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We are

the world's largest provider of hands-on training in qPCR, Europe's leading provider of nucleic acid analysis services by qPCR, and Sweden's most comprehensive distributor of qPCR products

Two-tailed PCR for Precision Diagnostics

TATAA Biocenter

TATAA Biocenter was founded in 2001 by pioneers in qPCR, and have extensive knowledge and hands-on experience within nucleic acid analysis. TATAA Biocenter offers a full range of RT-qPCR and Next-Generation Sequencing research services, and develops and performs a broad spectrum of hands-on courses world-wide. TATAA also offers a carefully chosen selection of high-quality products for qPCR and NGS applications. We are proud to provide expert support from our local specialists, from sample preparation to final result.

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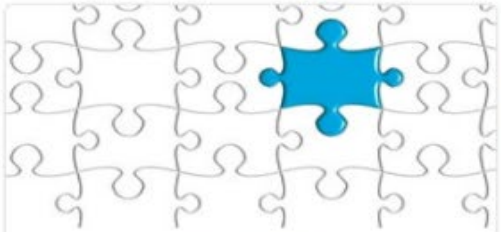
Latest news

12 DEC — Job Openings > Erfaren säljare till TATAA Bioc...
– READ MORE –

22 NOV — Molecular microbiological identification and typin...
– READ MORE –

16 NOV — Offer: Pick products from at least 2 of the workfl...
– READ MORE –



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Challenges analyzing miRNAs (and other short NA)

- microRNAs are short (most 21-22 nt) and cannot fit two conventional PCR primers
- There is no common sequence feature to use for the enrichment and amplification.
- The mature miRNA sequence is present also in the pre- and the pri-miRNAs
- miRNA isoforms (isomiRs) might evade capture, due to terminal heterogeneity

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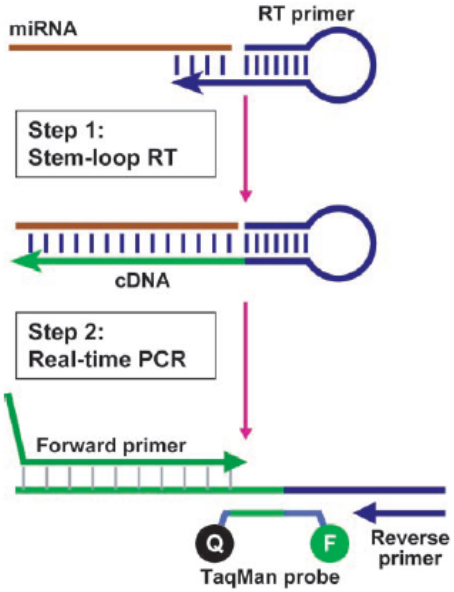
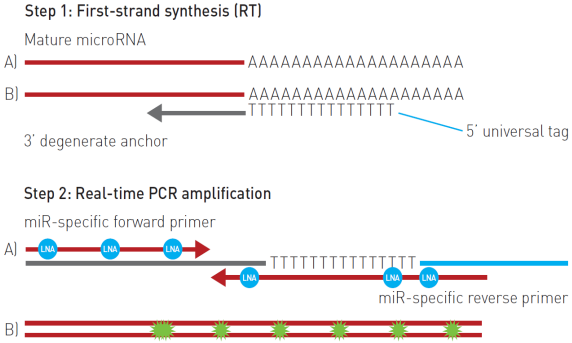
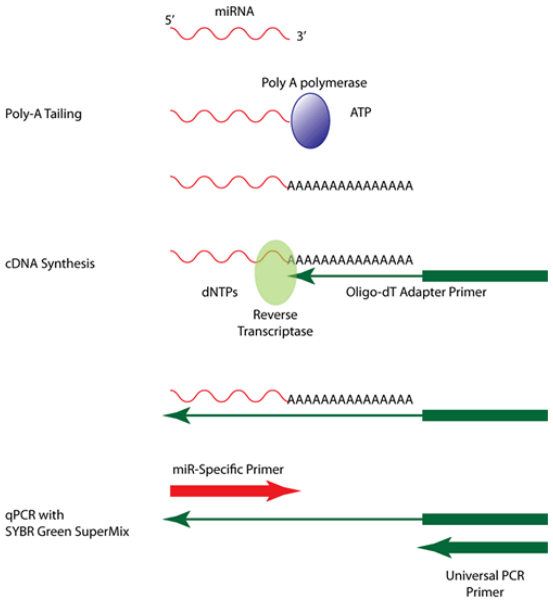
Current methods make the microRNA longer







ThermoFisher SCIENTIFIC

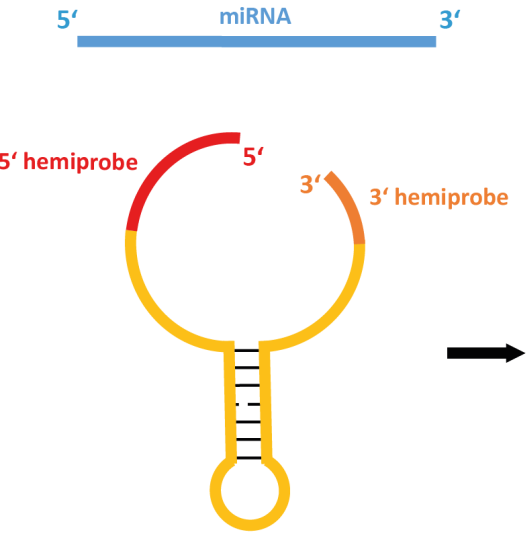
EXIQON
Now a QIAGEN company
QIAGEN

Quantabio
QIAGEN
TaKaRa
SIGMA-ALDRICH®

- Extension reduces sensitivity
- One probe only limits specificity

Two-tailed RT-qPCR

A



Two-tailed RT primer

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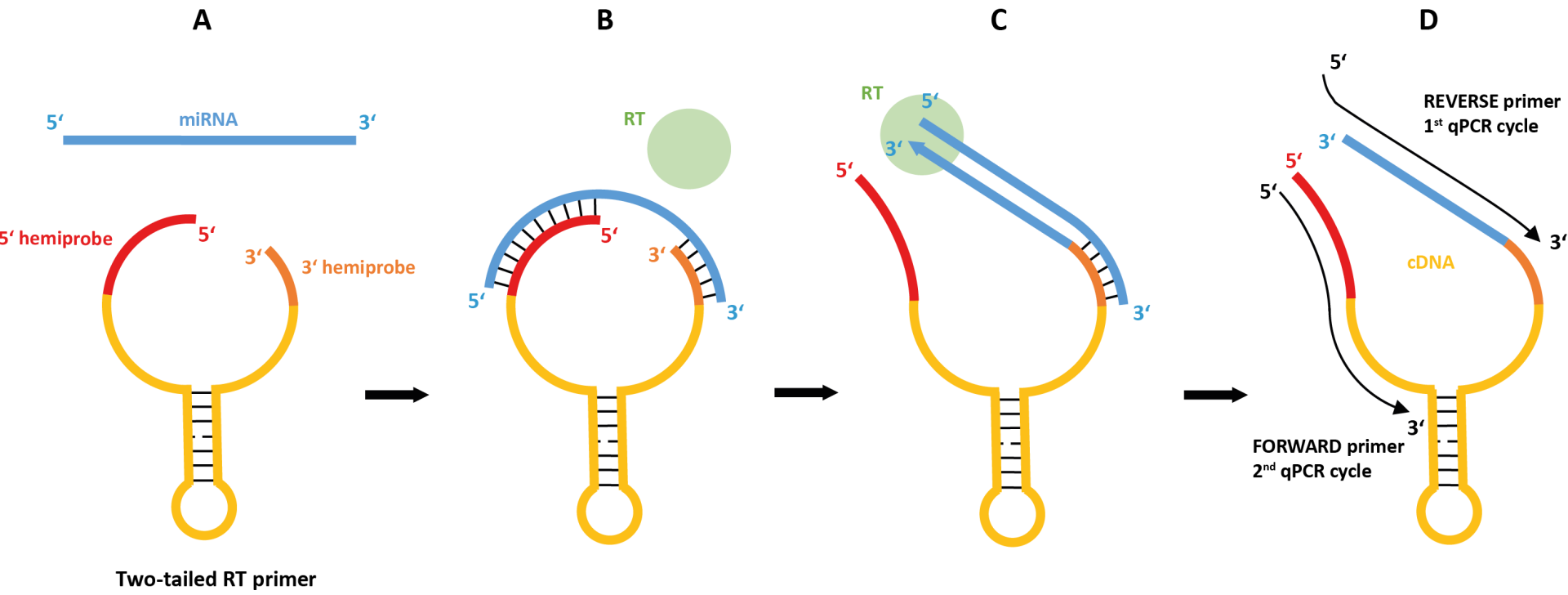
Two-tailed RT-qPCR



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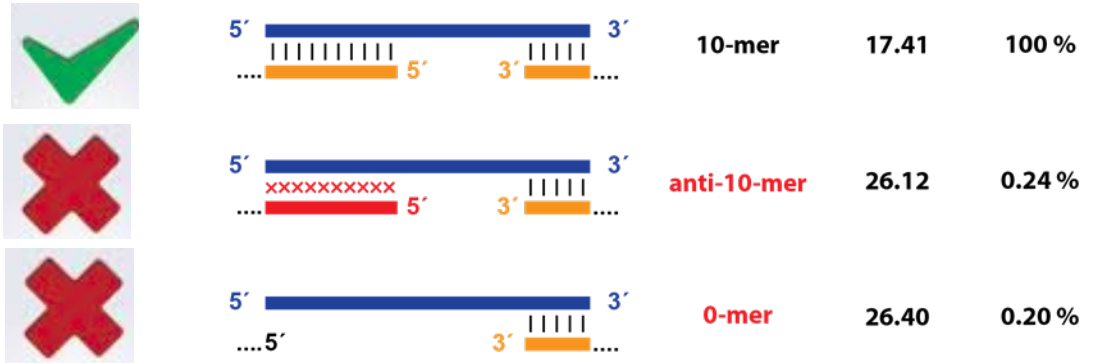
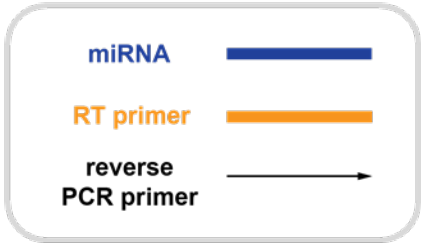
Design concept

- 5‘ complementary segment contributes to the **sensitivity** of the assays





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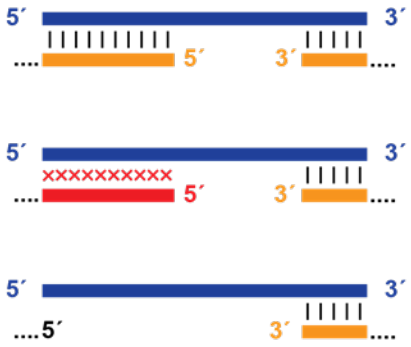
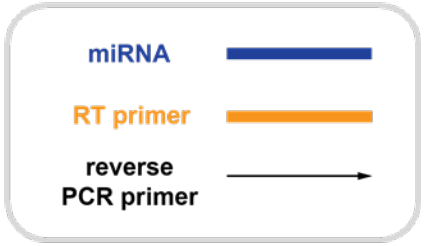
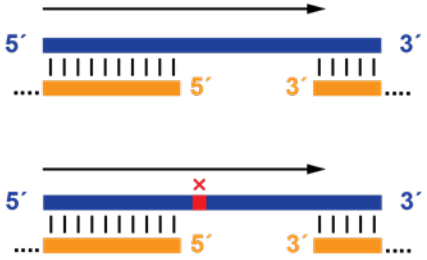


5´segment	Cq	Relative detection
10-mer	17.41	100 %
anti-10-mer	26.12	0.24 %
0-mer	26.40	0.20 %



- 5‘ complementary segment contributes to the **specificity** of the assays



Cq	Relative detection
17.41	100 %
22.07	3.96 %

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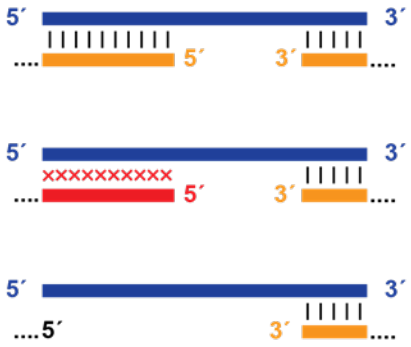

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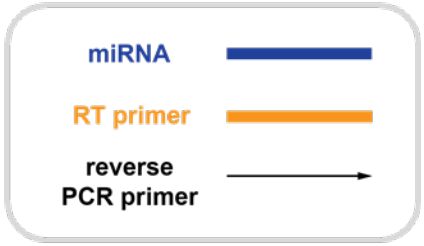

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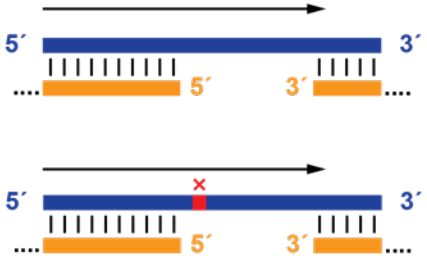

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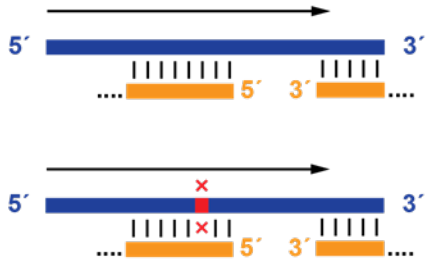

Cq	Relative detection
17.61	100 %
28.68	0.04 %

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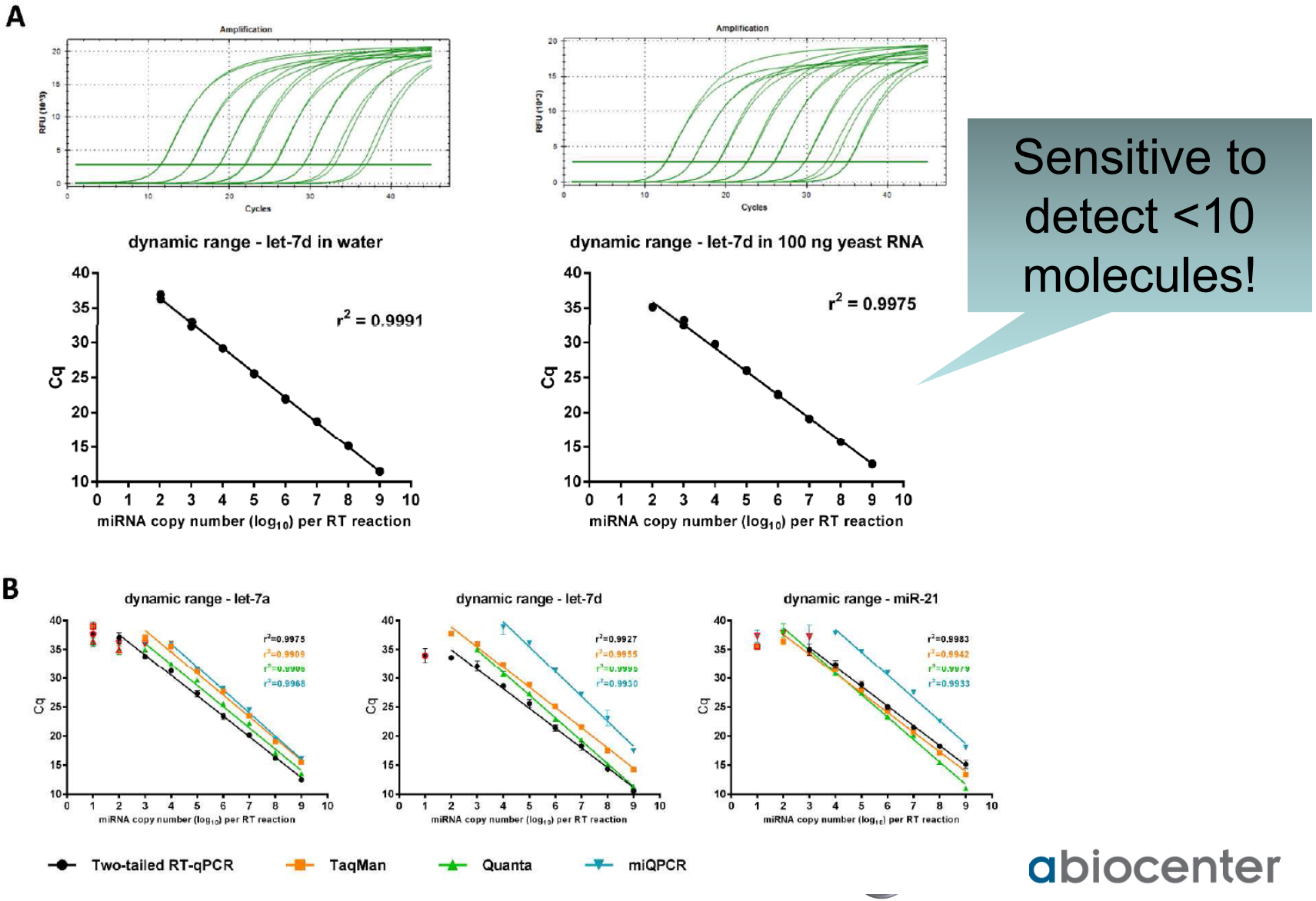
Sensitivity and dynamic range



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Sequence specificity across the entire microRNA

Relative detection (%)

Assays	*let-7a*	*let-7b*	*let-7c*	*let-7d*	*let-7e*	*let-7f*	*let-7g*	*let-7i*
A	100.00	0.07	0.46	0.14	0.31	0.01	0.00	0.00
B	0.00	100.00	0.61	0.00	0.00	0.00	0.00	0.00
C	0.01	0.18	100.00	0.00	0.00	0.00	0.00	0.00
D	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
E	0.15	0.00	0.00	0.01	100.00	0.00	0.00	0.00
F	0.18	0.00	0.01	0.00	0.00	100.00	0.02	0.00
G	0.00	0.00	0.00	0.00	0.00	0.01	100.00	0.00
I	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

Two-tailed RT-qPCR

Relative detection (%)

Assays	*let-7a*	*let-7b*	*let-7c*	*let-7d*	*let-7e*	*let-7f*	*let-7g*	*let-7i*
A	100.00	0.44	20.89	2.20	3.68	8.38	0.37	0.00
B	0.19	100.00	22.48	0.00	0.00	0.01	0.00	0.01
C	0.09	1.77	100.00	0.00	0.00	0.01	0.00	0.00
D	2.59	0.01	1.37	100.00	0.01	0.01	0.00	0.00
E	9.88	0.07	7.87	0.09	100.00	0.40	0.03	0.00
F	2.00	0.16	0.22	0.12	0.01	100.00	0.15	0.00
G	0.96	0.00	0.32	0.01	0.01	2.72	100.00	0.02
I	0.00	0.00	0.00	0.00	0.00	0.00	0.01	100.00

TaqMan

Relative detection (%)

Assays	*let-7a*	*let-7b*	*let-7c*	*let-7d*	*let-7e*	*let-7f*	*let-7g*	*let-7i*
A	100.00	0.27	50.71	2.17	1.58	2.47	1.55	0.00
B	0.09	100.00	32.84	0.00	0.00	0.00	0.00	0.02
C	48.91	27.00	100.00	0.31	0.56	0.95	0.06	0.00
D	0.12	0.33	0.07	100.00	0.00	0.00	0.00	0.00
E	0.13	0.13	0.13	0.00	100.00	0.03	0.03	0.02
F	0.73	0.85	0.72	0.02	0.00	100.00	0.05	0.04
G	0.02	0.00	0.01	0.00	0.00	0.26	100.00	16.84
I	0.00	0.00	0.00	0.00	0.00	0.00	0.38	100.00

Quanta

Relative detection (%)

Assays	*let-7a*	*let-7b*	*let-7c*	*let-7d*	*let-7e*	*let-7f*	*let-7g*	*let-7i*
A	100.00	12.64	55.52	101.75	122.47	76.72	48.68	0.69
B	7.78	100.00	45.46	1.08	0.06	0.08	0.01	1.39
C	66.40	75.14	100.00	28.76	1.13	9.15	0.45	0.01
D	14.84	0.00	0.09	100.00	0.21	0.19	0.03	0.00
E	51.07	0.04	20.96	27.57	100.00	6.52	0.99	0.00
F	54.28	0.01	0.56	11.85	3.28	100.00	14.45	0.05
G	0.07	0.00	0.00	0.00	0.00	0.18	100.00	0.91
I	0.00	0.00	0.00	0.00	0.00	0.00	7.43	100.00

miQPCR

B

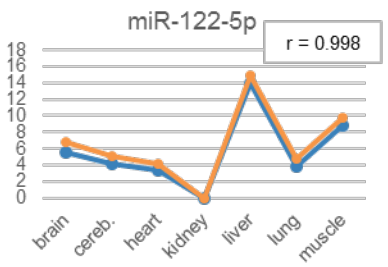
name	sequence
let-7a	UGAGGUAGUAGGUUGUAUAGUU
let-7b	UGAGGUAGUAGGUUGUGUGGUU
let-7c	UGAGGUAGUAGGUUGUAUGGUU
let-7d	AGAGGUAGUAGGUUGCAUAGUU
let-7e	UGAGGUAGGAGGUUGUAUAGUU
let-7f	UGAGGUAGUAGAUUGUAUAGUU
let-7g	UGAGGUAGUAGUUUGUACAGUU
let-7i	UGAGGUAGUAGUUUGUGCUGUU

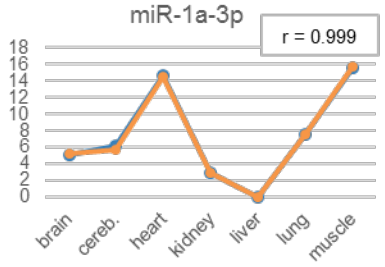
C

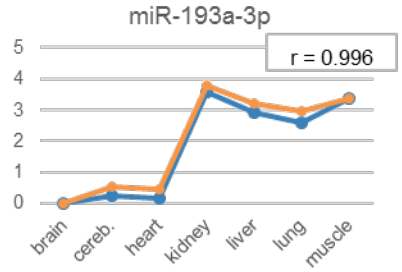
	Mature	Precursor	Relative detection
let-7a	17.74	21.31	6.98%
let-7b	16.98	21.22	5.31%
let-7f	16.85	23.78	0.82%

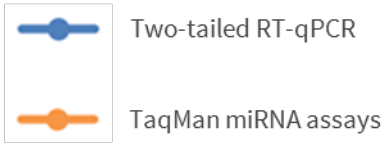
Benchmarking in biological samples

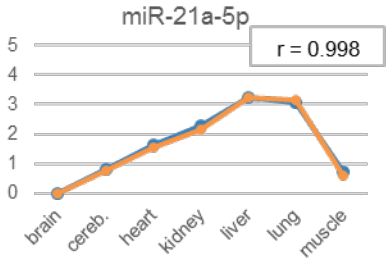
- Expression of 8 targets in 7 mouse tissues measured and compared with TaqMan miRNA assays
- Excellent correlation of relative expression profiles between the two methods

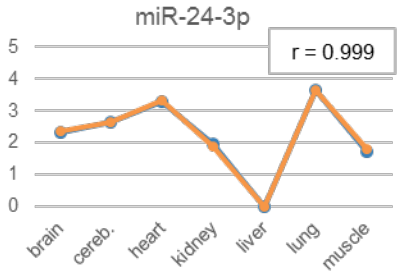


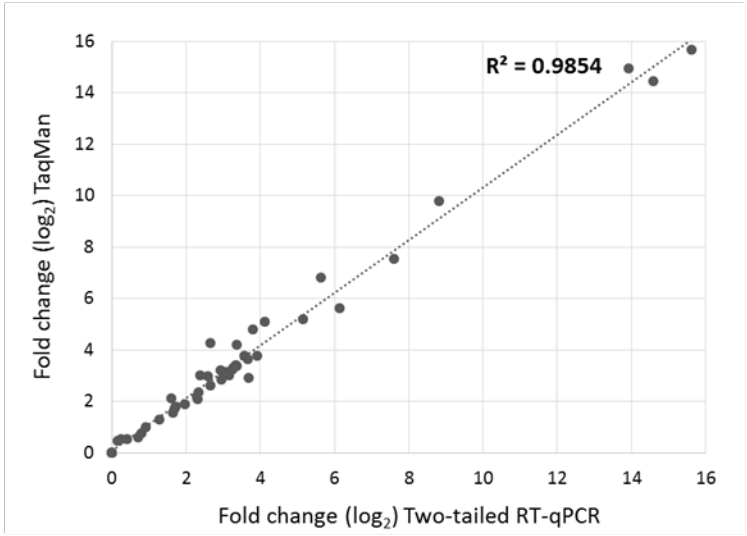


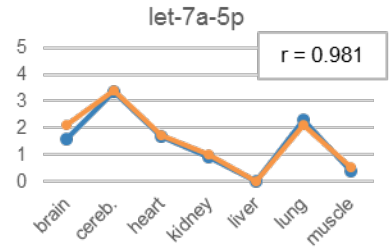


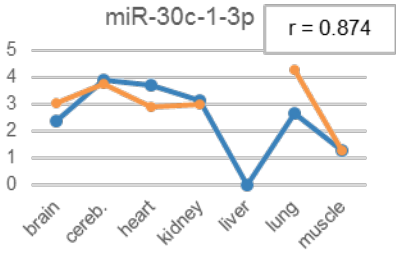












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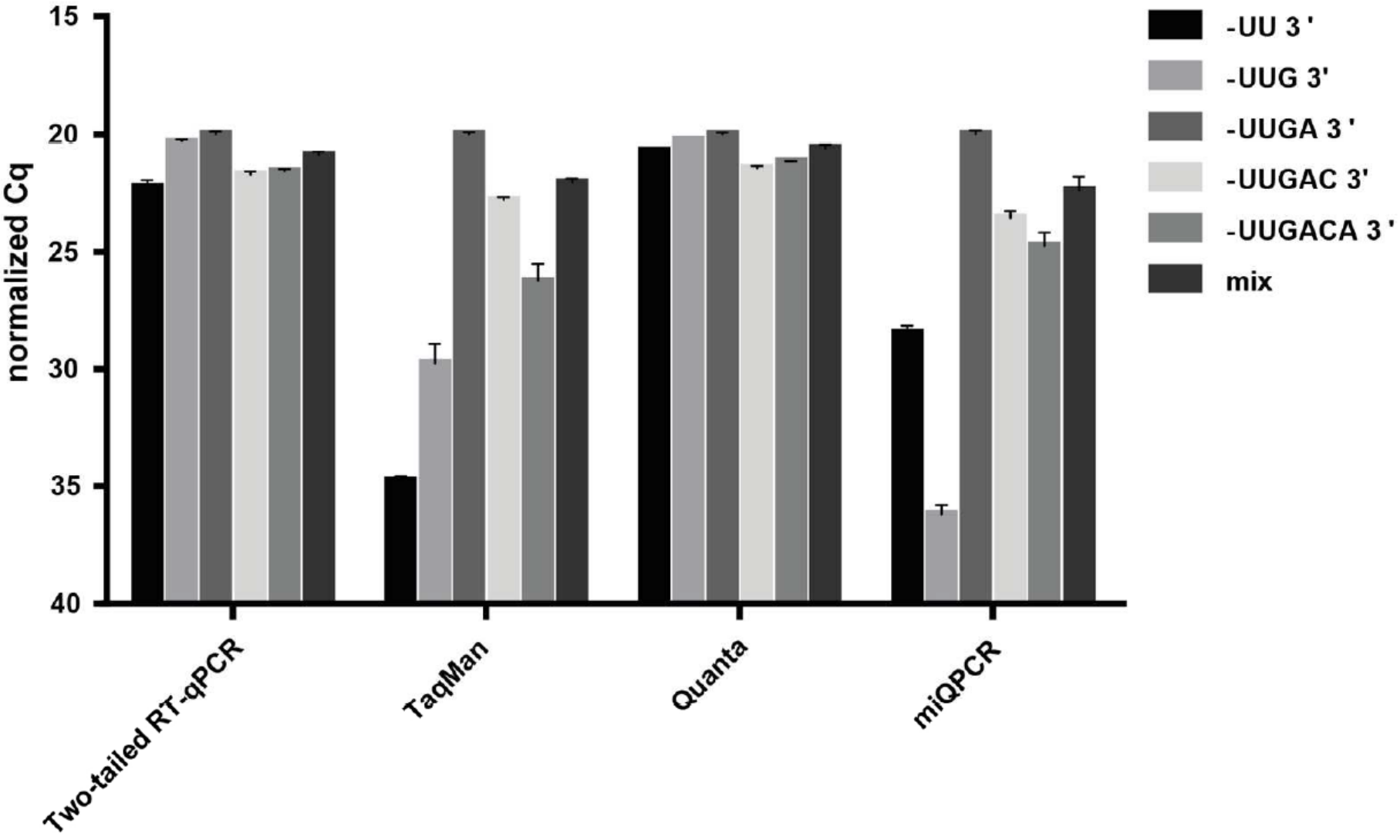
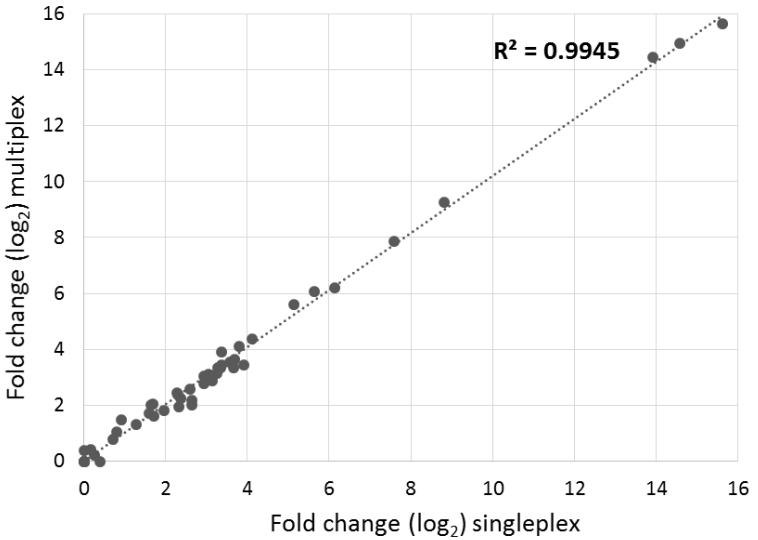
Discrimination of isomiRs



2-tube Multiplexing

8 different RT primers were pooled for multiplex reverse transcribed and subsequent singleplex qPCR

	Δ Cq (relative to singleplex protocol)						
Sample	*miR-122*	*miR-193a*	*miR-1a*	*miR-21a*	*miR-24*	*miR-30c*	*Let-7a*
brain	-0.12	0.93	1.26	2.41	0.11	-0.08	0.72
cereb.	0.09	0.99	1.67	2.17	0.20	0.28	0.85
heart	-0.21	0.67	1.38	2.06	-0.34	-0.13	0.50
kidney	0.32	0.95	1.90	2.26	-0.14	0.07	0.25
liver	-0.20	0.85	1.73	2.50	-0.28	-0.20	0.44
lung	0.02	0.96	1.47	2.36	0.04	0.44	0.76
muscle	-0.11	0.87	1.70	2.33	-0.17	-0.23	1.24
average	-0.03	0.89	1.59	2.30	-0.08	0.02	0.68
st.dev.	**0.19**	**0.11**	**0.22**	**0.15**	**0.20**	**0.25**	**0.32**



1-tube RT-qPCR multiplexing is also possible using probes

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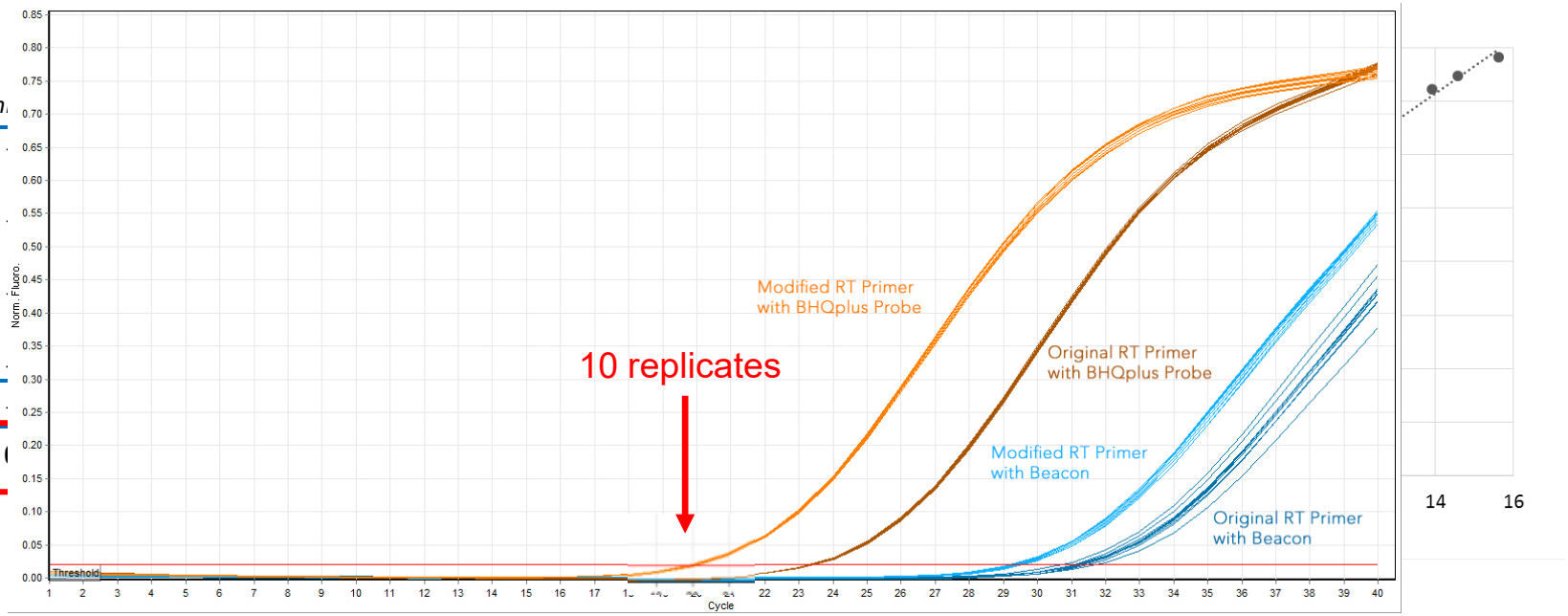
2-tube Multiplexing

8 different RT primers were pooled for multiplex reverse transcribed and subsequent singleplex qPCR

Sample	m
brain	
cereb.	
heart	
kidney	
liver	
lung	
muscle	
average	
st.dev.	



1-tube RT-qPCR multiplexing is also possible using probes

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Summary: Two-Tailed RT-PCR for microRNA

- New RT-qPCR method
- High sensitivity
- Wide dynamic range
- Very high specificity
- Unlimited multiplexing in RT with downstream singleplex qPCR
- RT-qPCR multiplexing with probes

Nucleic Acids Research, 2017 **1**
doi: 10.1093/nar/gkx588

Two-tailed RT-qPCR: a novel method for highly accurate miRNA quantification

Peter Androvic[1,2], Lukas Valihrach[1], Julie Elling[3], Robert Sjoback[3] and Mikael Kubista[1,3,*]

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SEVENTH FRAMEWORK PROGRAMME

ABOUT SPIDIA AND SPIDIA4P

SPIDIA was a 4.5-year project funded by the European Union FP7 programme. It brought together 16 leading academic institutions, international organisations and life sciences companies, coordinated by QIAGEN GmbH. The project tackled the standardisation and improvement of pre-analytical procedures for in-vitro diagnostics. Various new pre-analytical technologies were developed. Within the CEN/Technical Committee 140 for "In vitro medical devices", SPIDIA's results enabled to develop and introduce the first 9 CEN Technical Specifications (CEN/TS) for pre-analytical workflows in Europe.

The SPIDIA4P project builds on SPIDIA's results and is funded by the European Union's Horizon 2020 research and innovation programme. The consortium of 19 highly experienced partners from private industry including SMEs, public institutions and one European Standards Organisation is again coordinated by QIAGEN GmbH. It plans to initiate, develop and implement a comprehensive portfolio of an additional 14 pan-European pre-analytical CEN/TS and ISO/IS documents as well as external quality assessment schemes (EQAs), addressing the important pre-analytical workflows applied to personalised medicine.

+++ LATEST NEWS +++ LATEST NEWS +++ LATEST NEWS +++ LATEST NEWS +++ LATEST NEWS +++

Quality control tool box for microRNA

5’-Phos for sequencing

40 < GC/% < 64

Usage	Name	Sequence	GC %	Origin
Isolation spike-ins	cel-miR-54-3p	/5Phos/UACCCGUAAUCUUCAUAAUCCGAG	41.7	*C. elegans*
	miR-spike-A	/5Phos/UGCAGCCCUACCGACACGUUCC	63.6	artificial
	miR-spike-B	/5Phos/ACUCAGGUUGUAGGAGCGGUCUU	52.2	artificial
RT spike-ins	cel-miR-76-3p	/5Phos/UUCGUUGUUGAUGAAGCCUUGA	40.9	*C. elegans*
	cel-miR-2-3p	/5Phos/UAUCACAGCCAGCUUUGAUGUGC	47.8	*C. elegans*

Endogenous controls
mir-451a
mir-23a

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Test system for optimization

- Human plasma (K_2EDTA BD Vacutainer tubes; 1500g/3000g)
- Human serum (8.5 ml, vacutainer SST II Advanced tubes)
- Rat serum (1ml Eppendorf tube; 1000g/3000g)

- Extraction: miRNeasy Serum/Plasma Advance kit (Qiagen)

- RT: GrandScript FreePrime (TATAA)
- qPCR: GrandMaster SYBR (TATAA)

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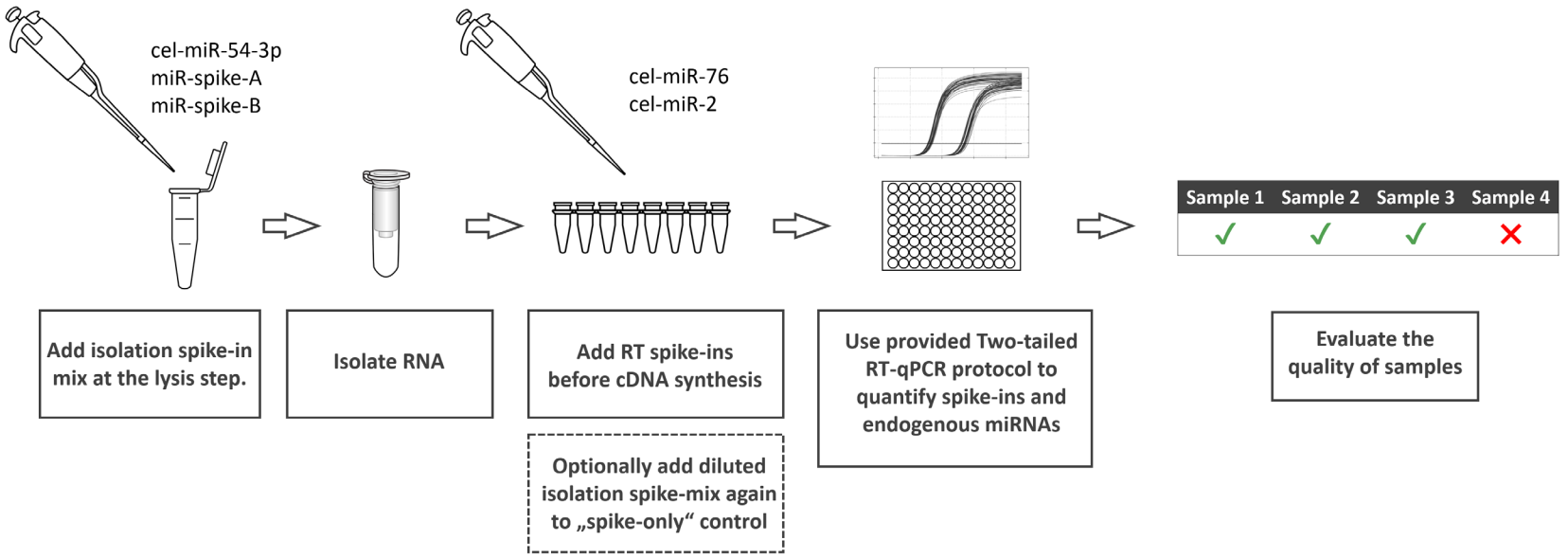
Workflow



Isolation spike-in mix

RNA oligo	Final concentration (copies/µl)	
cel-miR-54	1.00E+07	200x
spike_A	2.00E+05	200x
spike_B	4.00E+03	

RT spike-in mix

RNA oligo	Final concentration (copies/µl)	
cel-miR-76	1.00E+07	40000x
cel-miR-2	4.00E+03	

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Factors tested/optimized

- Initial **input volume** used for RNA isolation. Risk for carry over of contaminants. Saturation of column. Most vendors recommend: 200 µl. However, optimum volume seem to depend on:
  - isolation protocol
  - sample type
  - organism.

- **Hemolysis** was prepared by addition of lysed erythrocytes (by freeze-thawing) in a serial dilution. Ratio mir-451a:mir-23a is tested as indicator for hemolysis
  - Mir-451a is highly abundant in erythrocytes
  - Mir-23a is abundant in serum/plasma, but not in erythrocytes

- Effect of **glycogen** as carrier

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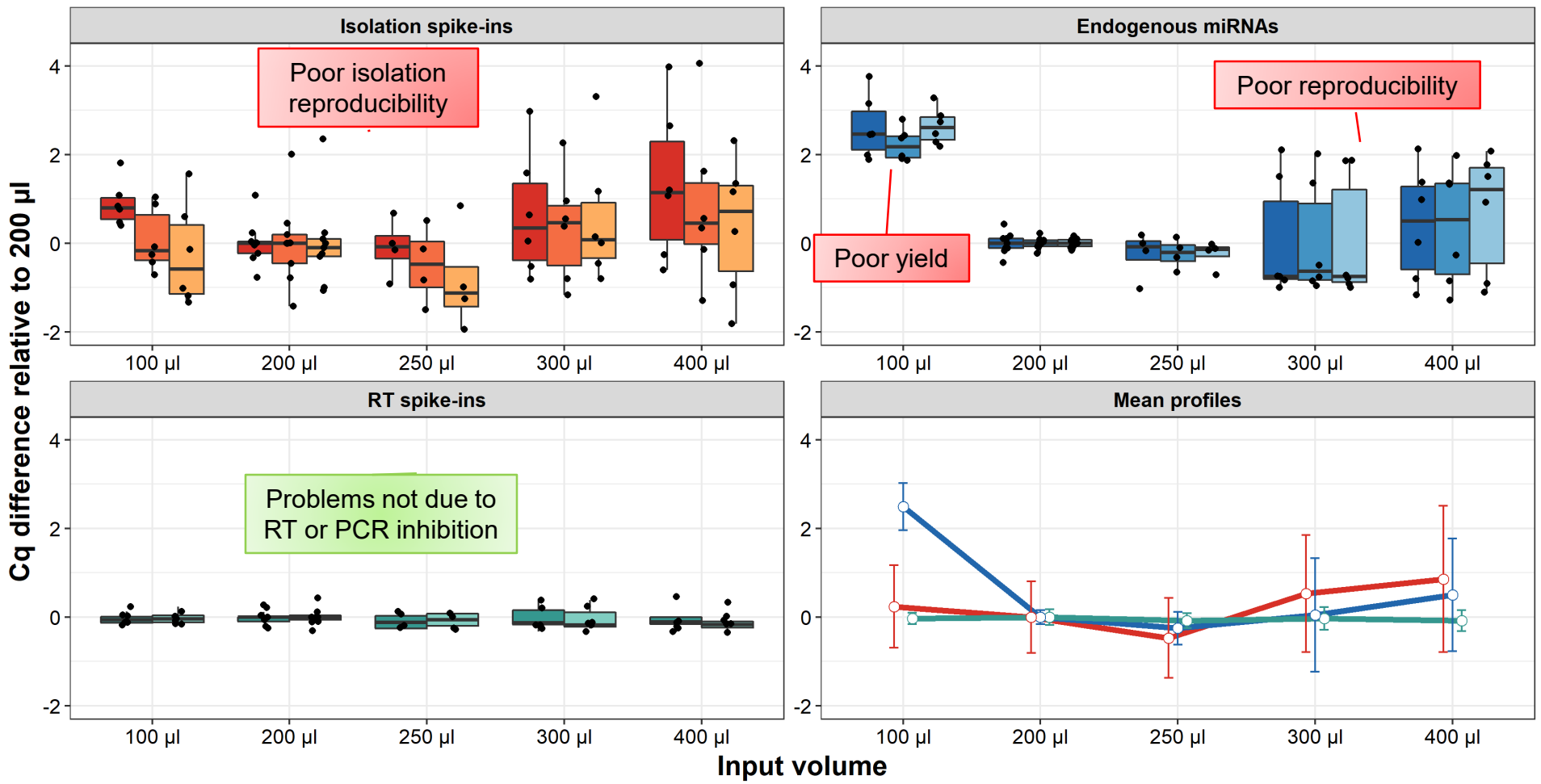
Human plasma

miRNeasy Serum/Plasma Advanced kit (Qiagen)



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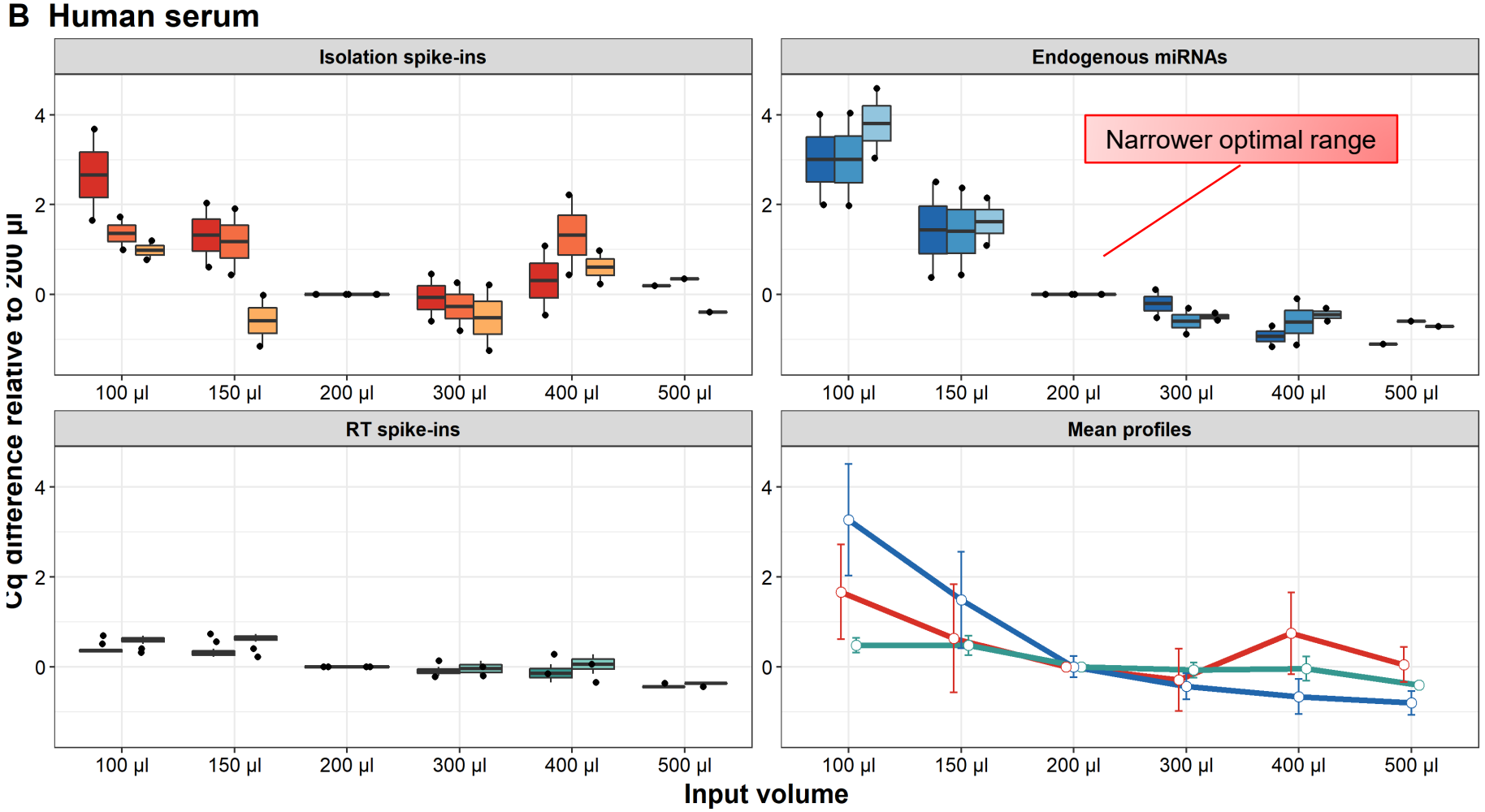
Human serum

miRNeasy Serum/Plasma Advanced kit (Qiagen)



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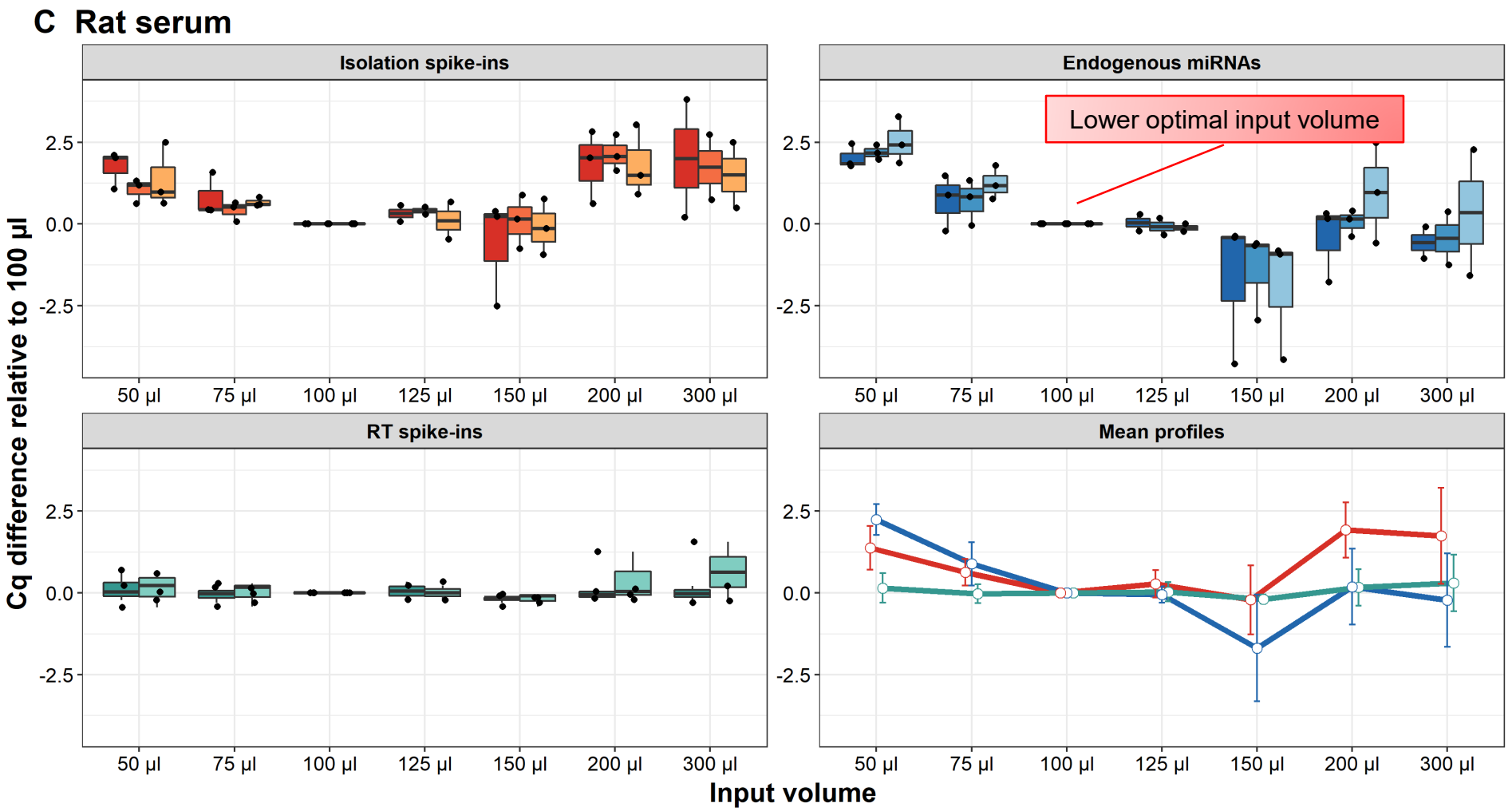
Rat serum

miRNeasy Serum/Plasma Advanced kit (Qiagen)



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Conclusions

Extracting with the miRNeasy Serum/Plasma Advanced kit (Qiagen) we find:

- Relation between input sample volume and amount of cDNA is **non-linear** due to extraction issues.

- Poor yields are observed with low as well as high input volumes. Working volumes are:
  - Human plasma: 250 µl
  - Human serum: 300 – 500 µl
  - Rat serum: 150 µl

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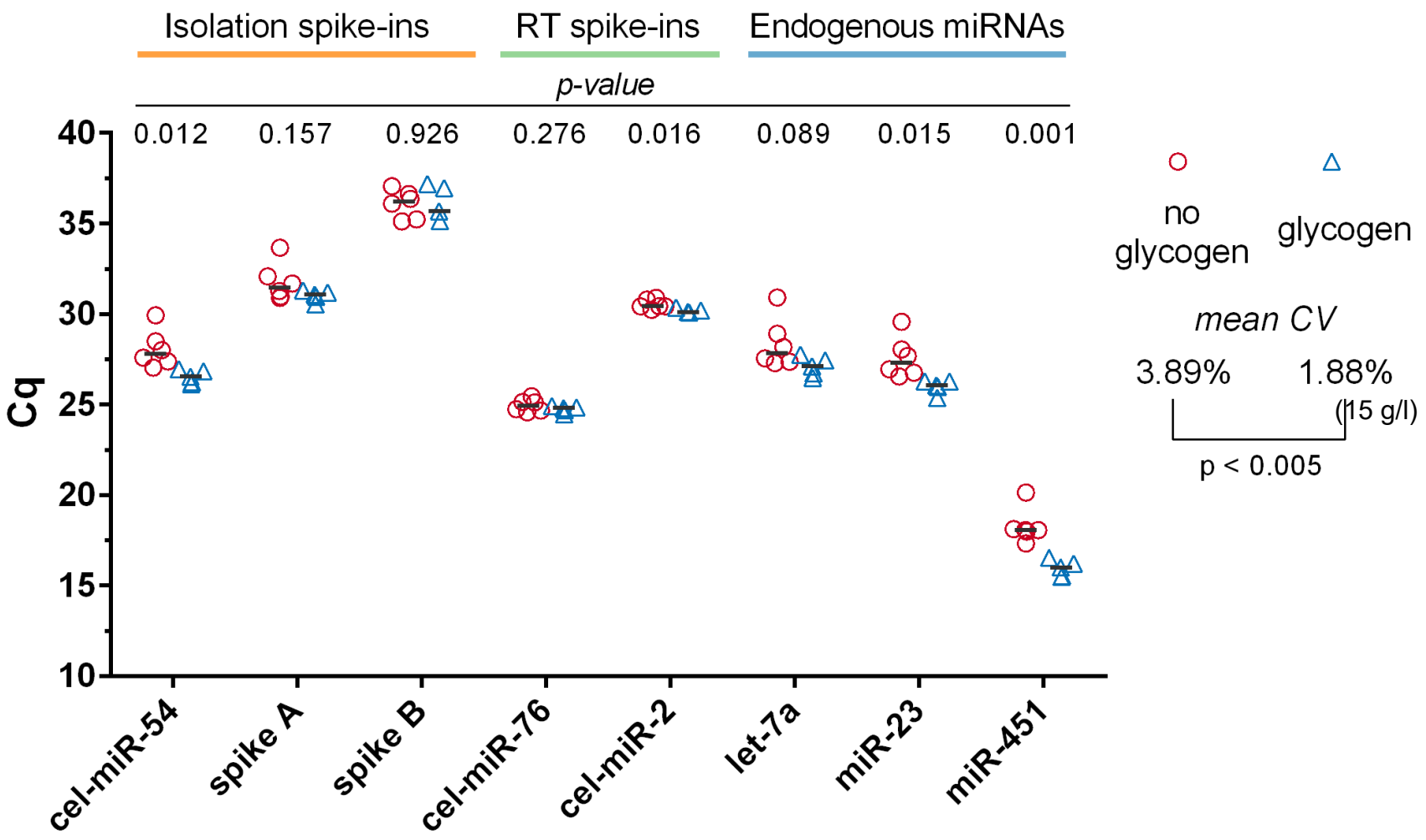
Effect of glycogen (human plasma)



Higher reproducibility: F-test, p<0.001)
Higher yield: ΔCq=1.25, paired t-test, p<0.011)

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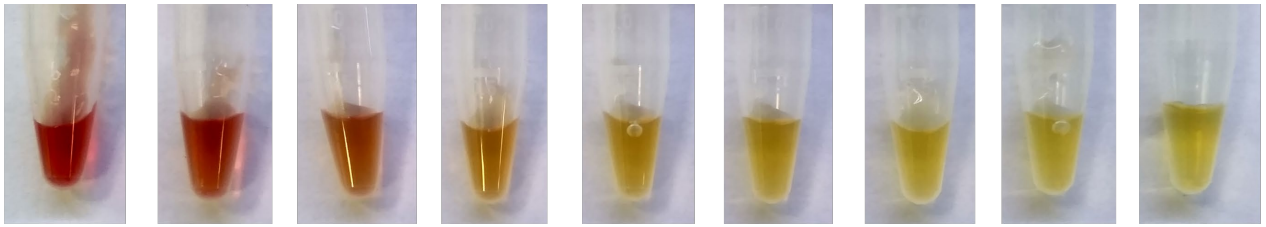
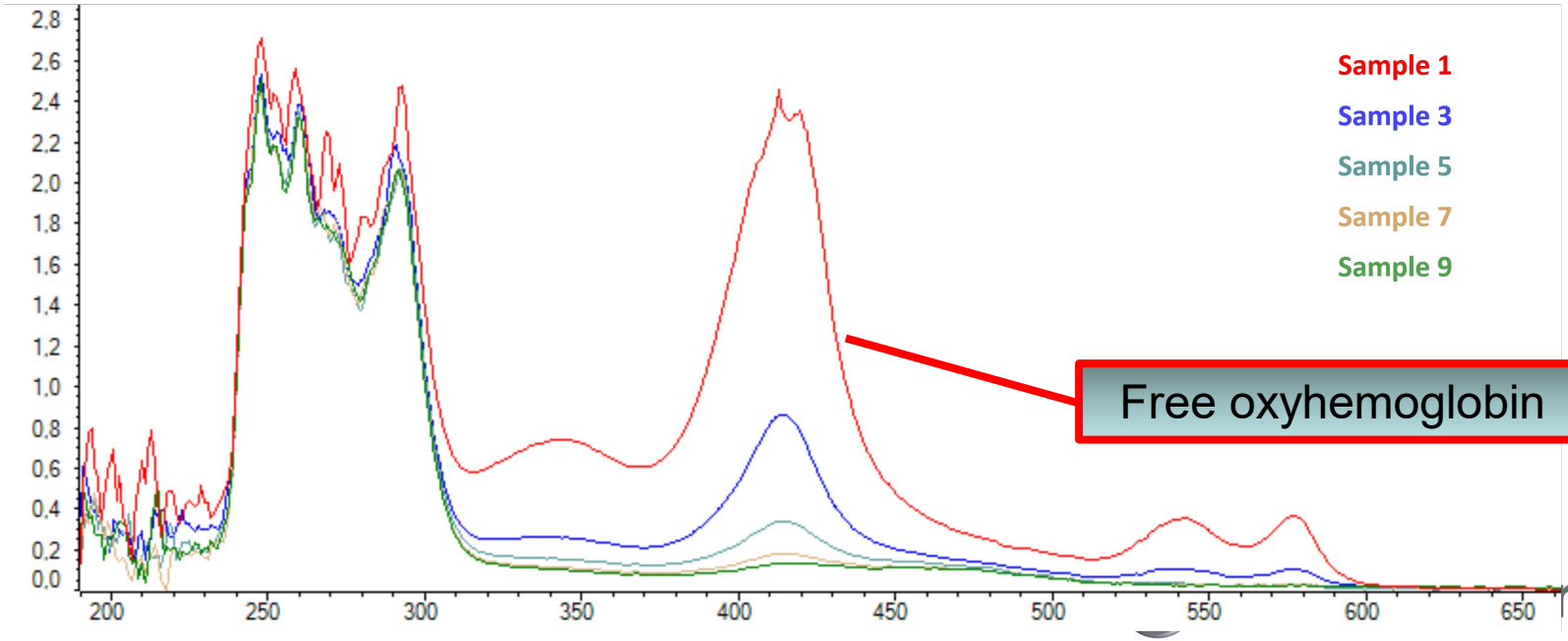
Hemolysis

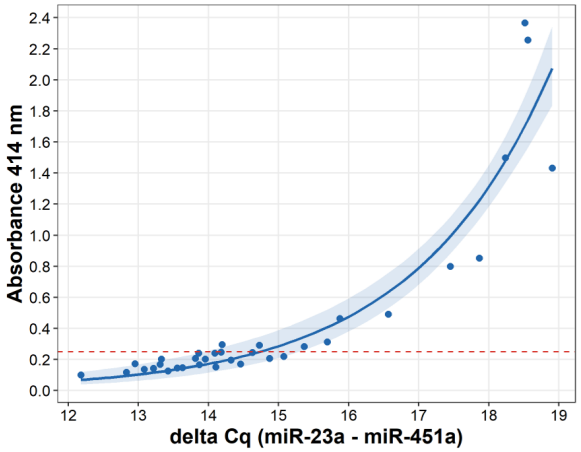


Sample	1	2	3	4	5	6	7	8	9
Erythrocyte (v/v)	1%	0.5%	0.25%	0.125%	0.063%	0.031%	0.016%	0.008%	0%
Absorb. 414nm	2.367	1.498	0.852	0.491	0.313	0.220	0.172	0.146	0.118
Absorb. 540nm	0.339	0.174	0.098	0.055	0.033	0.025	0.021	0.015	0.018
Absorb. 578 nm	0.354	0.178	0.100	0.053	0.029	0.020	0.019	0.011	0.013
ΔCq (miR-23a – miR-451a)	18.51	18.24	17.86	16.57	15.70	15.07	14.46	13.55	12.83

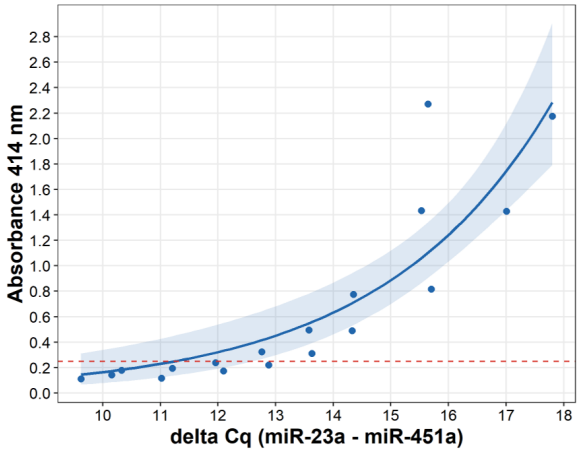


mir-451a:mir-23a as indicator for hemolysis

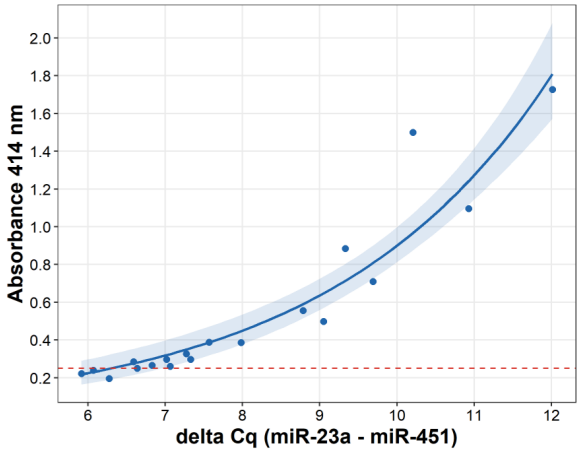
1) Human plasma

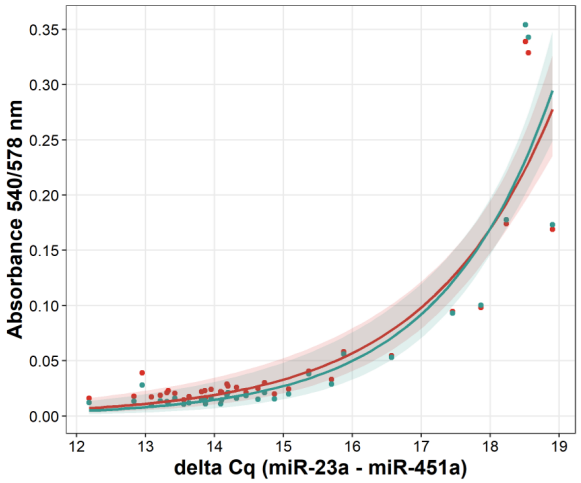


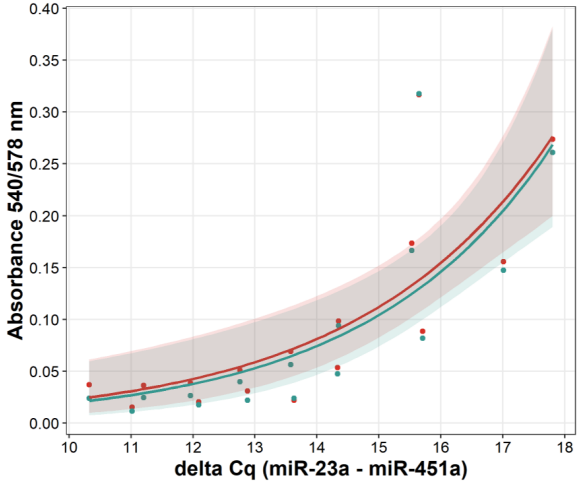
2) Human serum

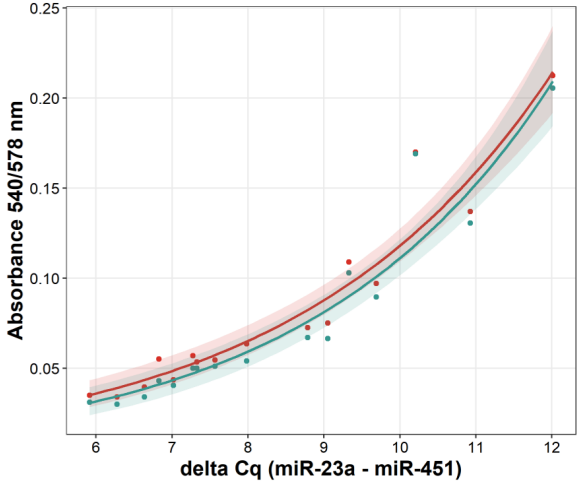


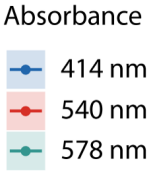
3) Rat serum











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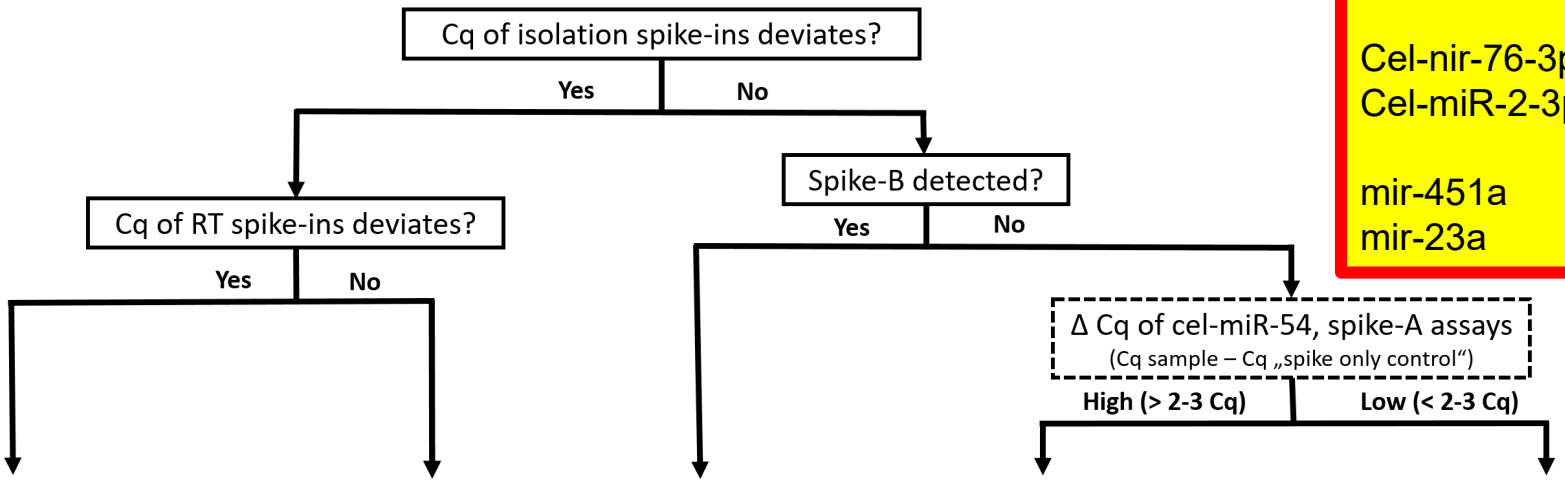
Decision workflow

Cel-miR-54-3p
miR-spike-A
miR-spike-B

Cel-nir-76-3p
Cel-miR-2-3p

mir-451a
mir-23a



Interpretation				
Inhibition of reverse transcription and/or PCR amplification	Suboptimal isolation efficiency	No technical errors	Isolation efficiency comparable between samples, but overall poor and low-abundant miRNAs may have been lost	Low amount of isolation spike-ins added before isolation / stock is degraded
Suggested action				
Re-isolate the samples/purify or dilute the template RNA and check again for inhibition. Alternatively exclude affected samples from the study	Re-isolate samples or exclude them from the study	Proceed with the experiment	Increase the RNA input and volumes of RT and qPCR reactions. Consider using more efficient isolation protocol. Check for RNase contamination	RNA eluates are of good quality. Next time increase the amount of isolation spike-in added per isolation / prepare fresh stock solution

P. Androvic, L. Valihrach, N. Romanyuk, M. Kubista, Nature Scientific Reports (accepted)

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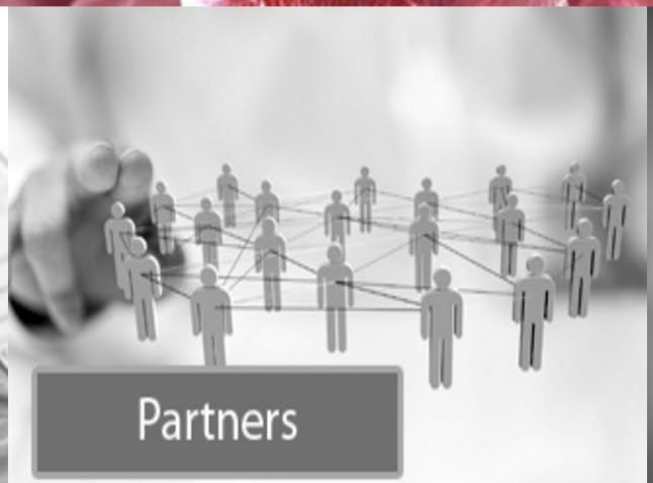
CANCER ID

Cancer treatment and monitoring through identification of circulating tumor cells and tumor related nucleic acids in blood



TATAA Alu QC assays

The Alu element is the most abundant sequence being present in over 1 million copies comprising ~11% of the genome

TATAA has developed three Alu assays

- TATAA Alu-60 (60 bp)
- TATAA Alu-135 (135 bp)
- TATAA Alu-187 (187 bp)

Application 1: Super sensitive assay to measure total amount of genomic DNA in a sample.

Application 2: Test for gDNA contamination in master mixes, primer mixes and other reagents.

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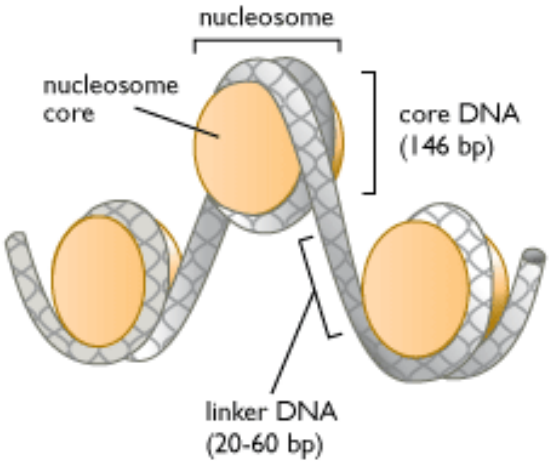
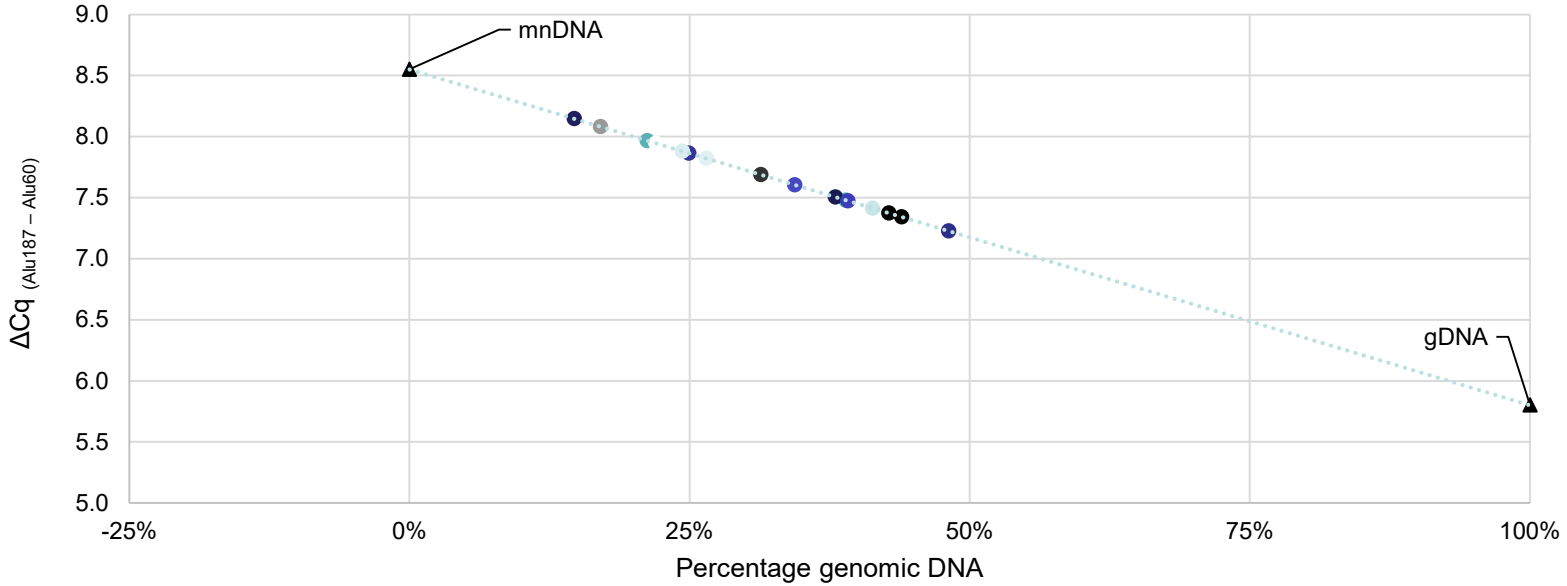
https://webshop.tataa.com/product.html/tataa-alu-assay?category_id=66

Applications using TATAA Alu assays



Application 3: Test for gDNA contamination due to e.g., cell lysis in serum/plasma samples



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https://webshop.tataa.com/product.html/human-genomic-dna-standard-calibrated?category_id=43