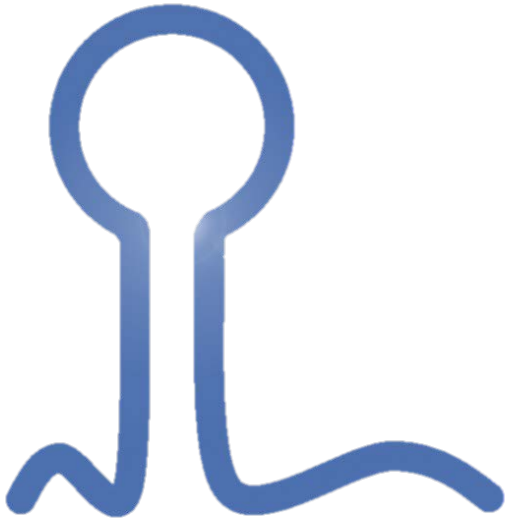
Qiagen Investigator Forum
Young Investigator Session
5/2/18

Little Stains, Lots of Problems



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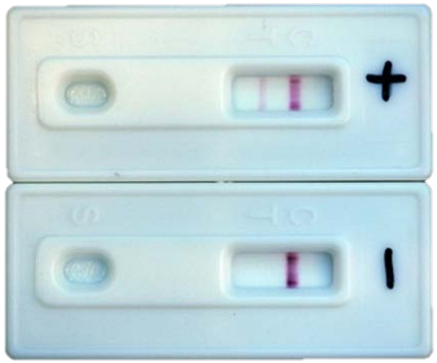
Statement of the problem

- Body fluid identification (BFID) often undervalued in crime labs
- Current methods either consume or dilute sample
  - Small samples are not being tested for body fluid
- Limited number of body fluids currently being tested
  - Blood, semen, and saliva



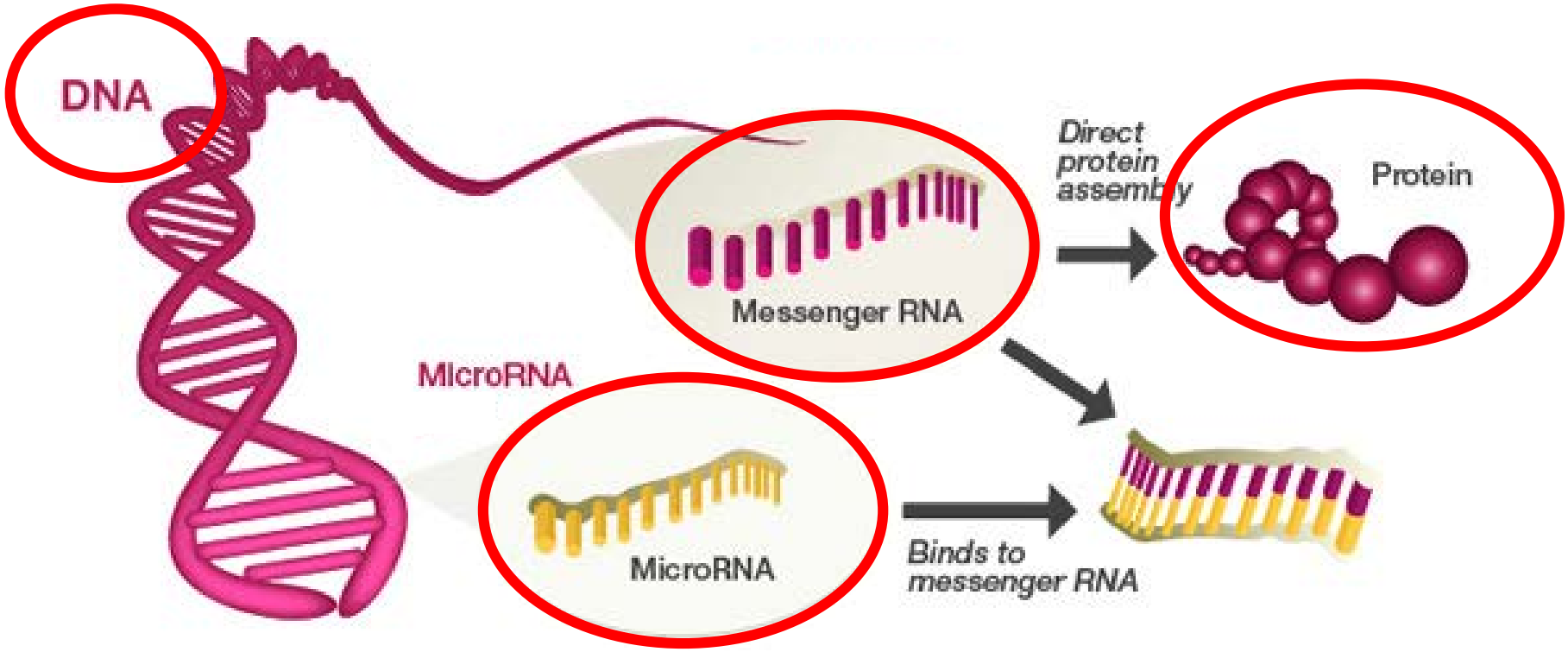
Statement of the problem

Current Techniques	Problems
Heme-antibody cards	False positives, ambiguous readings, consumption of sample
Luminol, phenolphthalein	Not human specific, not blood specific, dilution of sample
Acid Phosphatase	Not semen specific, susceptible to storage conditions, dilution of sample
P30/PSA cards	Not semen specific, ambiguous readings, consumption of sample
Visualization of Spermatozoa	Oligo/azoospermic individuals, diluted samples

Impromptu game show

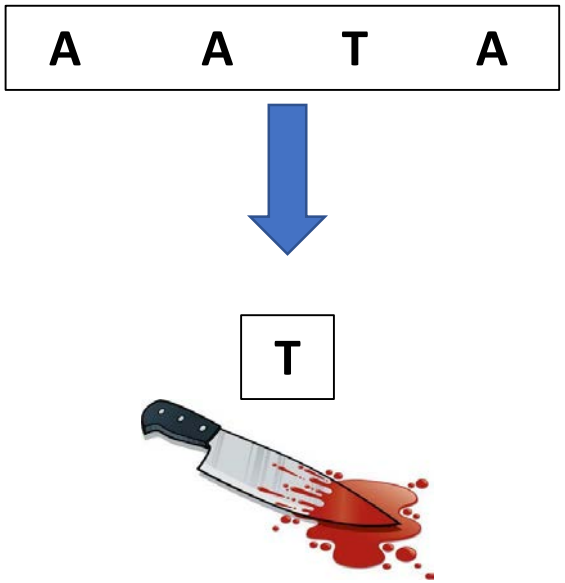
Is there a line?

Lines of Research into BFID



mRNA based BFID

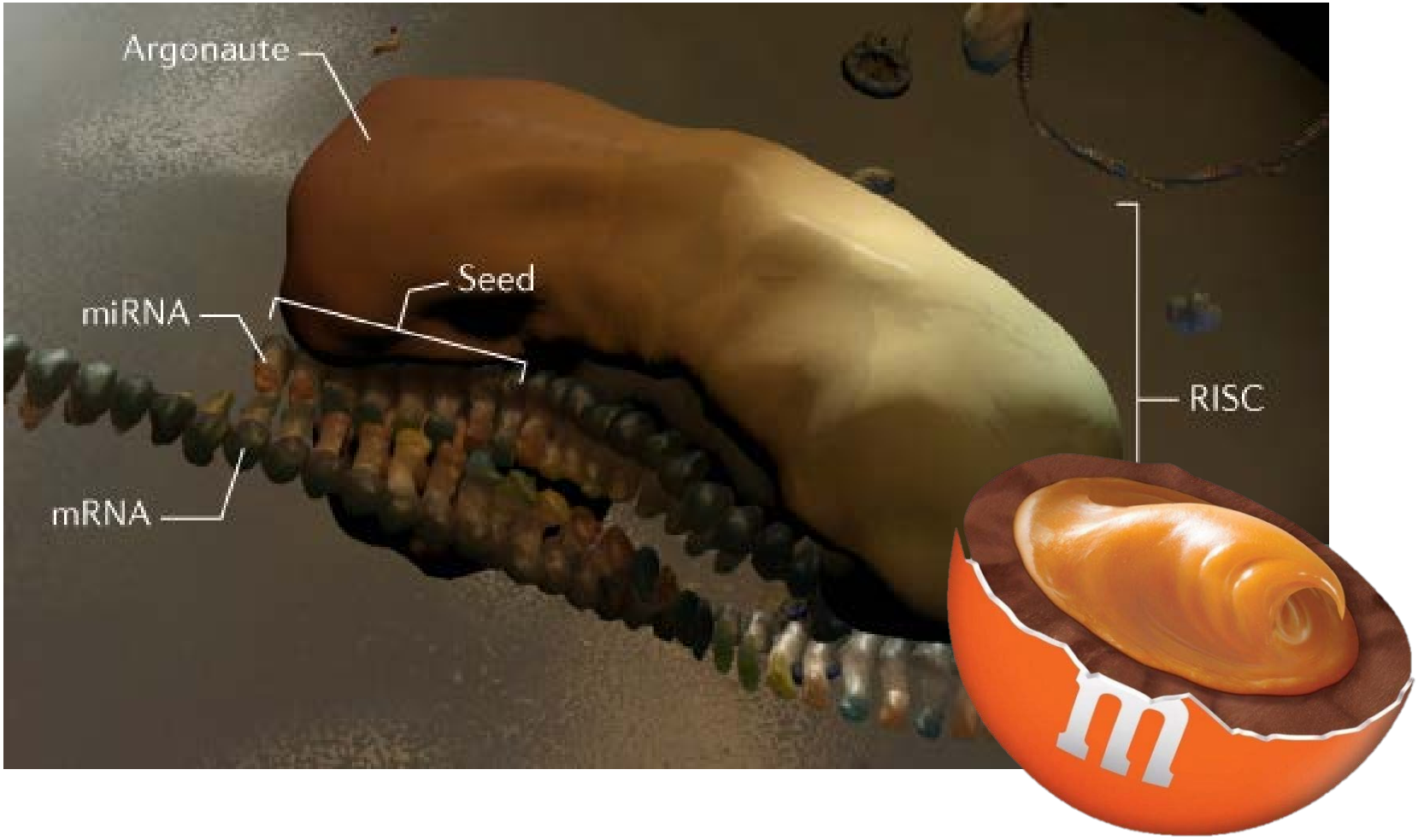
- Co-extracted with DNA
- Instrumentation and techniques
- mRNA BFID CE-based assay already implemented (NFI, ESR)
- Persistence
  - Setzer et al. (2008)
  - Zhao et al. (2017)
  - Kulstein & Wiegand (2017)
- SNP analysis



miRNA based BFID

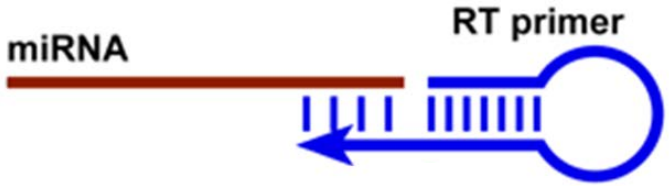
- Co-extracted with DNA
  - Observed in DNA extracts
- Instrumentation
- Similar techniques
- No consensus on markers
- Persistence
  - Grabmüller et al. (2017)
- Stability is theoretical

miRNA Stability



Analysis of miRNAs

- Difficult due to their small size (20-25 nucleotides)
- Stem-loop primers or polyadenylation
- Reverse Transcription Quantitative PCR (RT-qPCR)
  - Limited number of miRNAs that can be tested due to instrumentation
- Capillary Electrophoresis
  - Primer dimers



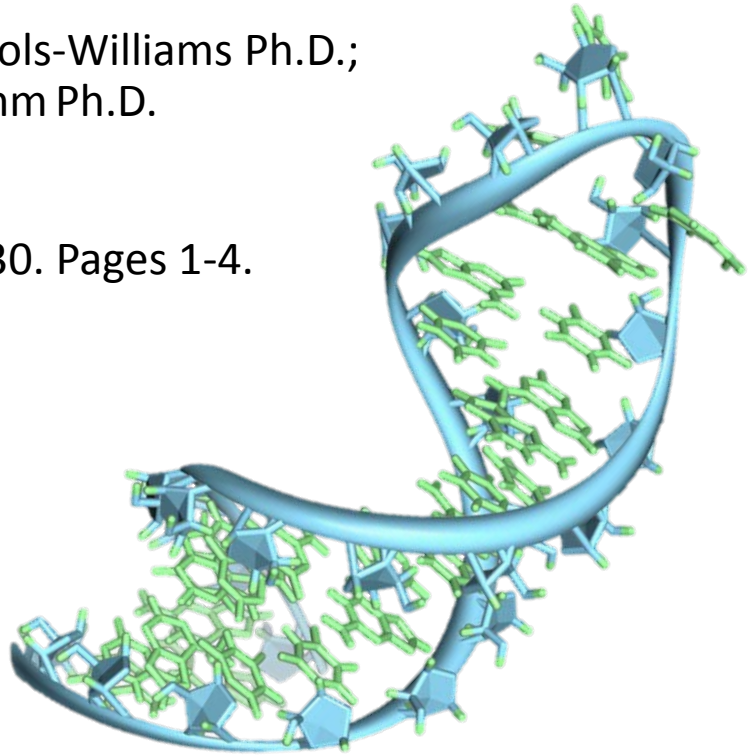
Analysis of miRNAs

- System of linear reverse transcription, specific, and universal primers
  - Paired sequences on reverse transcription and universal primers
  - Developed by Li et al. (2014)
- No primer dimers
- Ability to multiplex
  - Only one dye channel utilized

A capillary electrophoresis method for identifying forensically relevant body fluids using miRNAs

Carrie Mayes B.S.; Sarah Seashols-Williams Ph.D.;
Sheree Hughes-Stamm Ph.D.

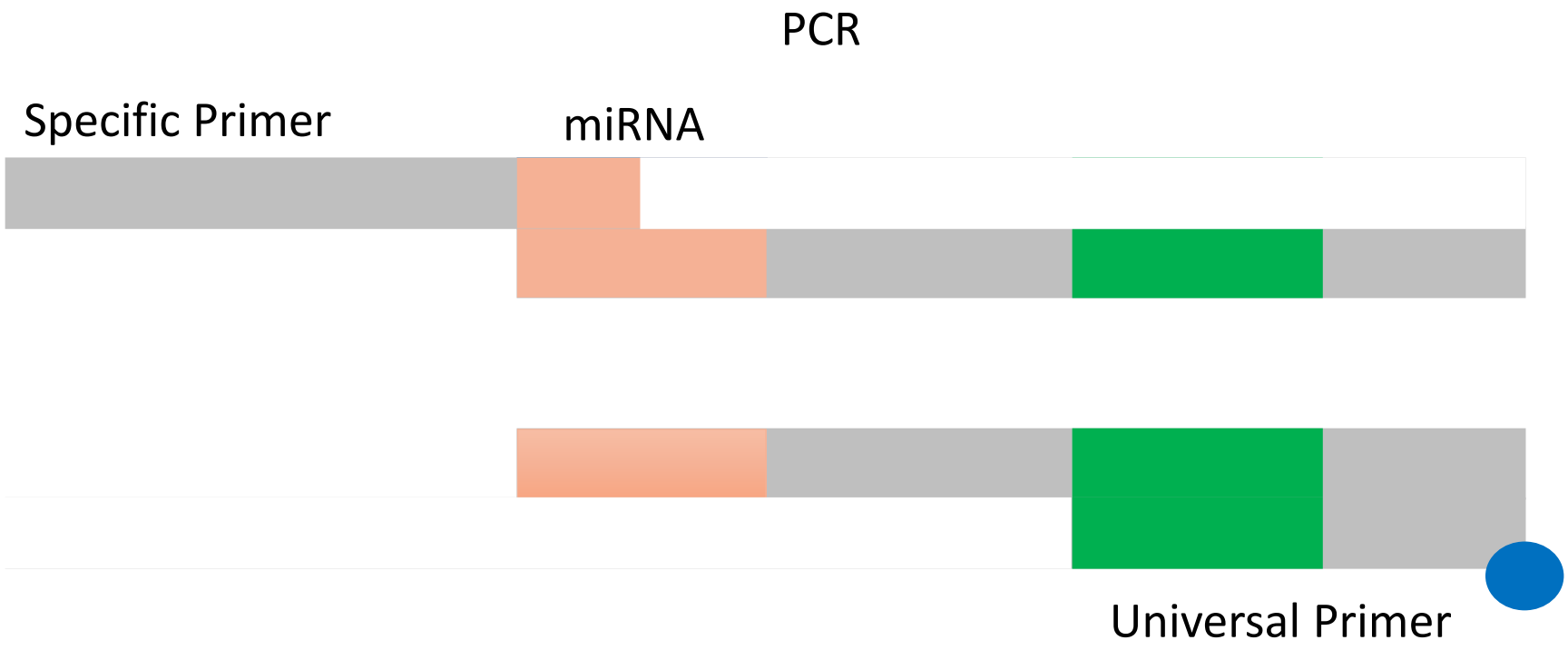
Legal Medicine. Volume 30. Pages 1-4.



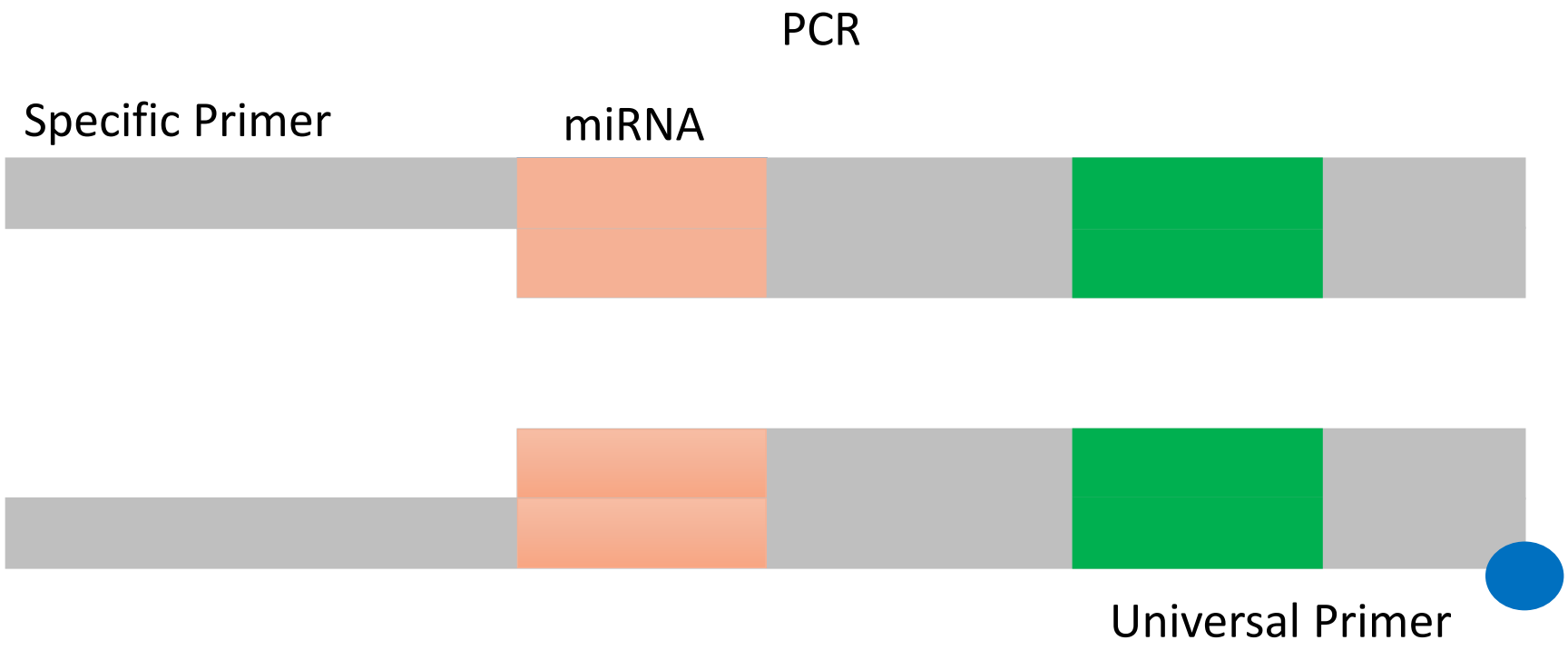
Linear Primer System

Reverse Transcription

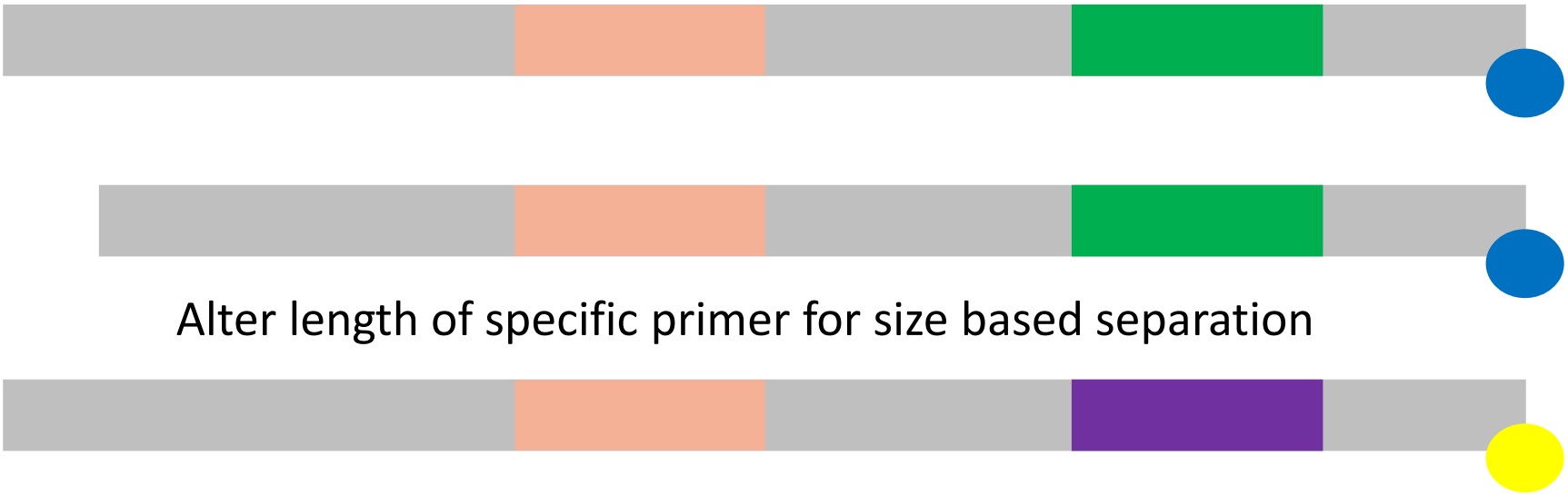
Linear Primer System



Linear Primer System



Linear Primer System



Alter length of specific primer for size based separation

Different paired binding sequences correspond with dye

Body Fluid	Marker	RT Primer	Specific Primer	Universal Primer
Venous Blood	miR-451	GTTCTTGCTGTCAACGATACGCTACGT TTTCTTTTCTTTT-AACTCAGT	GTTCTTCTTTTTCTTTTTCTTTTTC-AAACCGTTACCATT	FAM'GTTCTTCTTTTTCTTTTTCT-GCTGTCAACGATACGCTACG
	miR-142-3p	GTTCTTGCTGTCAACGATACGCTACGT TTTCTTTTCTTTT-TCCATAAA	GTTCTTCTTTTTCTTTTTCTTTTTCTTT TTCT-TGTAGTGTTTCCTAC	
Ref. Gene	let-7g	GTTCTTGCTGTCAACGATACGCTACGT TTTCTTTTCTTTTAACTGTAC	GTTCTTCTTTTTCTTTTTCTTTTCTTTC TTTCTTTCTTTTGAGGTAGTAGTTT	
Menstrual Blood	miR-141-3p	GTTCTT AACTGACTAAACTAGGTGCC TTTTCTTTTCTTTT-CCATCTTT	GTTCTTCTTTTTCTTTTTCT-TAACACTGTCTGGT	VIC'GTTCTTCTTTTTCTTTTTCT-AACTGACTAAACTAGGTGCC
	miR-412	GTTCTT AACTGACTAAACTAGGTGCC TTTTCTTTTCTTTT- ACGGCTAG	GTTCTTCTTTTTCTTTTTCTTTTTCTTT TTCT-ACTTCACCTGGTCCA	
Semen	miR-891a	GTTCTT ACGTCGTGAAAGTCTGACAA TTTTCTTTTCTTTT- TCAGTGGC	GTTCTTCTTTTTCTTTTTCTTTTTCTTT TTCTTTT-TGCAACGAACCTGA	NED'GTTCTTCTTTTTCTTTTTCT-ACGTCGTGAAAGTCTGACAA
	miR-10b	GTTCTT ACGTCGTGAAAGTCTGACAA TTTTCTTTTCTTTT- CACAAATT	GTTCTTCTTTTTCTTTTTCTTTTTCT-ATGGGACATCTTCCG	
Saliva	miR-205	GTTCTT GTAAAACGACGGCCAG TTTTCTTTTCTTTT- CAGACTCC	GTTCTTCTTTTTCTTTTTCTTTTTCTTT -TCCTTCATTCCACC	PET'GTTCTTCTTTTTCTTTTTCT-GTAAAACGACGGCCAG
	miR-658	GTTCTT GTAAAACGACGGCCAG TTTTCTTTTCTTTT- ACCAACGG	GTTCTTCTTTTTCTTTTT-GGCGGAGGGAAGTAGGT	

Materials and Methods

- Sample Collection
  - Donors (n=5) per body fluid
  - SHSU IRB approval #2015-09-26124

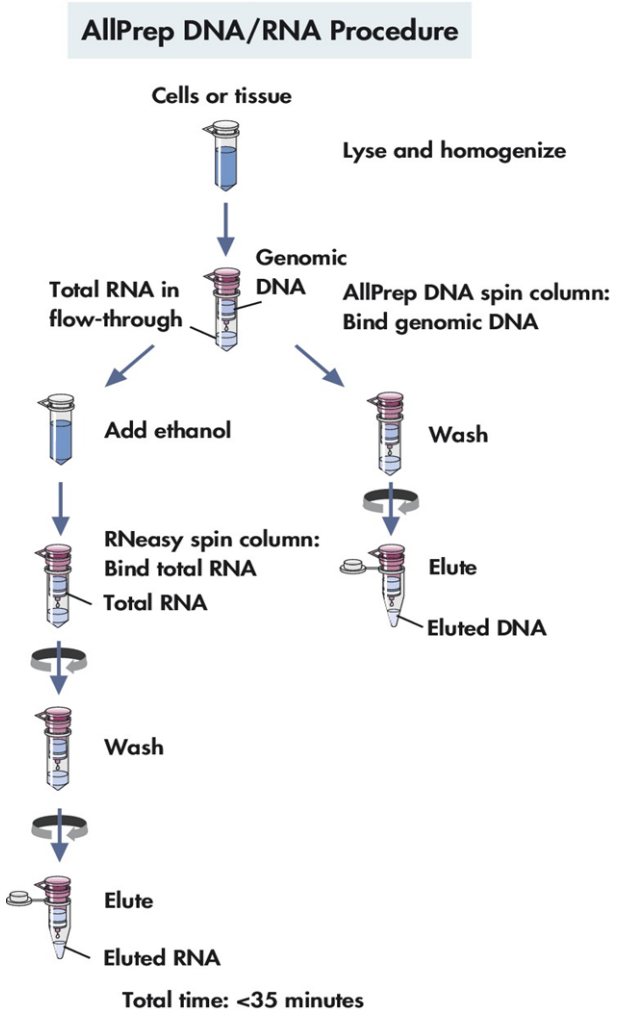
- Venous blood collected through venipuncture, saliva in tubes, menstrual blood on cotton swabs, and semen in specimen containers

- 50 µL of blood, saliva, and semen or 1 menstrual swab used for extractions

Materials and Methods

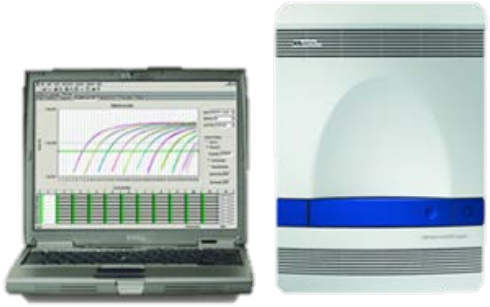
Extraction

DNA



Materials and Methods

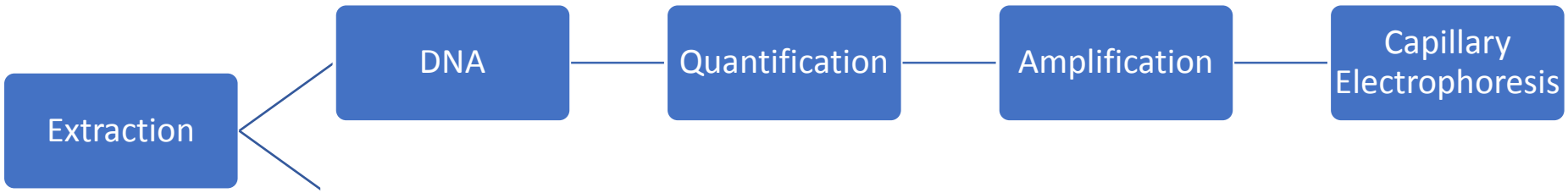
Extraction → DNA → Quantification



Materials and Methods

Extraction → DNA → Quantification → Amplification

Materials and Methods



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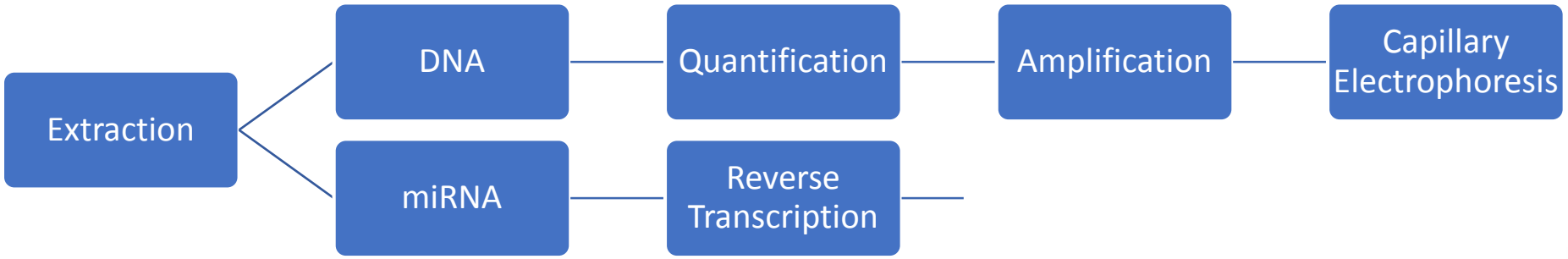

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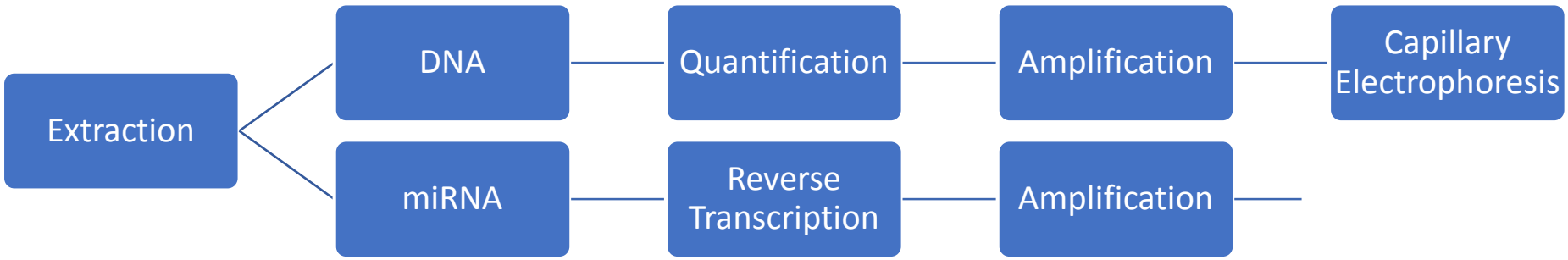

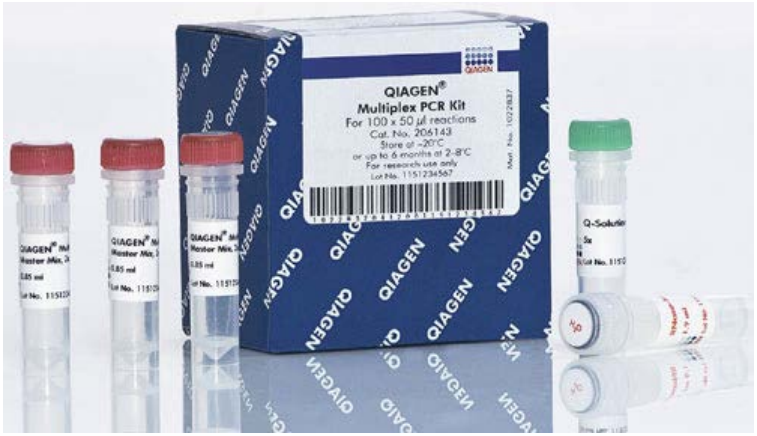

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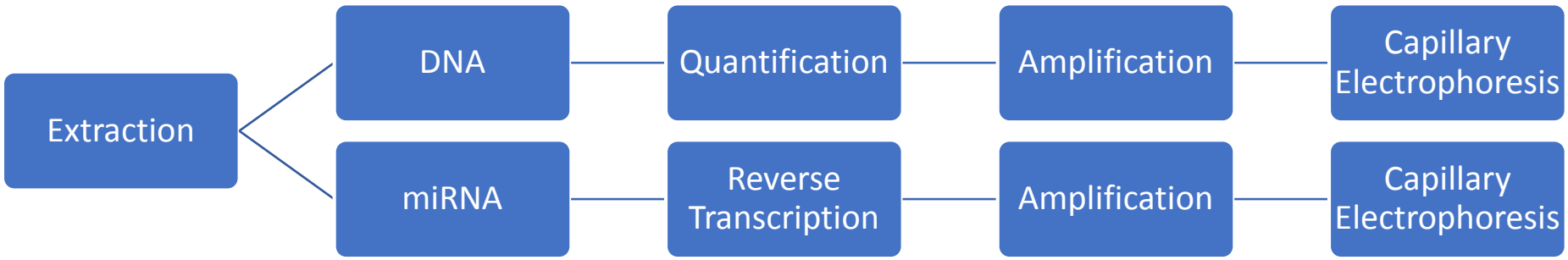
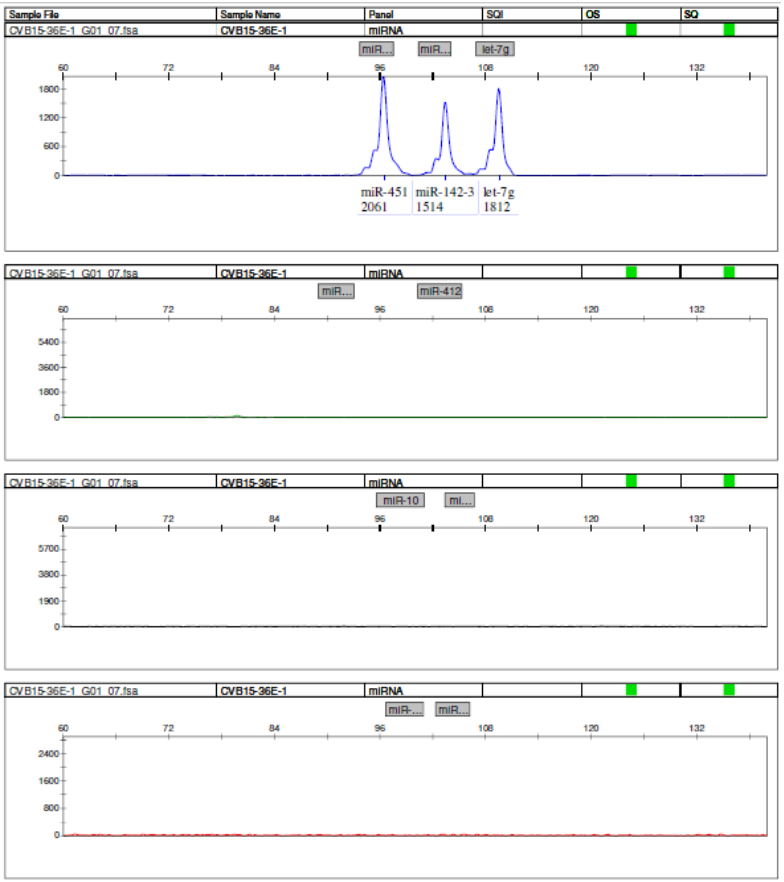


miRNA Profiles

VENOUS BLOOD

SALIVA

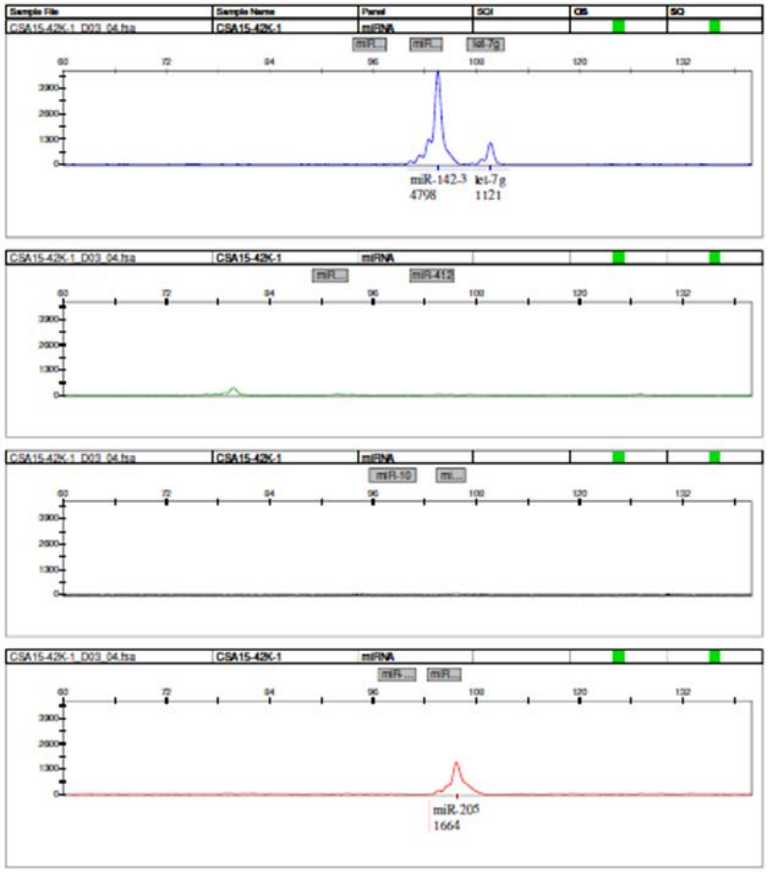


Venous Blood

Menstrual Blood

Semen

Saliva

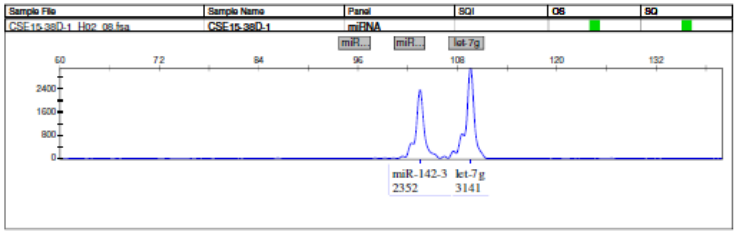


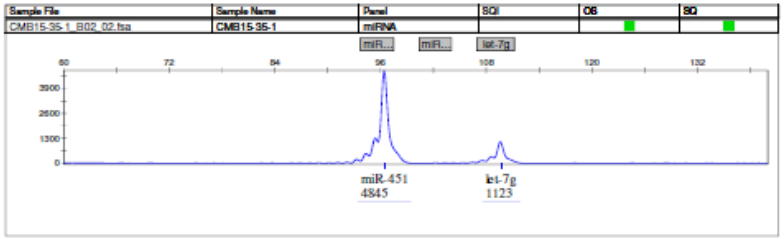
miRNA Profiles

SEMEN

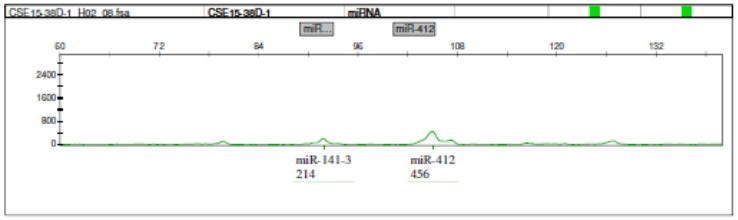
MENSTRUAL BLOOD

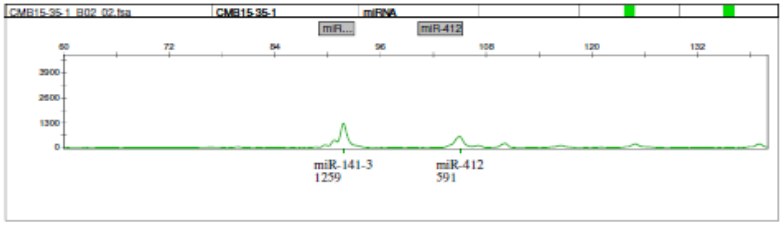
Venous Blood



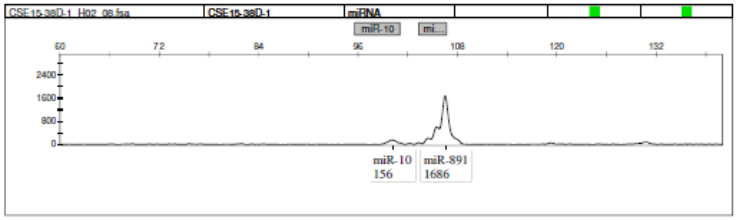


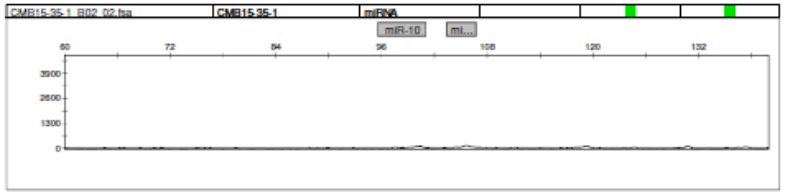
Menstrual Blood



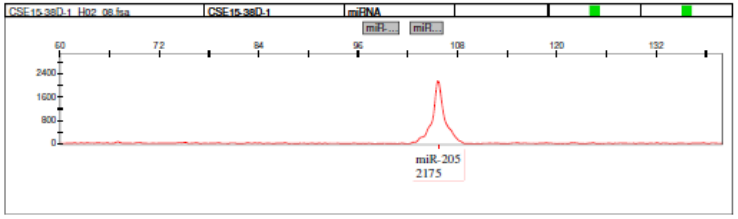


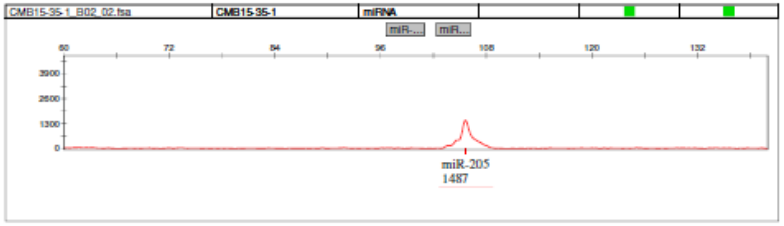
Semen





Saliva



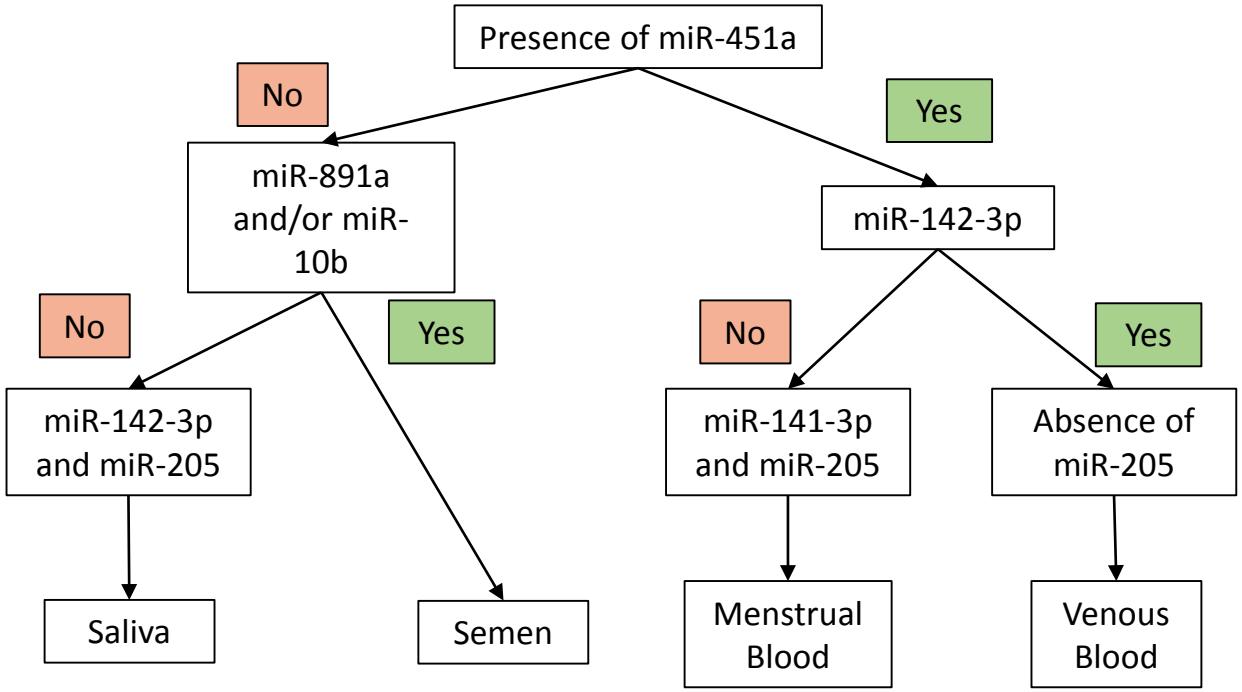


Results - Summary

Body Fluid	Reference	Venous Blood		Menstrual Blood		Semen		Saliva
	let-7g	miR-451a	miR-142-3p	miR-141-3p	miR-412-3p	miR-10b	miR-891a	miR-205
Venous Blood	1	1	1	0	0	0	0	0
Menstrual Blood	1	1	0	1	0.6	0	0	1
Semen	1	0	1	0.8	0.8	1	1	1
Saliva	1	0	1	0.2	0.2	0	0	1

- Proportion markers observed in each fluid (n=5)

Decision Tree for BFID Determination



Conclusions

All DNA profiles were concordant

An interpretation strategy was developed based on the presence/absence of markers.

Non-specific amplification was observed in the green channel in menstrual blood and saliva samples.

Cross-reactivity of miR-141-3p and miR-412-3p complicates mixture interpretation.

The multiplex was able to distinguish between venous blood, menstrual blood, semen, and saliva.

Comparing the stability and persistence of miRNA and mRNA for body fluid identification in forensic samples

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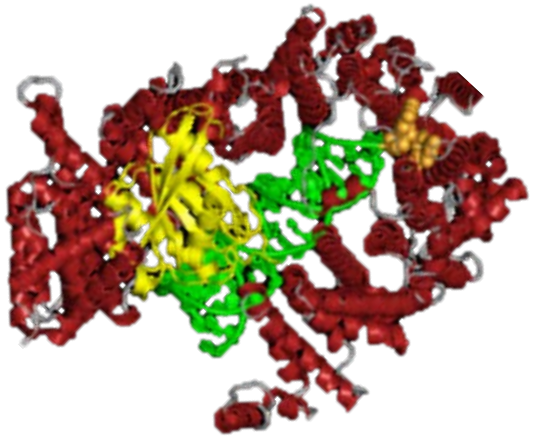
Sheree Hughes-Stamm Ph.D.



Blood
(n=3; 50µL)

Semen
(n=3; 50µL)

Cotton
Swatches

Temp, UV,
Humidity
Controlled

Temp, UV,
Humidity
Exposed

Collection on days:
0,1,7,14 30,60,90,180

RNA Extraction:
miRNeasy Kit

RNA Extraction:
miRNeasy Kit

Reverse Transcription:
miScript II RT Kit
QuantiTect® RT Kit

RNA Extraction:
miRNeasy Kit

Reverse Transcription:
miScript II RT Kit
QuantiTect® RT Kit

Real-Time PCR:
miScript SYBR® Green
PCR Kit (duplicate)

mRNA
QuantiTect®
Primer Assay

miRNA
miScript Primer
Assay

Ref.
B2M

Blood
HBB

Semen
PRM1

Ref.
let-7g

Blood
miR-451

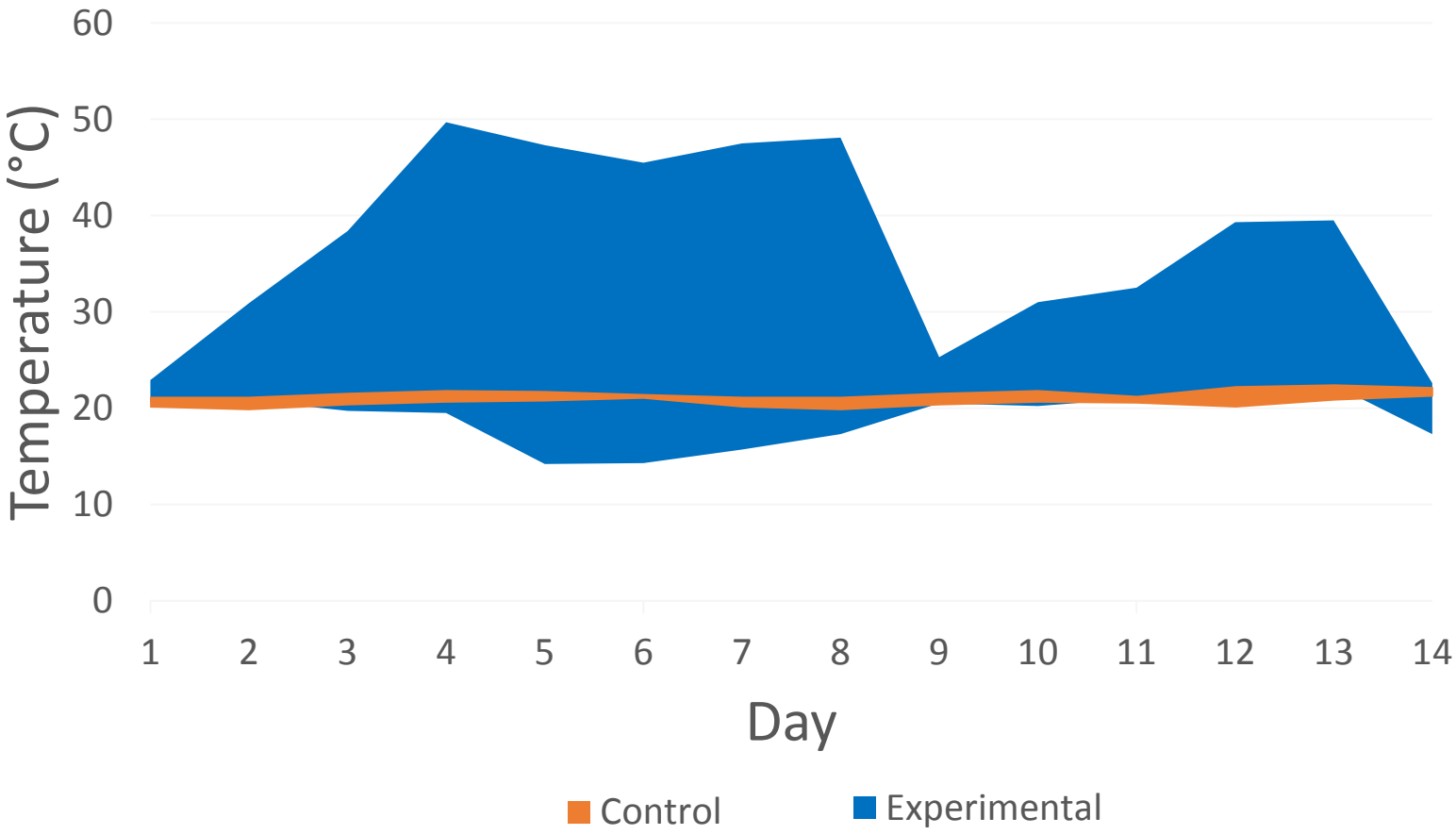
Semen
miR-891

Data Interpretation

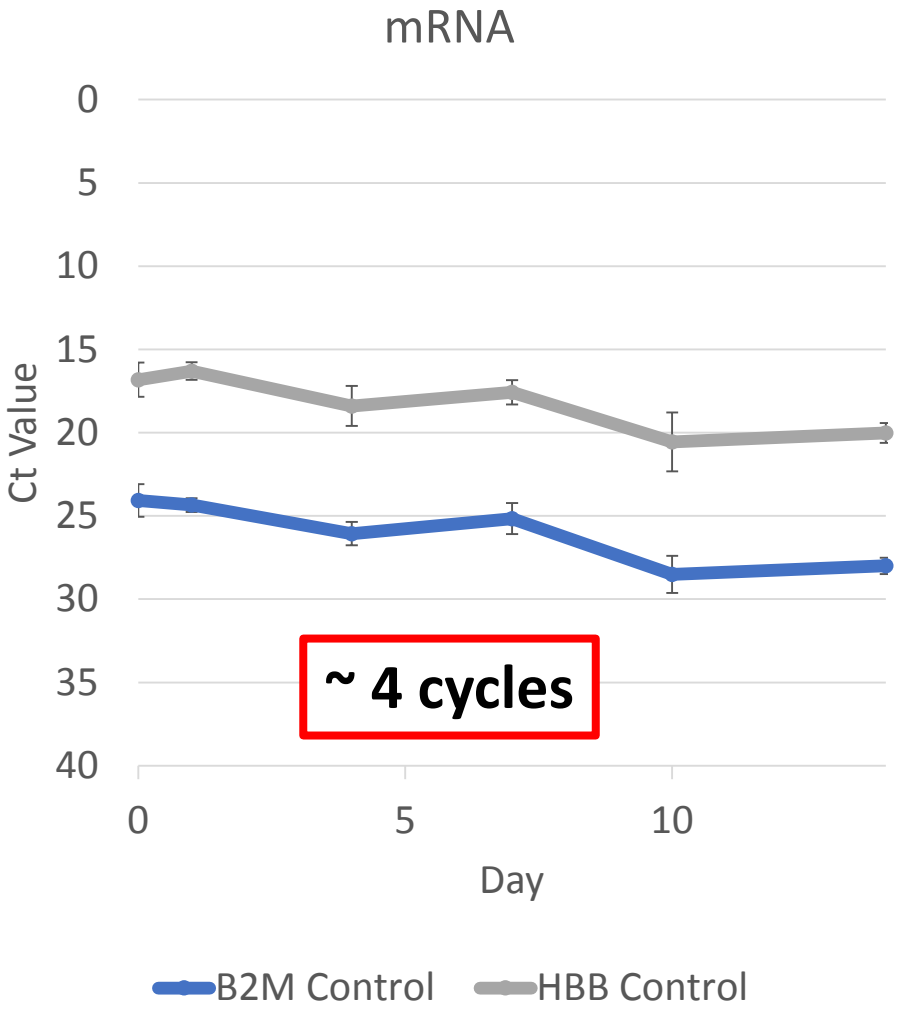
- Primer validation
  - Amplification efficiencies
  - Melt curve
  - Comparable scale between mRNA and miRNA assays
- ΔΔCt
- Drop in cycle number over time

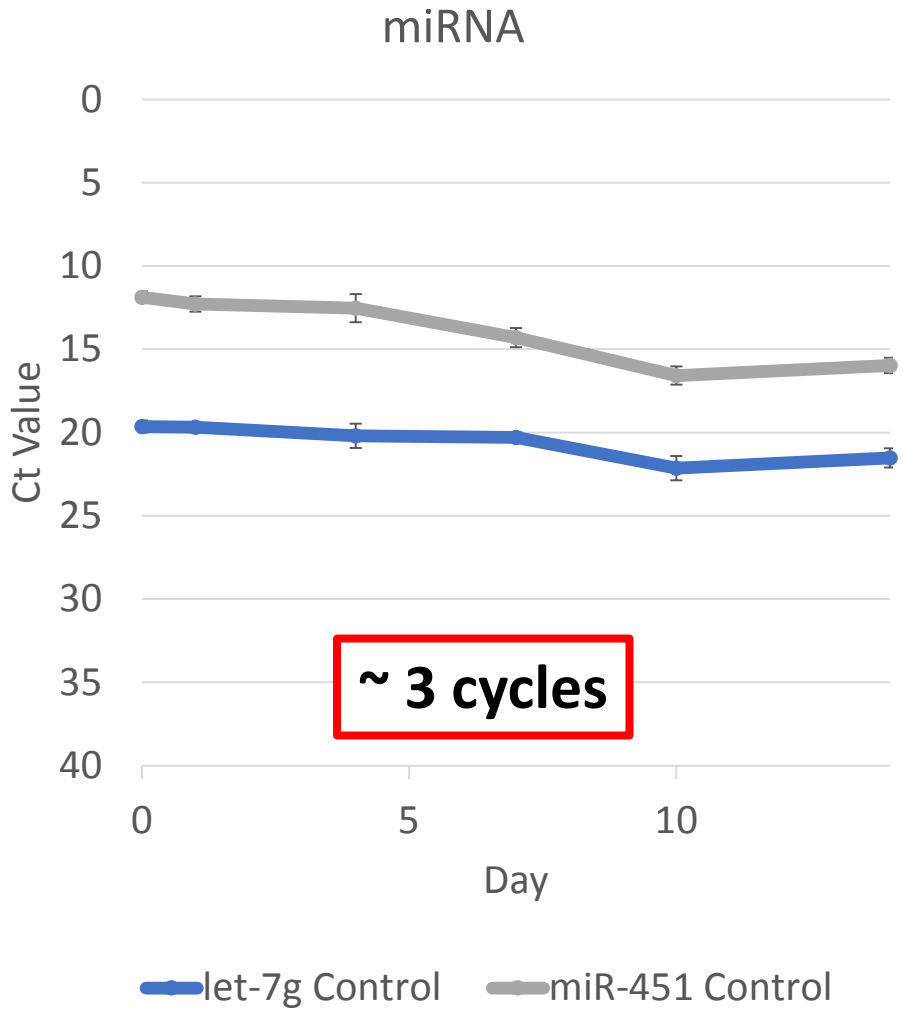
Fluid	Marker	Efficiency	R^2
Blood	let-7g	87.3%	0.999
	miR-451	89.2%	0.999
	B2M	95.3%	0.994
	HBB	93.2%	0.998
Semen	let-7g	96.6%	0.994
	miR-891	95.5%	0.988
	B2M	96.9%	1.000
	PRM1	98.0%	0.999

Temperature

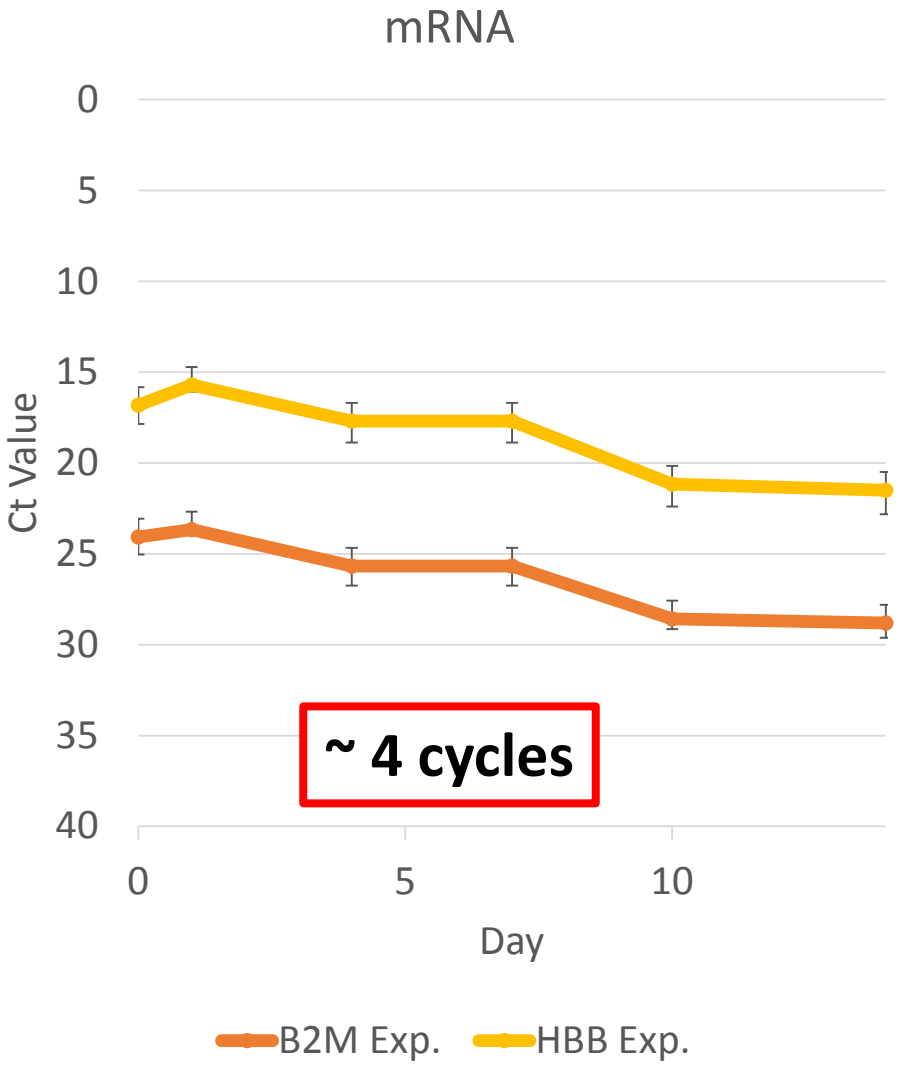


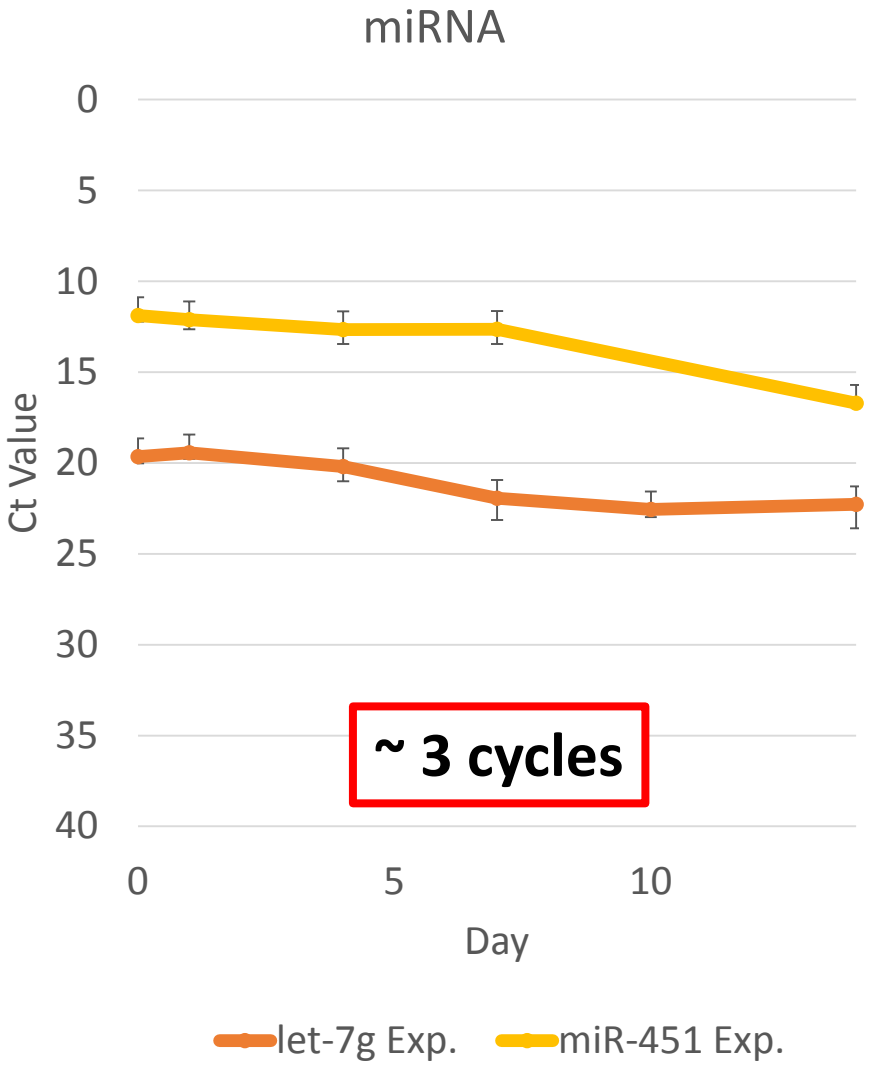
Blood – Controlled Conditions



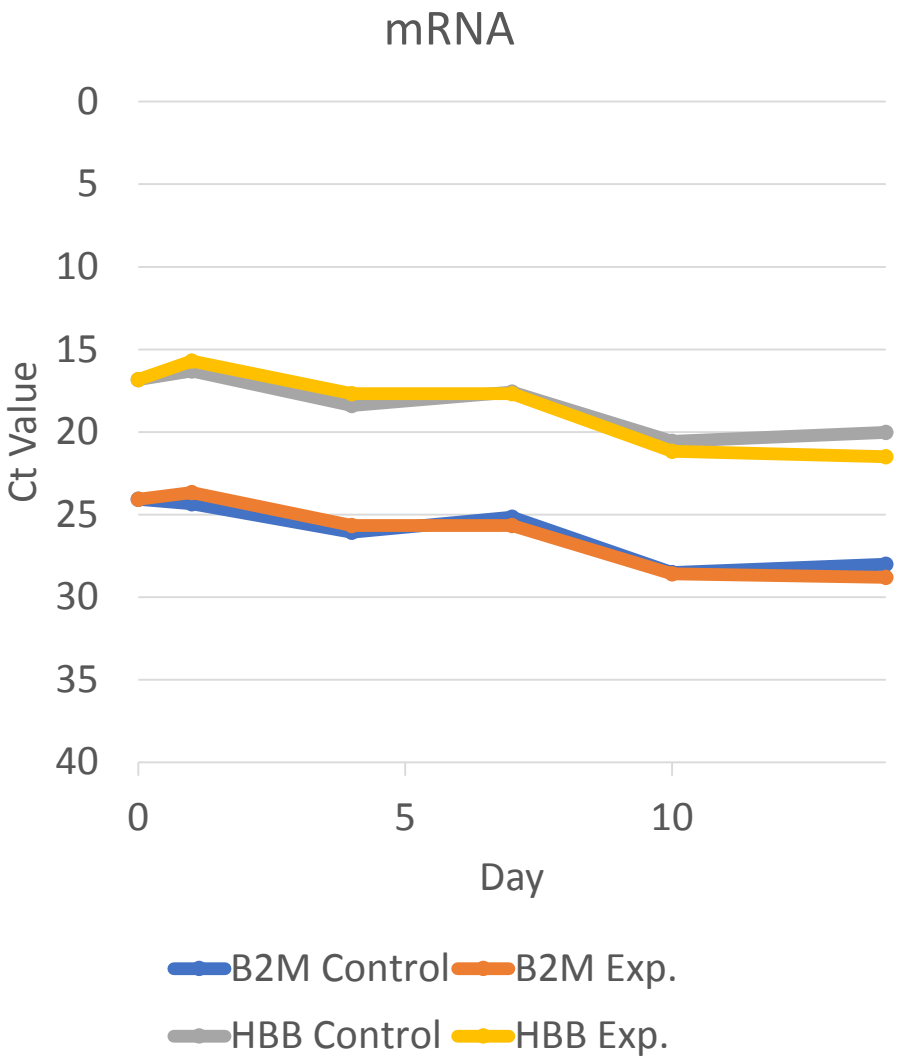


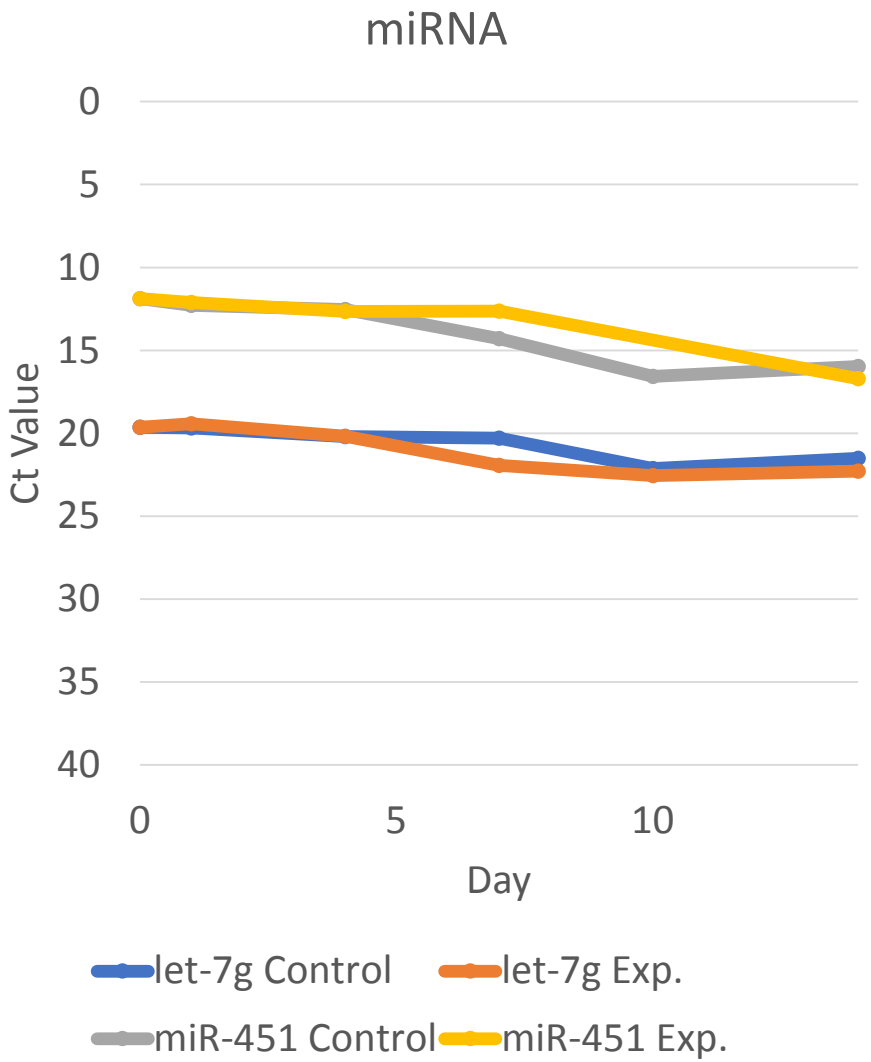
Blood – Experimental



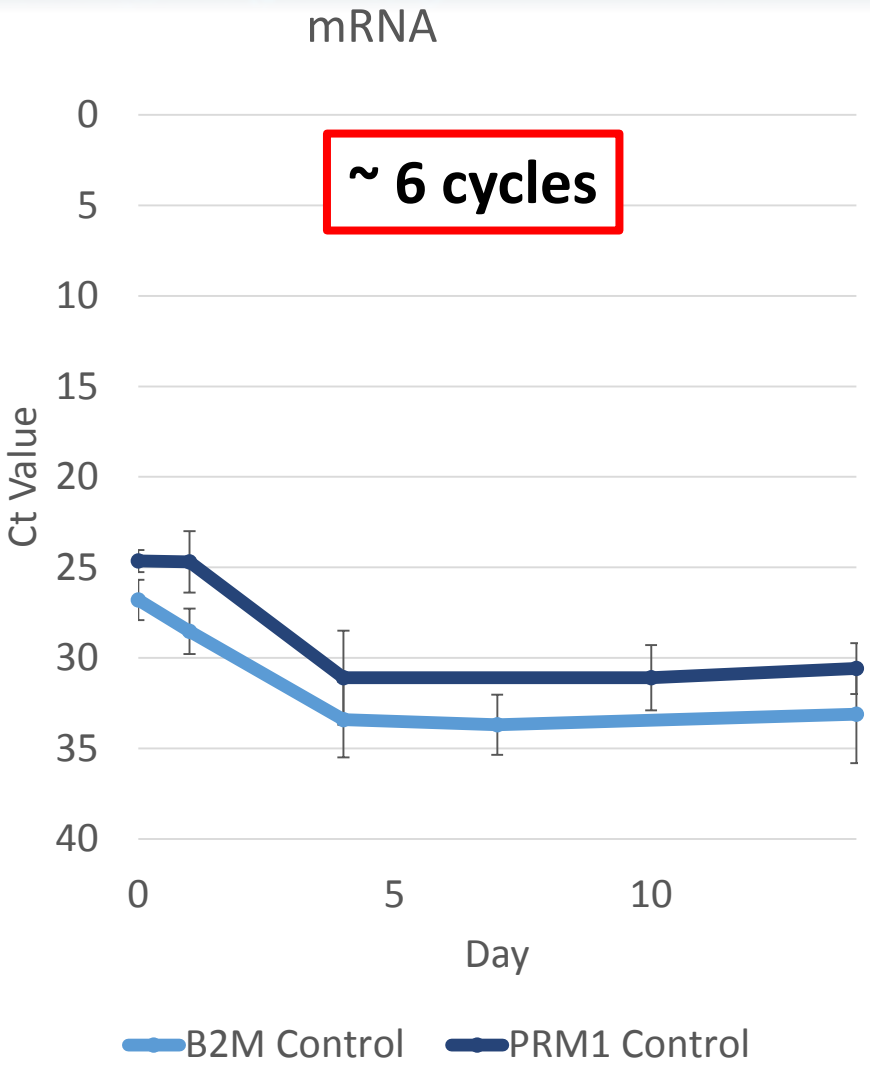


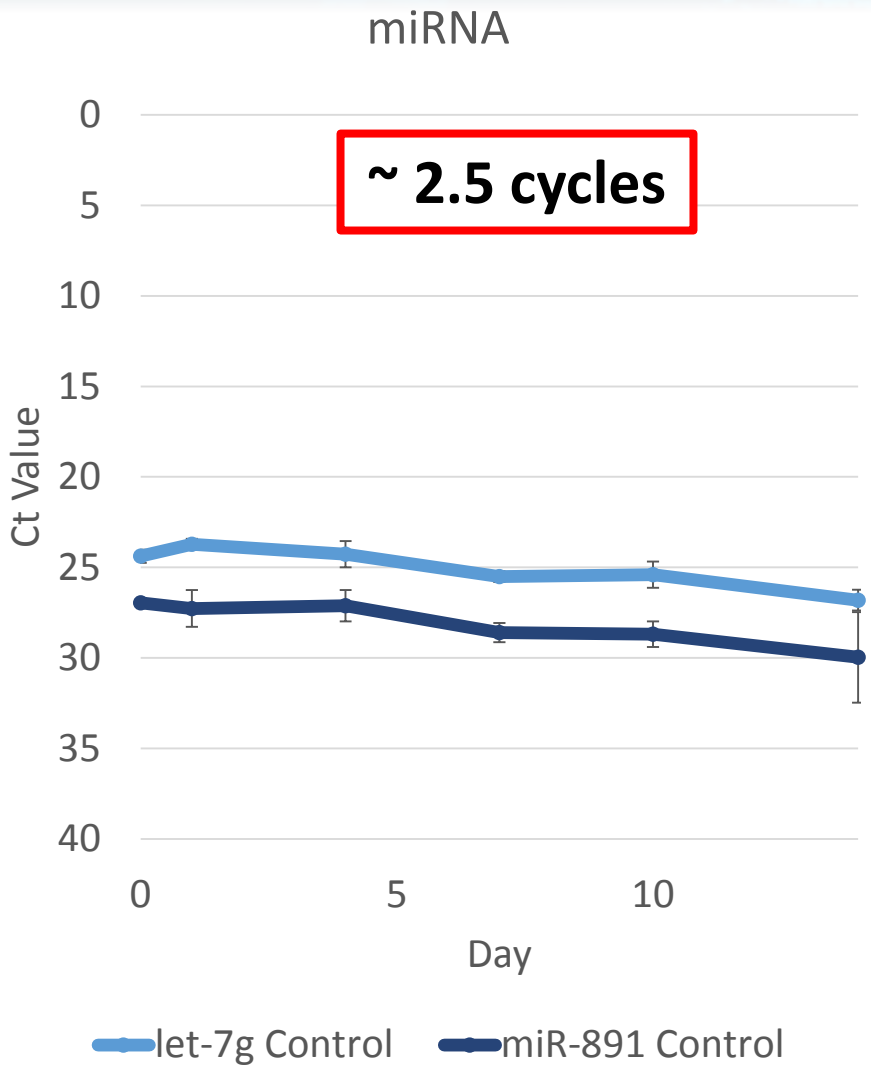
Blood – Overall



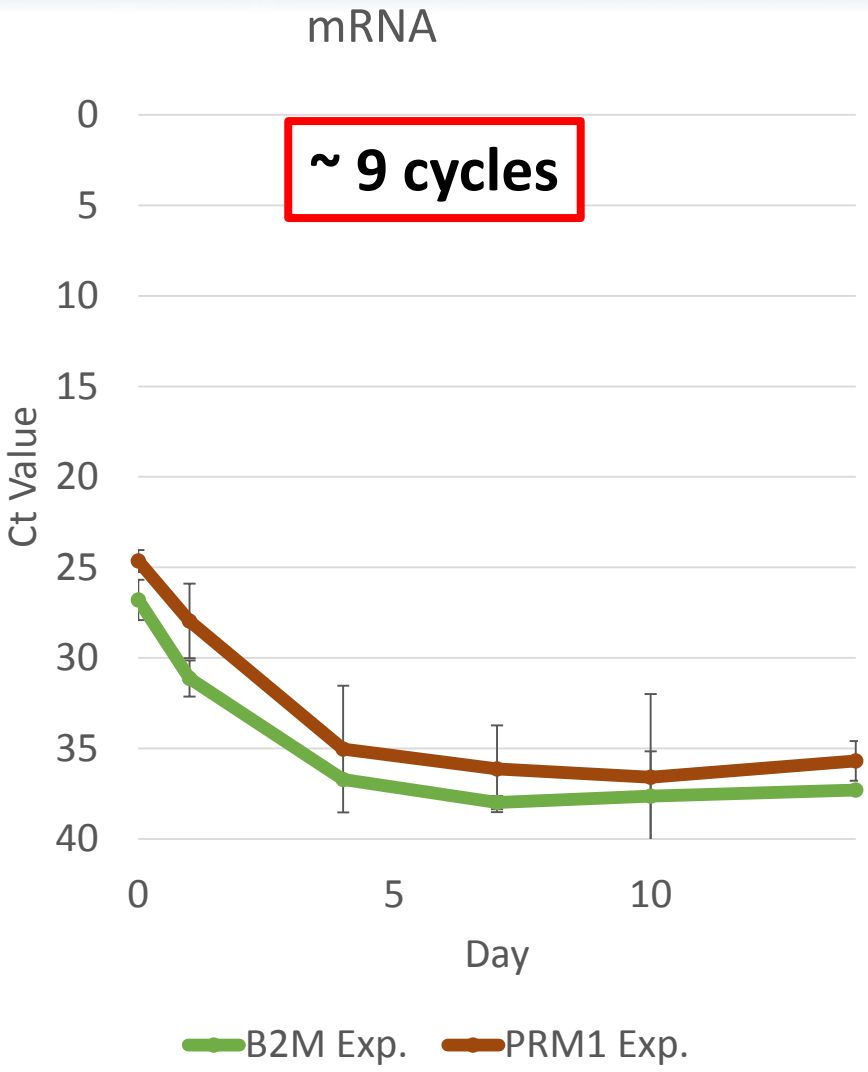


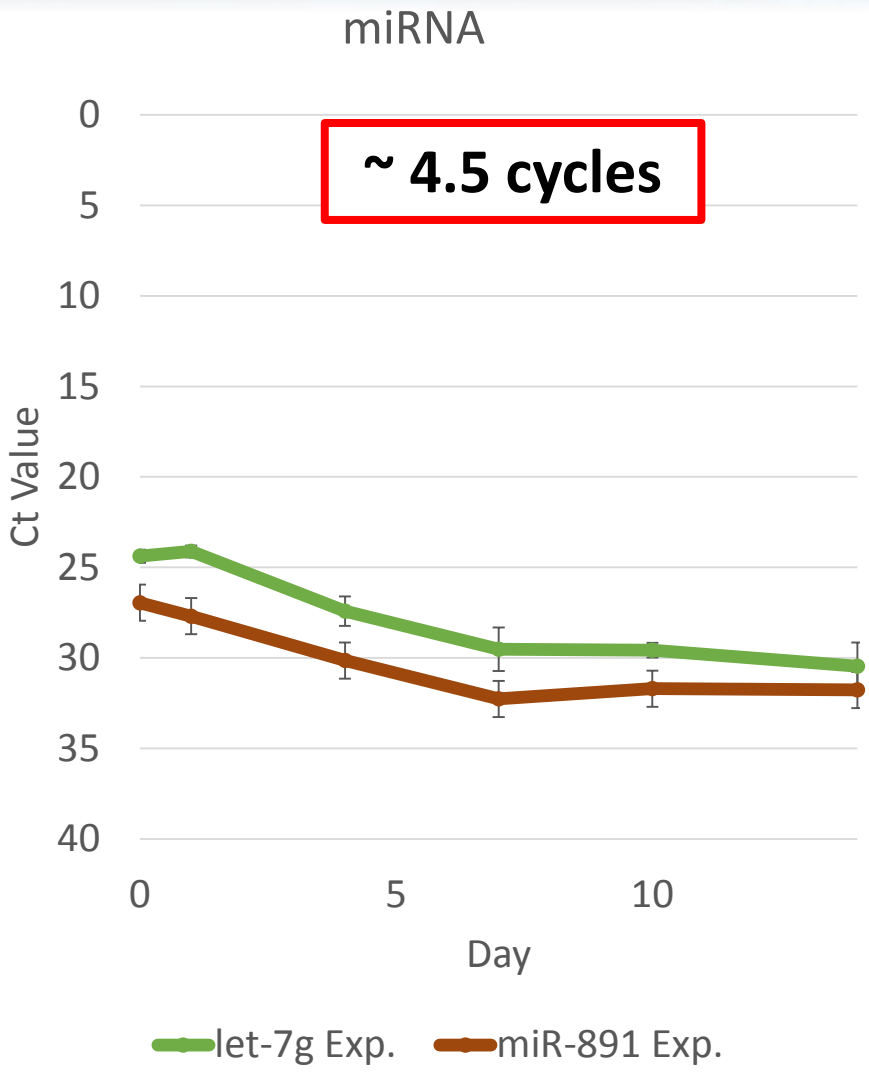
Semen – Controlled Conditions



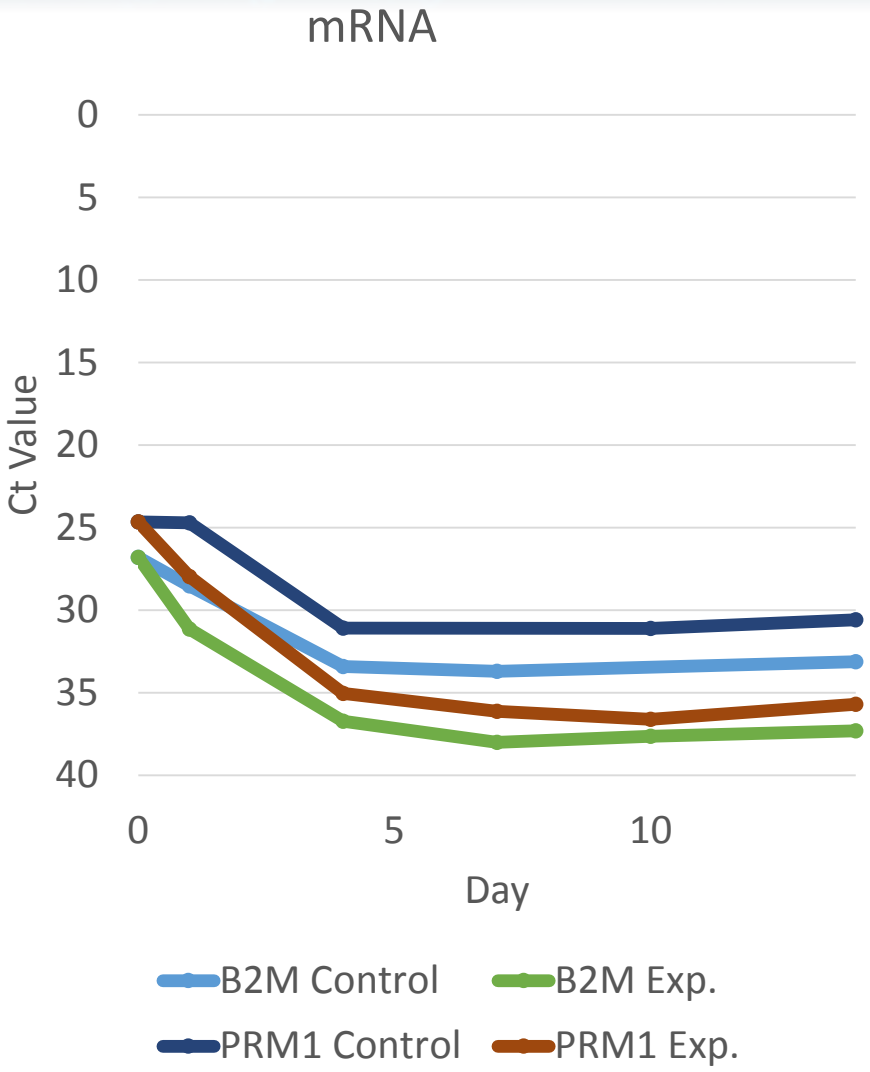


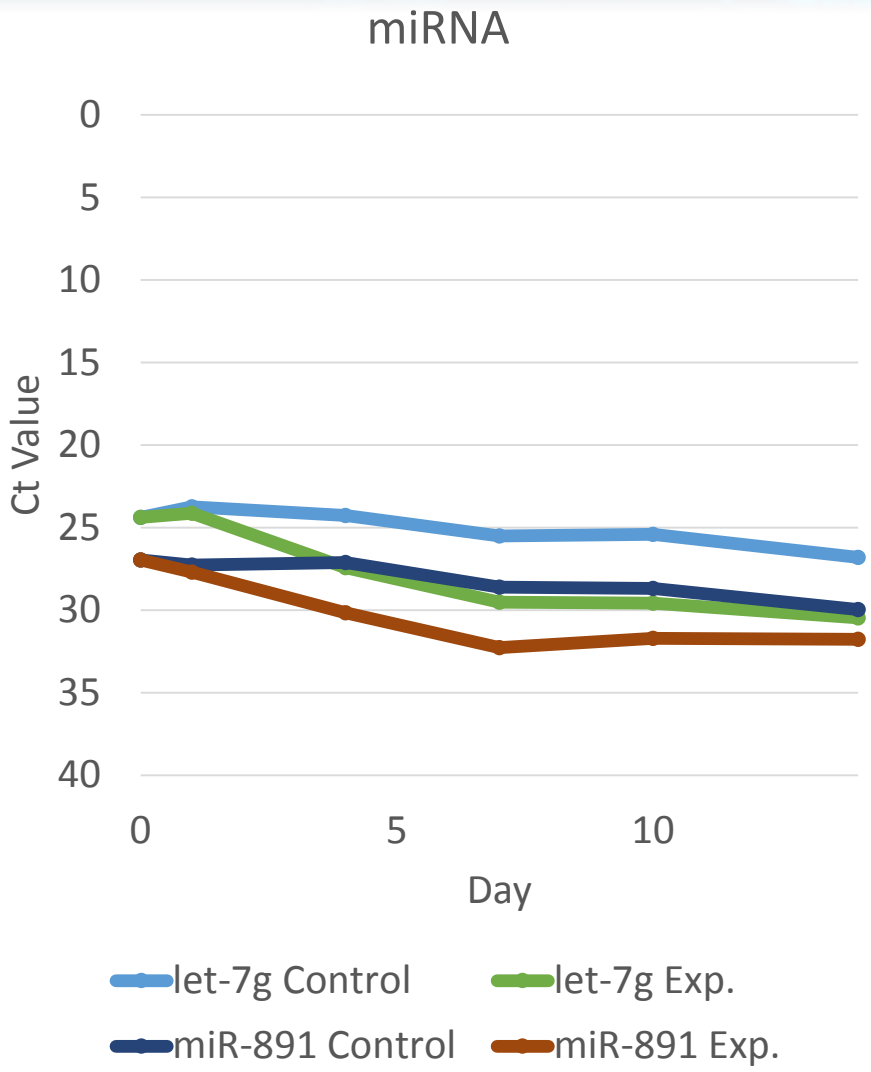
Semen – Experimental





Semen – Controlled Conditions





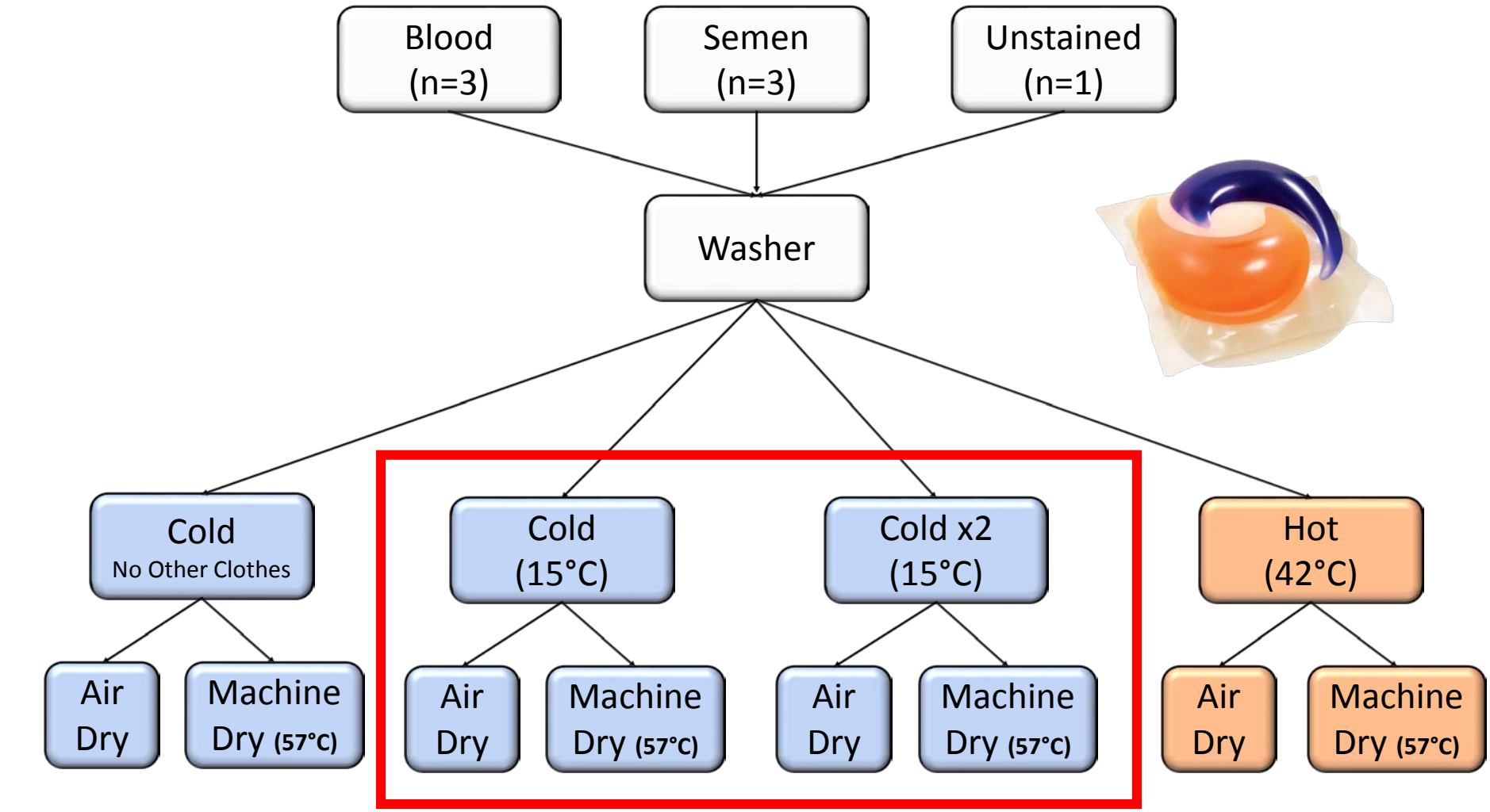
Preliminary Degradation Results

- Blood appears more stable
  - Anti-coagulant in venipuncture tubes
- mRNA drop-off between day 1 and day 4 in all semen samples
  - Similar trend observed in miRNA experimental samples
- Greater change in cycle number for mRNA
  - Suggests miRNA may be more stable
- Looking forward to later dates

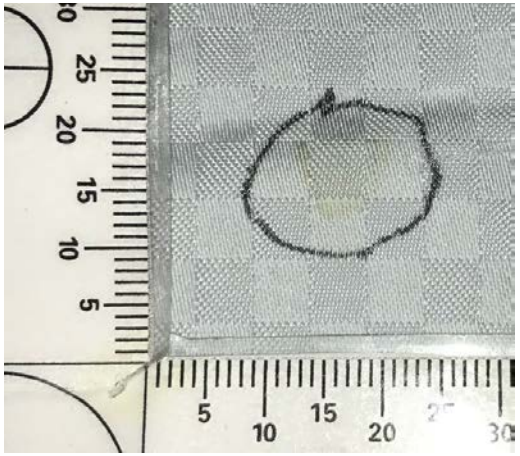
Laundered Items

- Transferred DNA has been observed in laundered items
  - Voskoboinik et al. (2017), Brayley-Mills et al. (2017).
- Conventional BFID techniques
  - Kulstein and Wiegand (2017), Edler et al. (2017)
- Spermatozoa identified post-laundry
  - Noel et al. (2016)

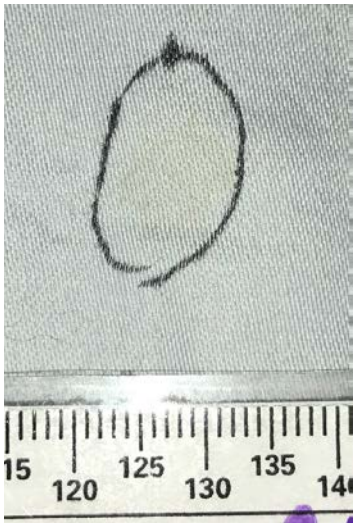
Laundered


Blood
(n=3)
Semen
(n=3)
Unstained
(n=1)
Washer
Cold
No Other Clothes
Air
Dry
Machine
Dry (57°C)
Cold
(15°C)
Air
Dry
Machine
Dry (57°C)
Cold x2
(15°C)
Air
Dry
Machine
Dry (57°C)
Hot
(42°C)
Air
Dry
Machine
Dry (57°C)

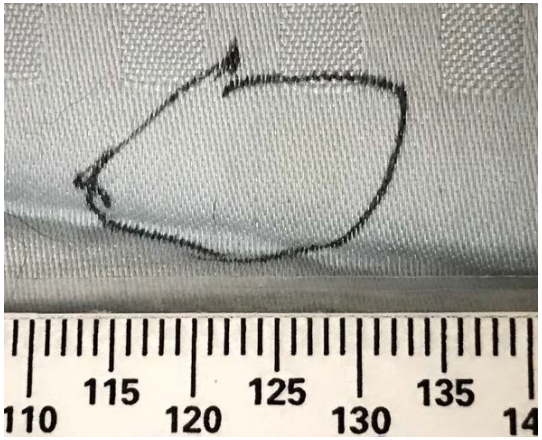
Rethinking my detergent…



Cold Wash
Air Dry

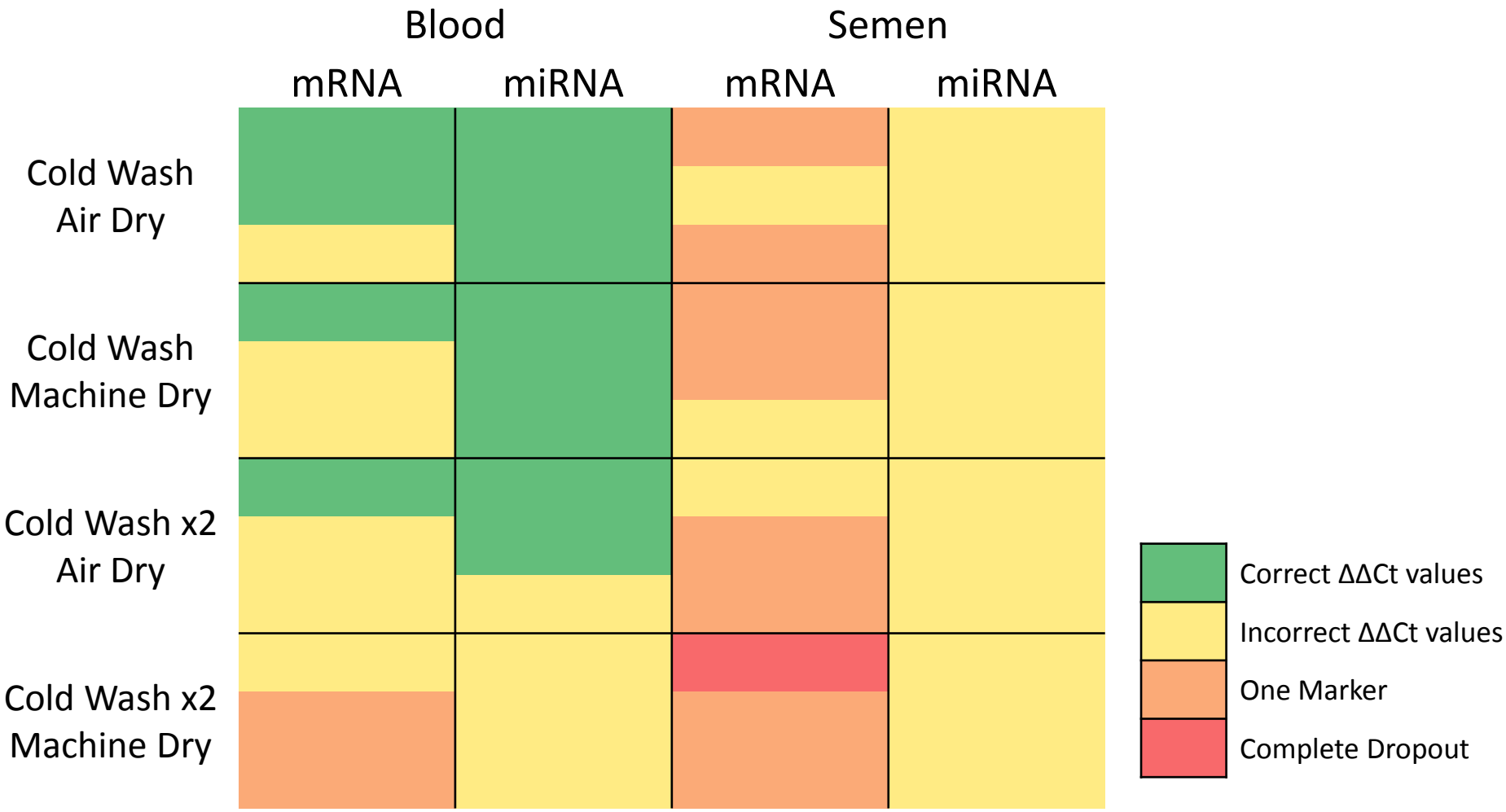


Cold Wash x2
Air Dry

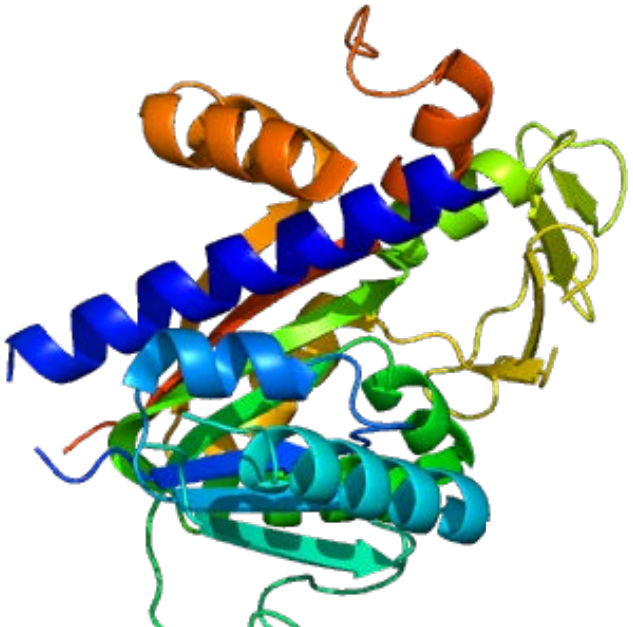


Cold Wash
Machine Dry

Laundered Samples



Evaluating the utility of massively parallel sequencing for mRNA for body fluid identification in challenging samples



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Questions?

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Extractimus Prime