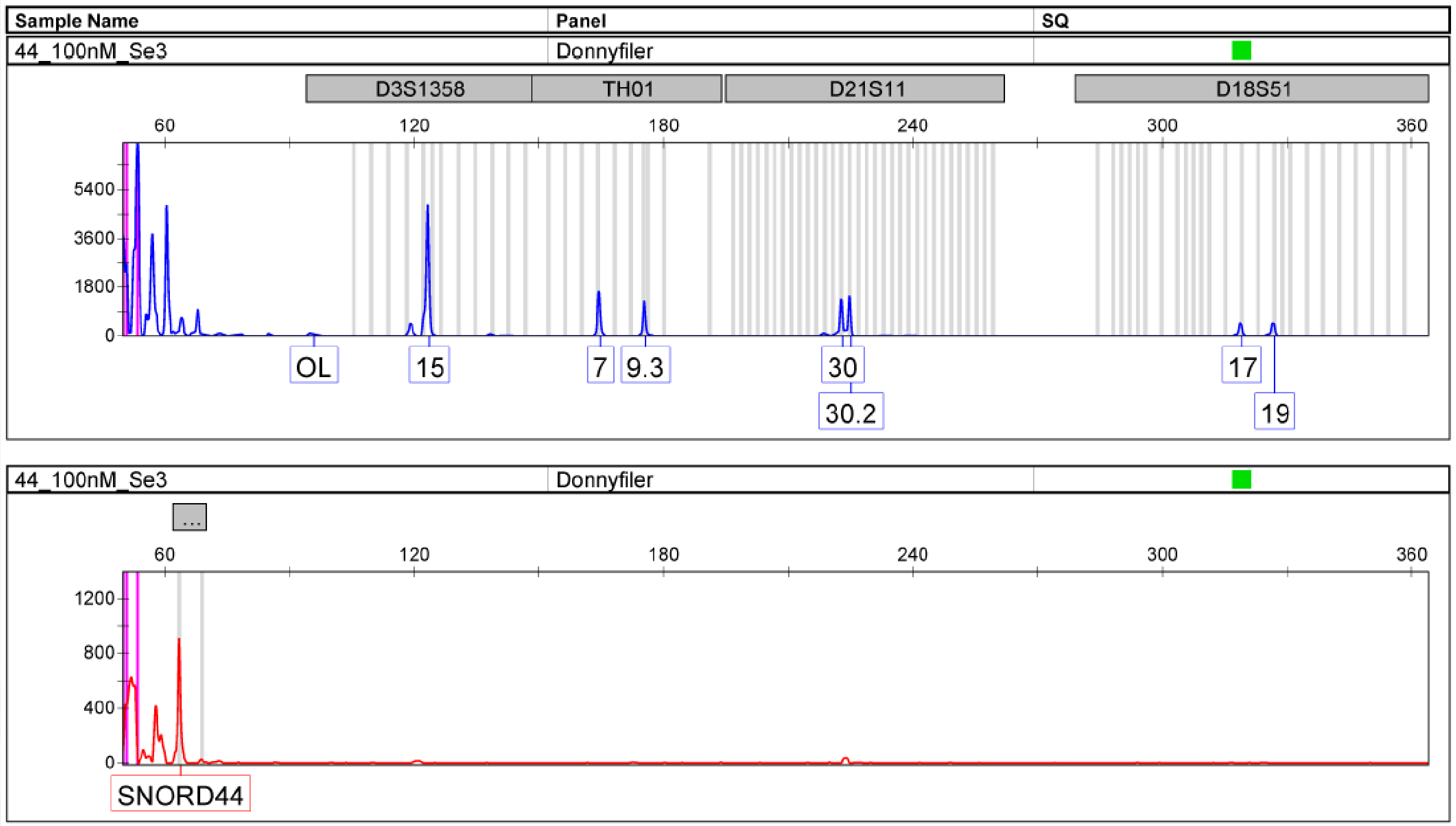
Blood



Saliva



Multiplex with STR markers



Sample Name	Panel	SQ
44_100nM_Se3	Donnyfiler	🟩

44_100nM_Se3	Donnyfiler	🟩

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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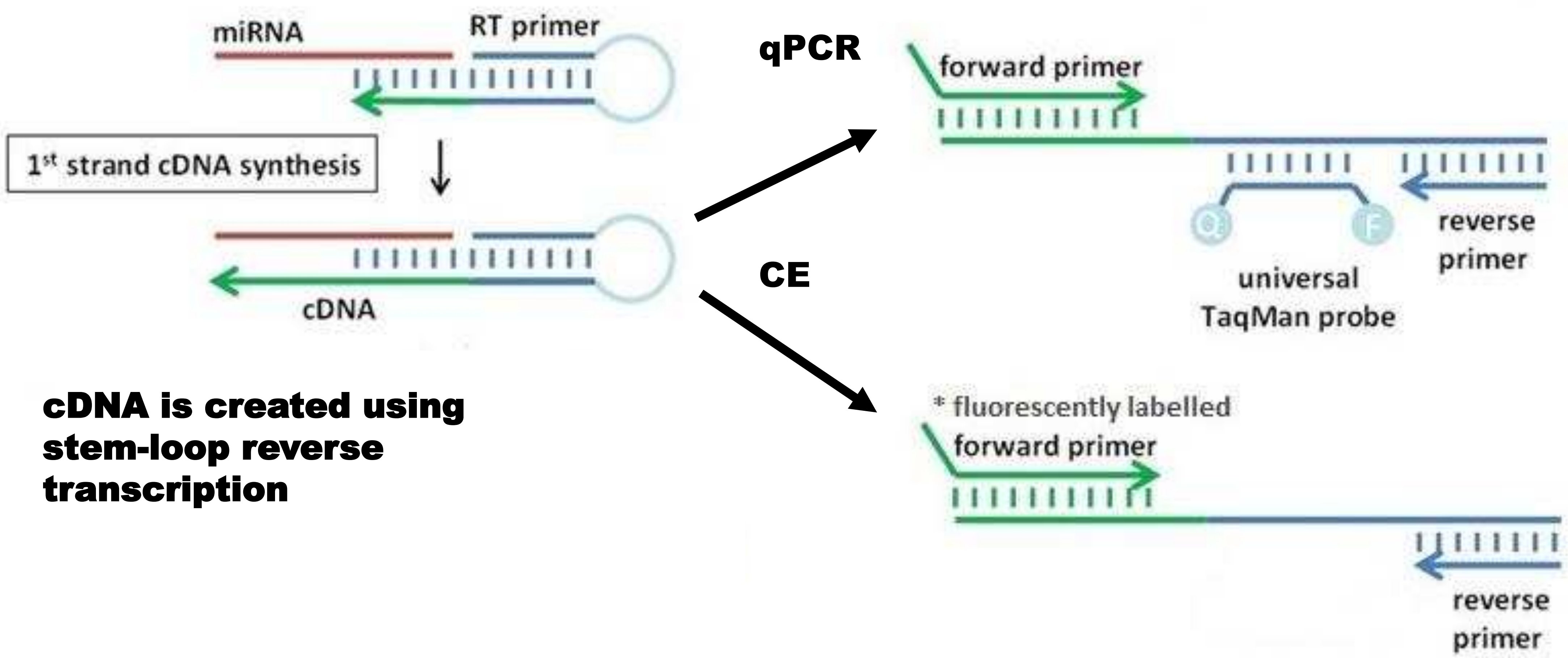
Our workflow



cDNA is created using stem-loop reverse transcription

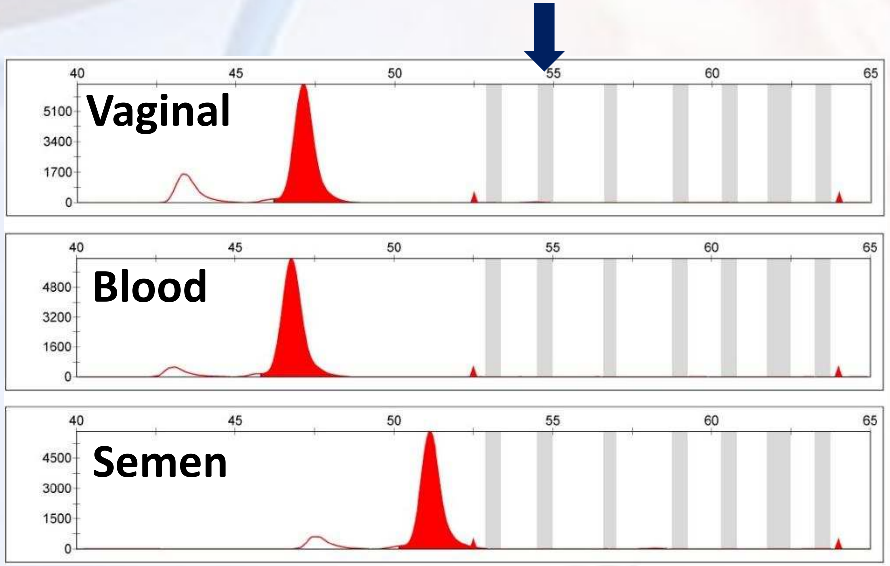
Chen, C. *et al.* (2005) *Nucl Acids Res*, *33*(20); Jung, U. *et al.* (2013) *RNA*, *19*, 1864-73

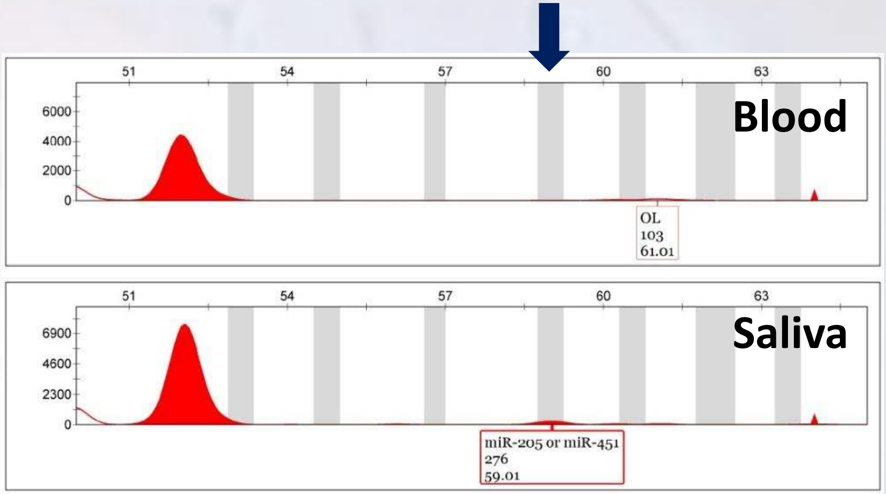
miR-1260b and miR-205 fail due to multiplexing reverse transcription

miR-1260b

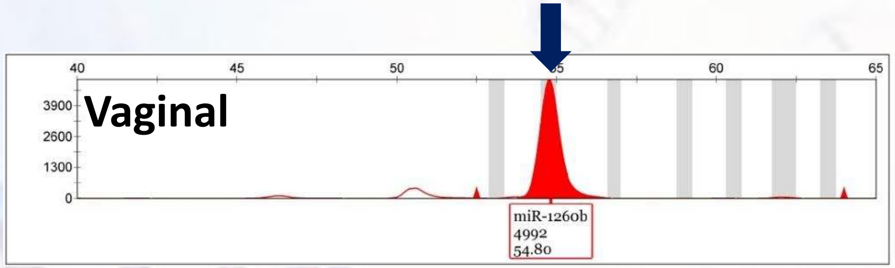
miR-205

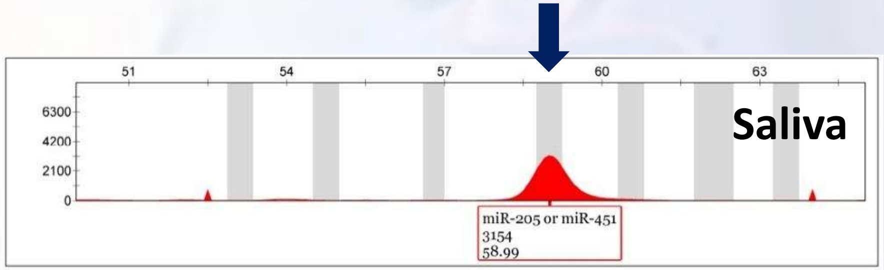
Multiplex RT





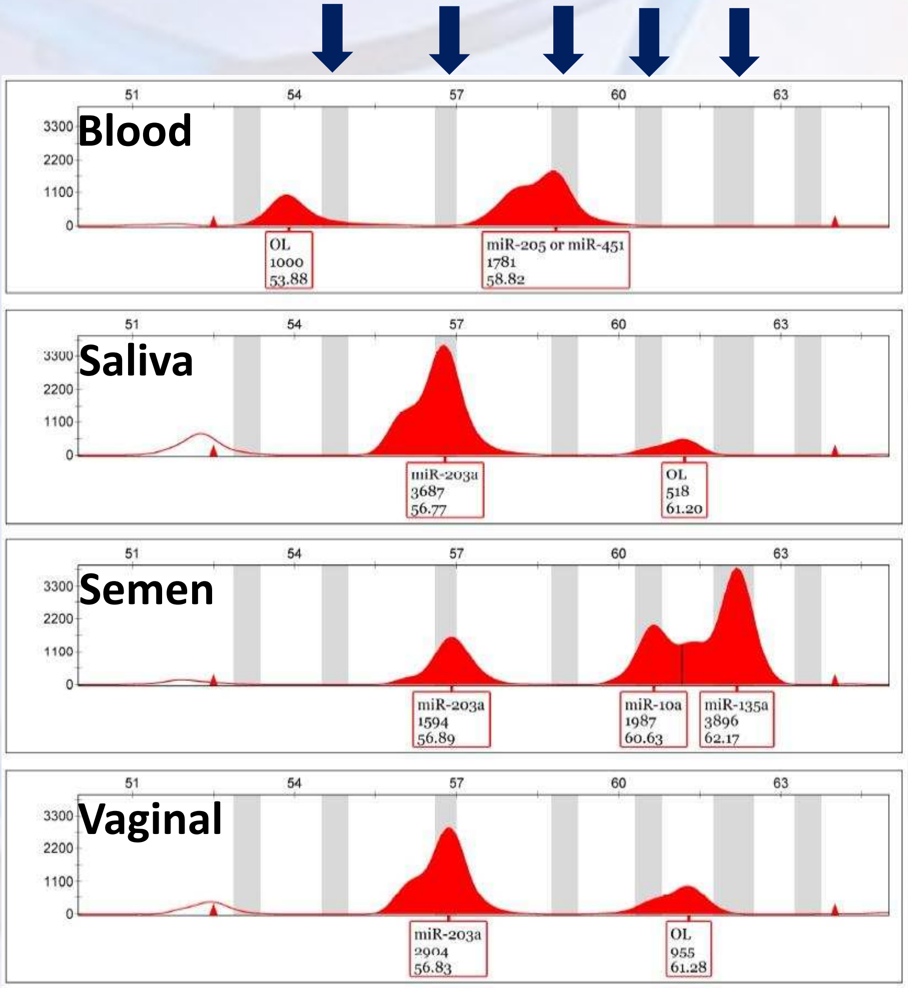
Singleplex RT





Multiplexing multiple markers yields expected results

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



Blood 16a
Saliva 205
Blood 142
Control SNORD44