tataabiocenter

COURSES SERVICES WEBSHOP CORE FACILITY RESEARCH INFO

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 and other methods 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.

– READ MORE –

ghp Global Health & Pharma

Search the site

Research & Development | Translation & Commercialisation | Funding & Investment | Education & Skills | Innovation & Technology

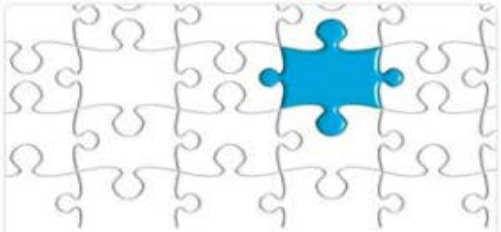
About Us | Circulation | Awards | Issues | Contact Us | Subscribe

TATAA Biocenter AB

March 8, 2019

TATAA Biocenter AB

Best Nucleic Acid Analysis Service Provider - Europe



COURSES

SERVICES

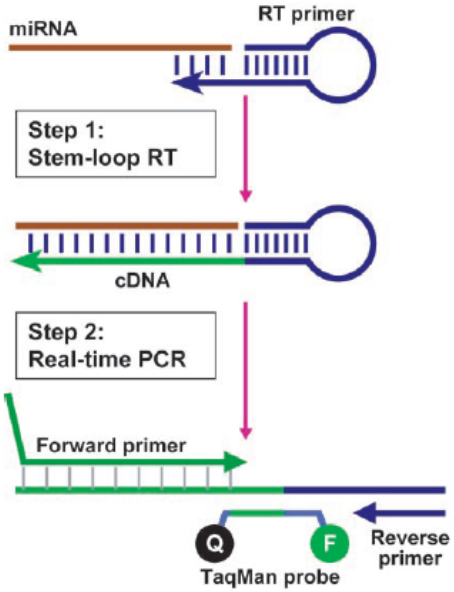
CORE FACILITY

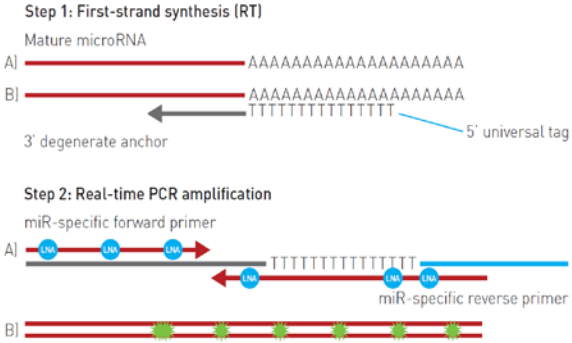
ibt

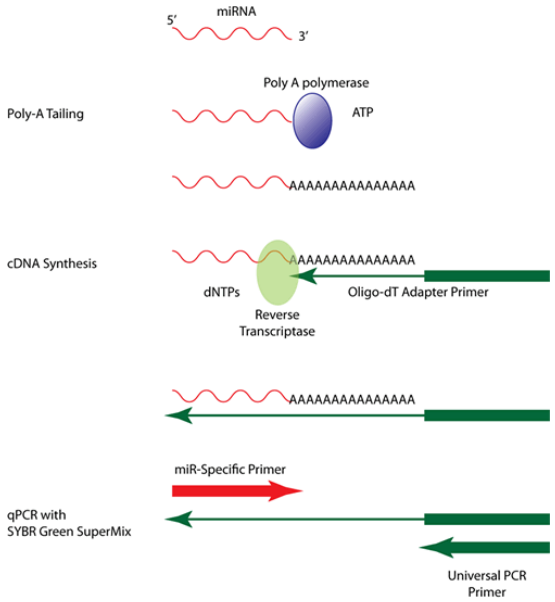
Challenges analyzing miRNAs (and other short NA)

- 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

Current methods make the microRNA longer







ThermoFisher SCIENTIFIC

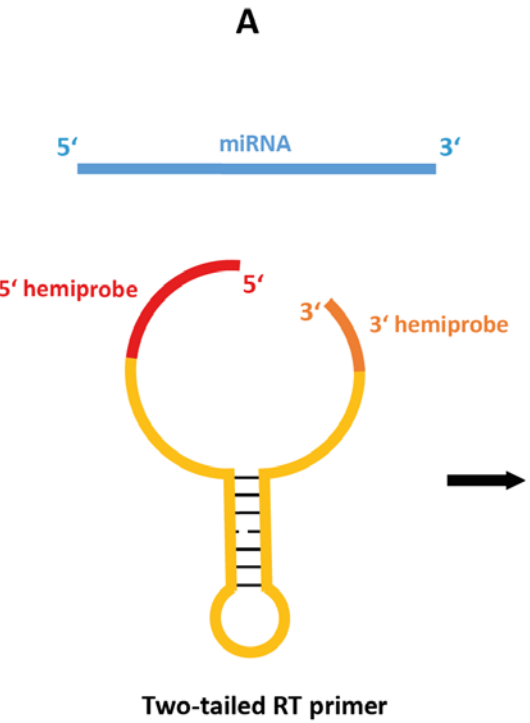
EXIQON
Now a QIAGEN company
QIAGEN

Quantabio
QIAGEN
TaKaRa
SIGMA-ALDRICH

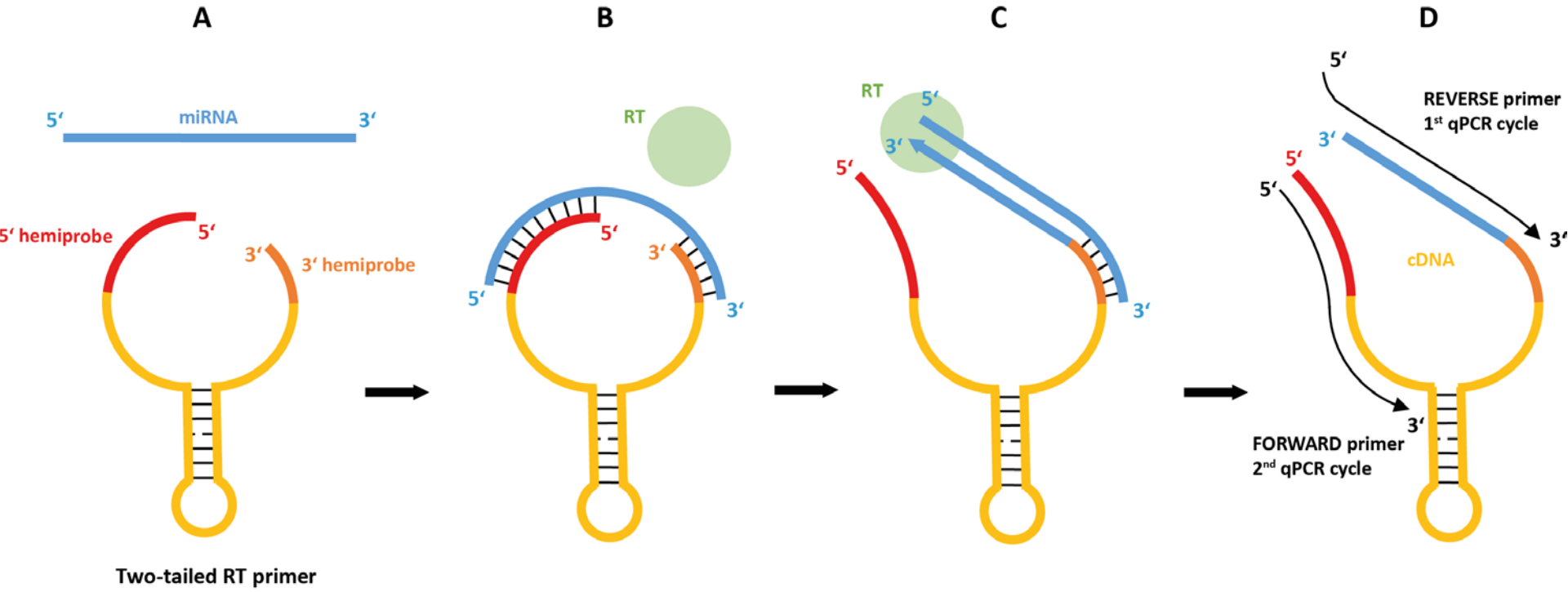
- Extension reduces sensitivity
- One probe only limits specificity

ibt

Two-tailed RT-qPCR



Two-tailed RT-qPCR

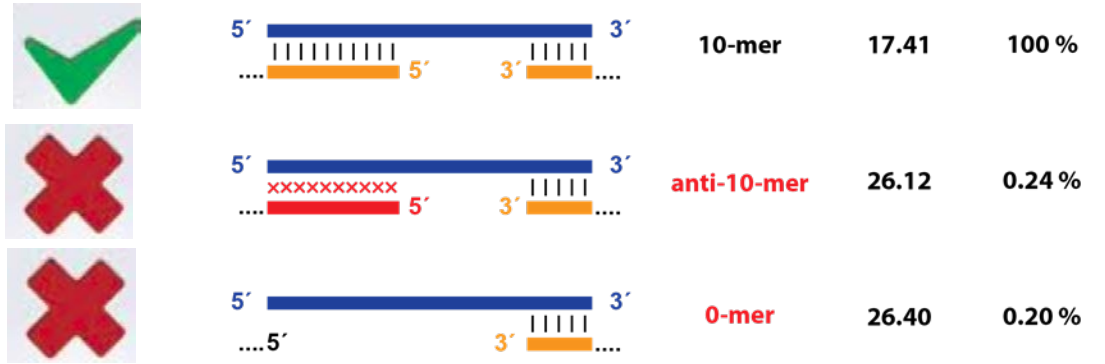


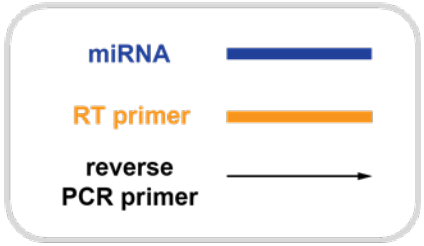
ibt

tataabiocenter

Design concept

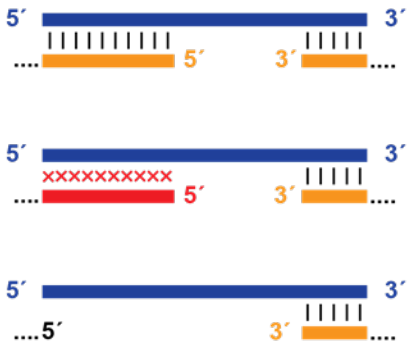
- 5‘ complementary segment contributes to the **sensitivity** of the assays



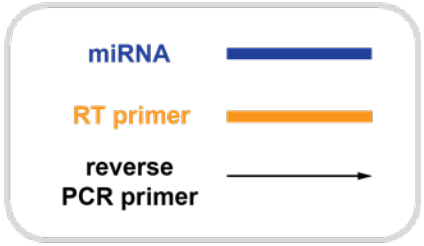


Design concept

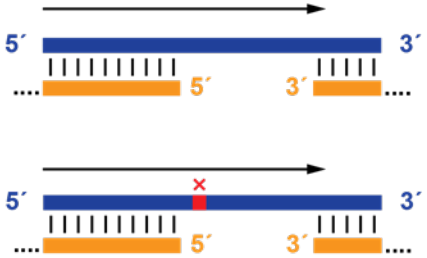
- 5‘ complementary segment contributes to the **sensitivity** of the assays



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



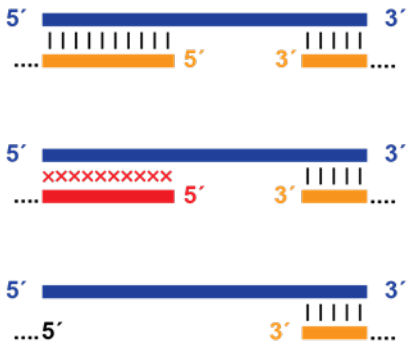
Cq	Relative detection
17.41	100 %
22.07	3.96 %

ibt

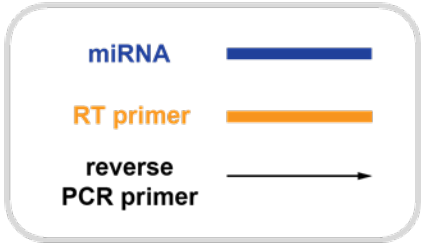
tataabiocenter

Design concept

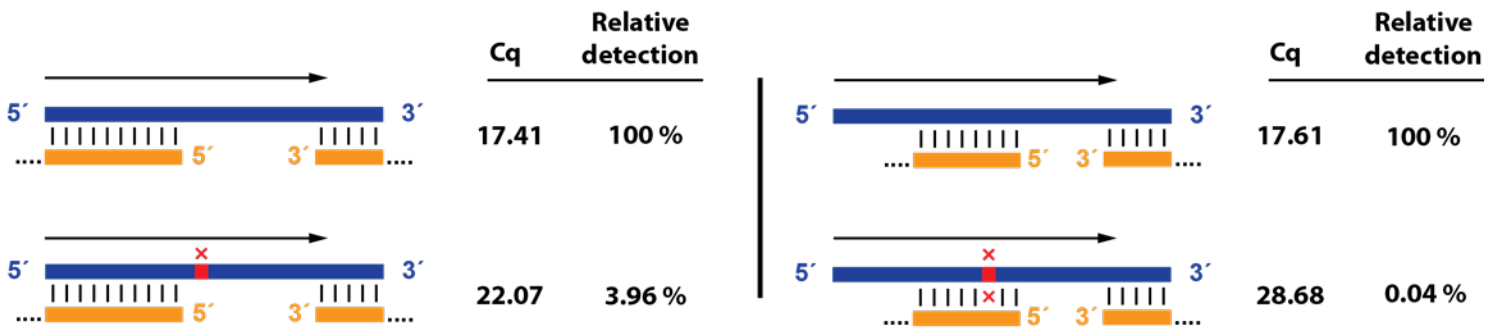
- 5‘ complementary segment contributes to the **sensitivity** of the assays



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



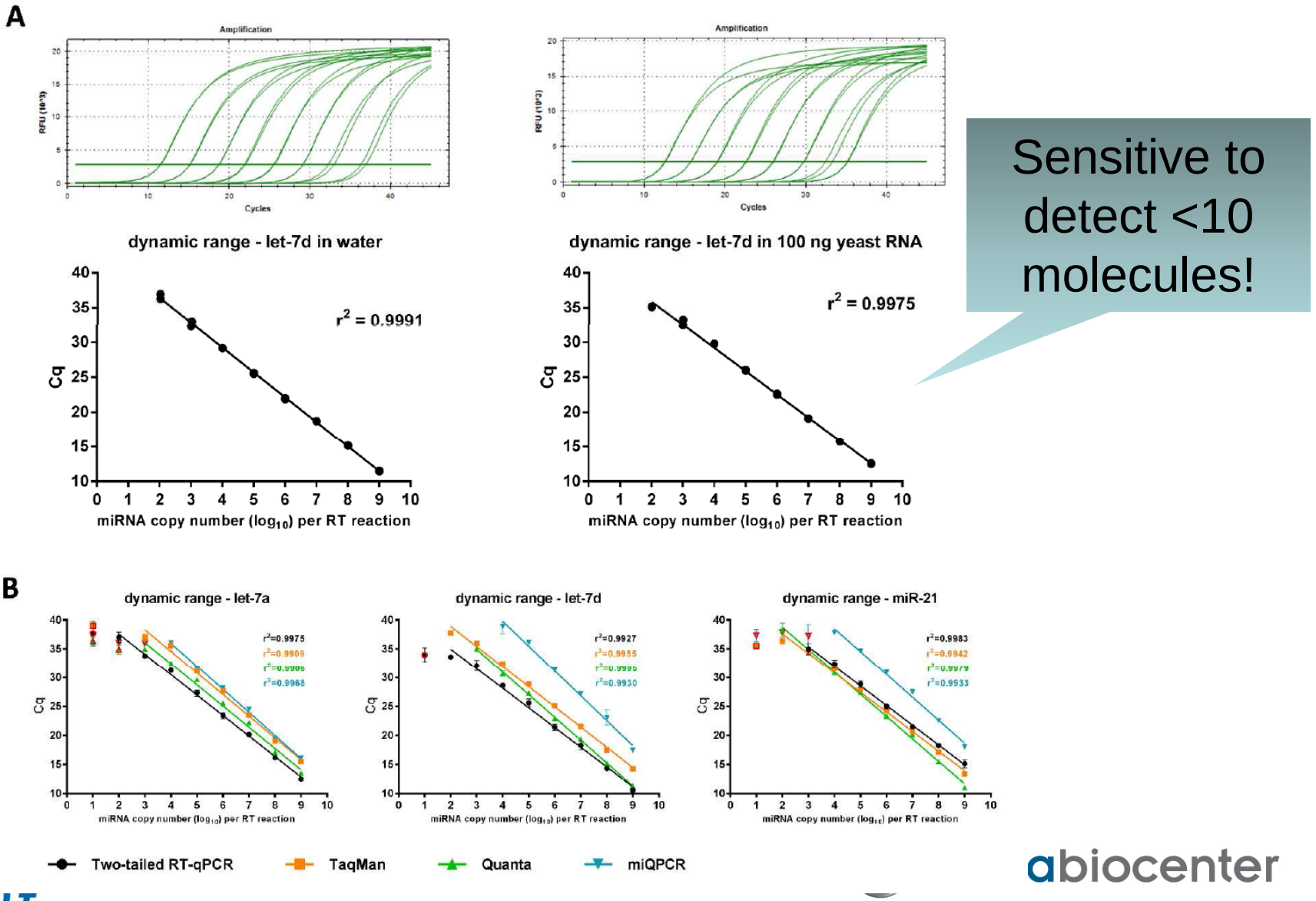
Cq	Relative detection
17.41	100 %
22.07	3.96 %

Cq	Relative detection
17.61	100 %
28.68	0.04 %

ibt

tataabiocenter

Sensitivity and dynamic range



Sensitive to detect <10 molecules!

abiocenter

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

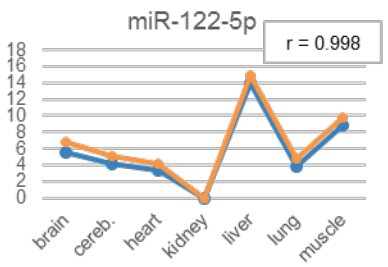
name	sequence
let-7a	UGAGGUAGUAGGUUGUAUAGUU
let-7b	UGAGGUAGUAGGUUGUGUGGUU
let-7c	UGAGGUAGUAGGUUGUAUGGUU
let-7d	AGAGGUAGUAGGUUGCAUAGUU
let-7e	UGAGGUAGGAGGUUGUAUAGUU
let-7f	UGAGGUAGUAGAUUGUAUAGUU
let-7g	UGAGGUAGUAGUUUGUACAGUU
let-7i	UGAGGUAGUAGUUUGUGCUGUU

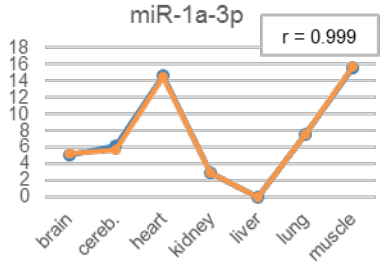
C

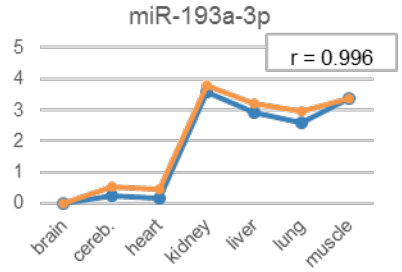
	Mature	Precursor	Relative detection
let-7a	17.74	21.31	6.98%
let-7b	16.98	21.22	5.31%
let-7f	16.85	23.78	0.82%

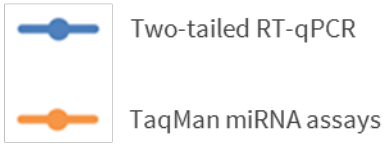
Benchmarking in biological samples

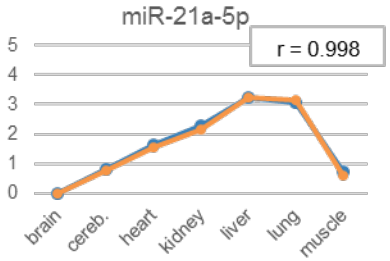
- Expression of 8 targets in 7 mouse tissues measured and compared with TaqMan miRNA assays
- Excellent correlation of relative expression profiles between the two methods

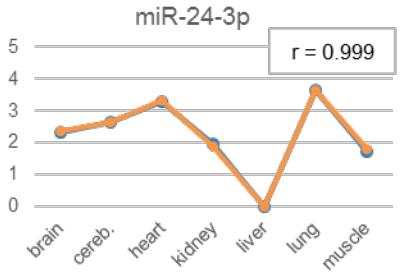


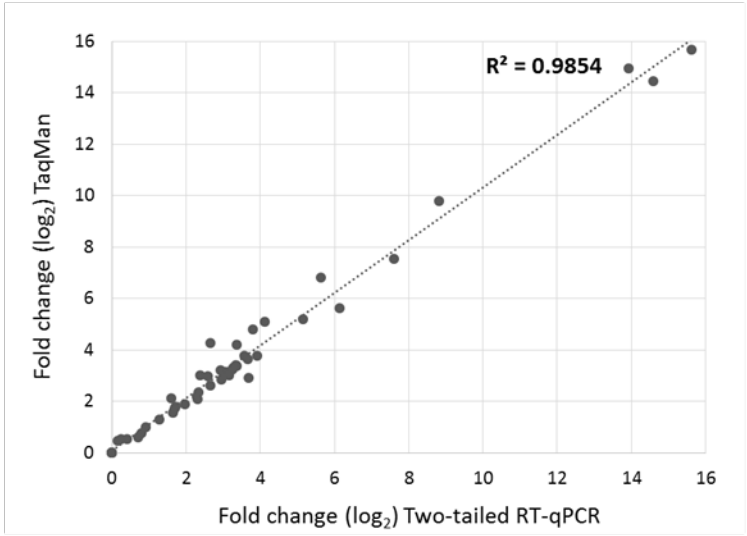


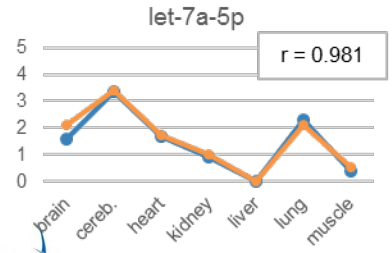


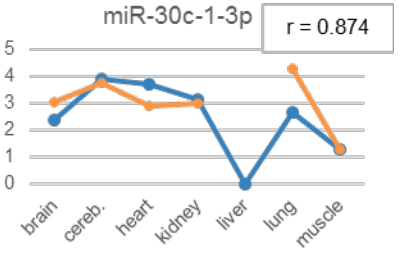








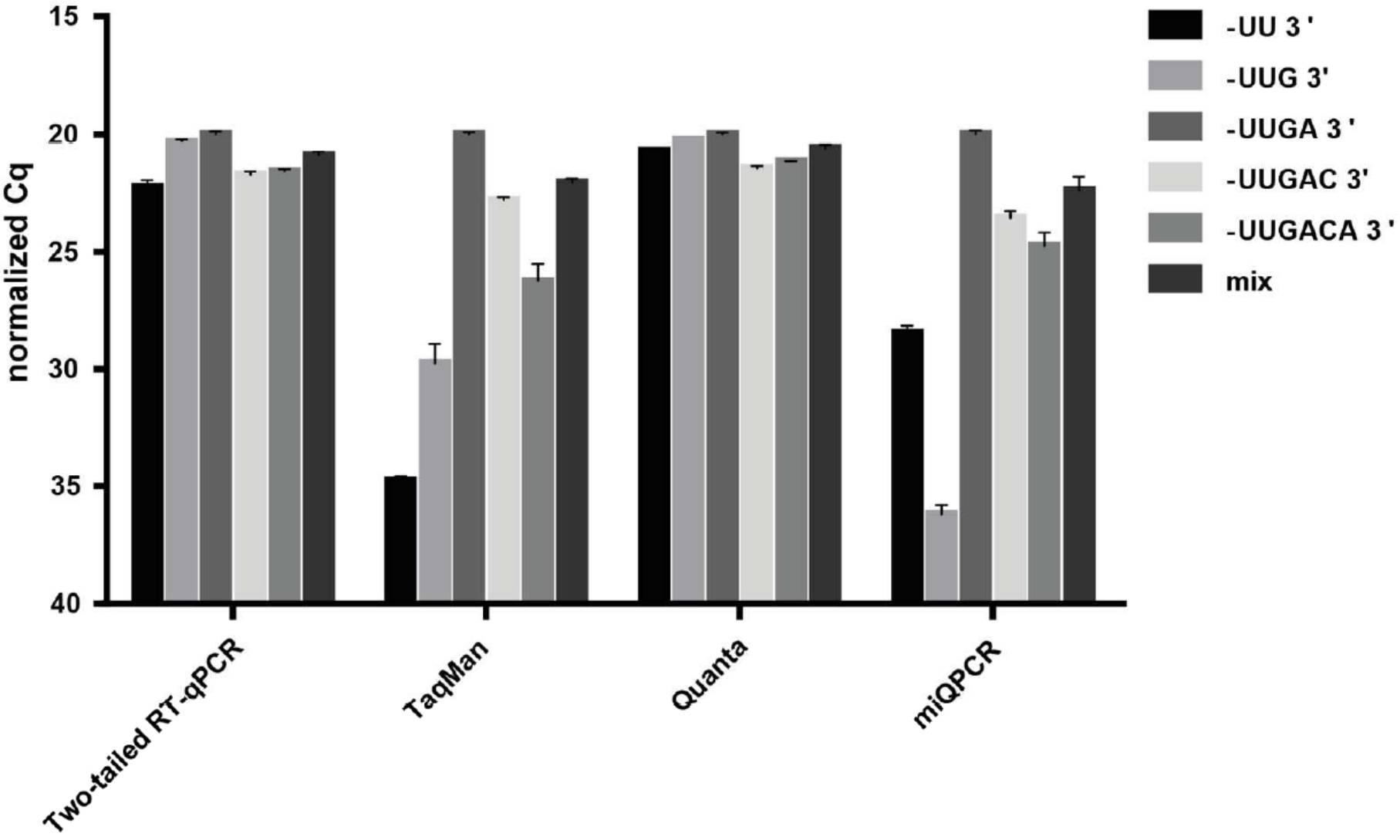




ibt

tataabiocenter

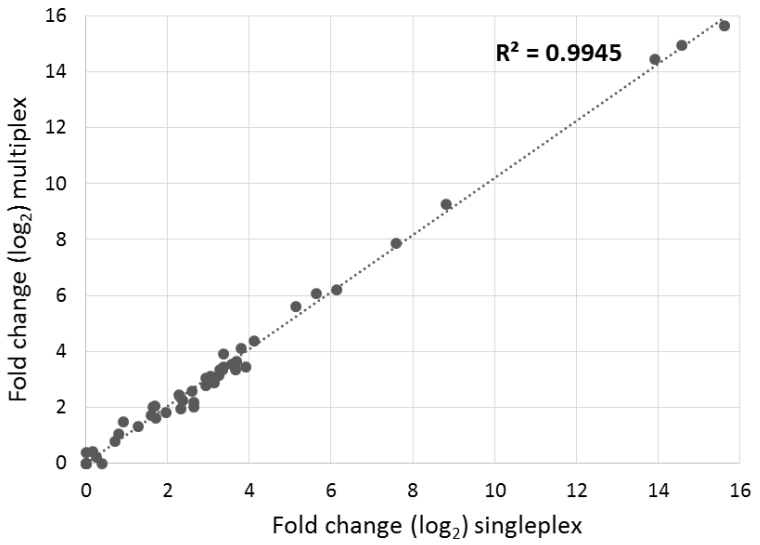
Discrimination of isomiRs



2-tube Multiplexing

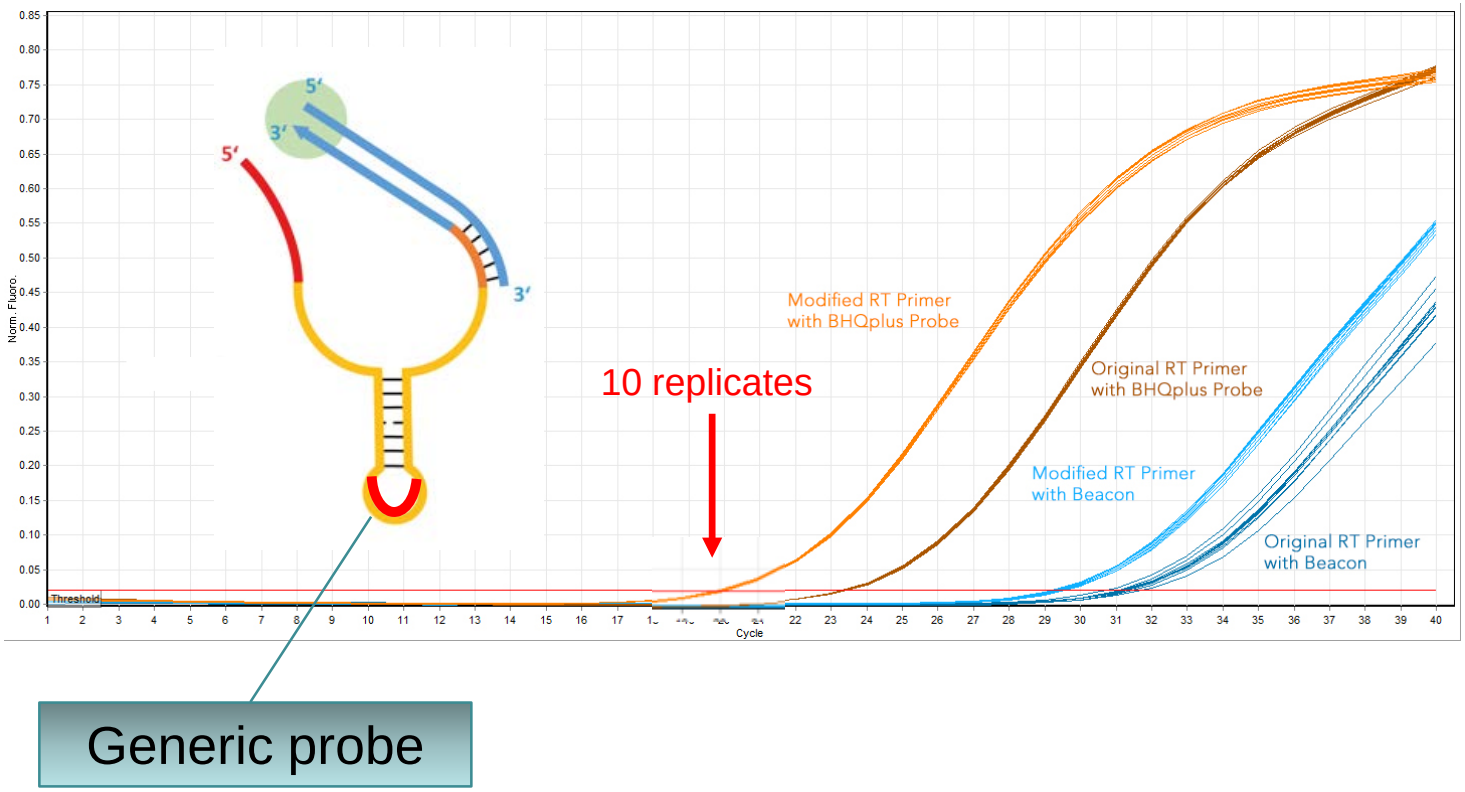
8 different RT primers were pooled for multiplex reverse transcribed and subsequent singleplex qPCR

	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**



tataabiocenter

1-tube Multiplexing



1-tube Multiplexing

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,*]

[1]Laboratory of Gene Expression, Institute of Biotechnology CAS, Biocev, Vestec 252 50, Czech Republic,
[2]Laboratory of Growth Regulators, Faculty of Science, Palacky University, Olomouc 783 71, Czech Republic and
[3]TATAA Biocenter AB, Gothenburg 411 03, Sweden

Received December 06, 2016; Revised June 07, 2017; Editorial Decision June 24, 2017; Accepted June 28, 2017

Generic probe

ibt

tataabiocenter

Home

NEWSLETTER

Subscribe to our newsletter to receive latest news about the project

CONTACT US

Visit our contact form to contact SPIDIA and to submit comments and questions about this website.

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

ibt

tataabiocenter

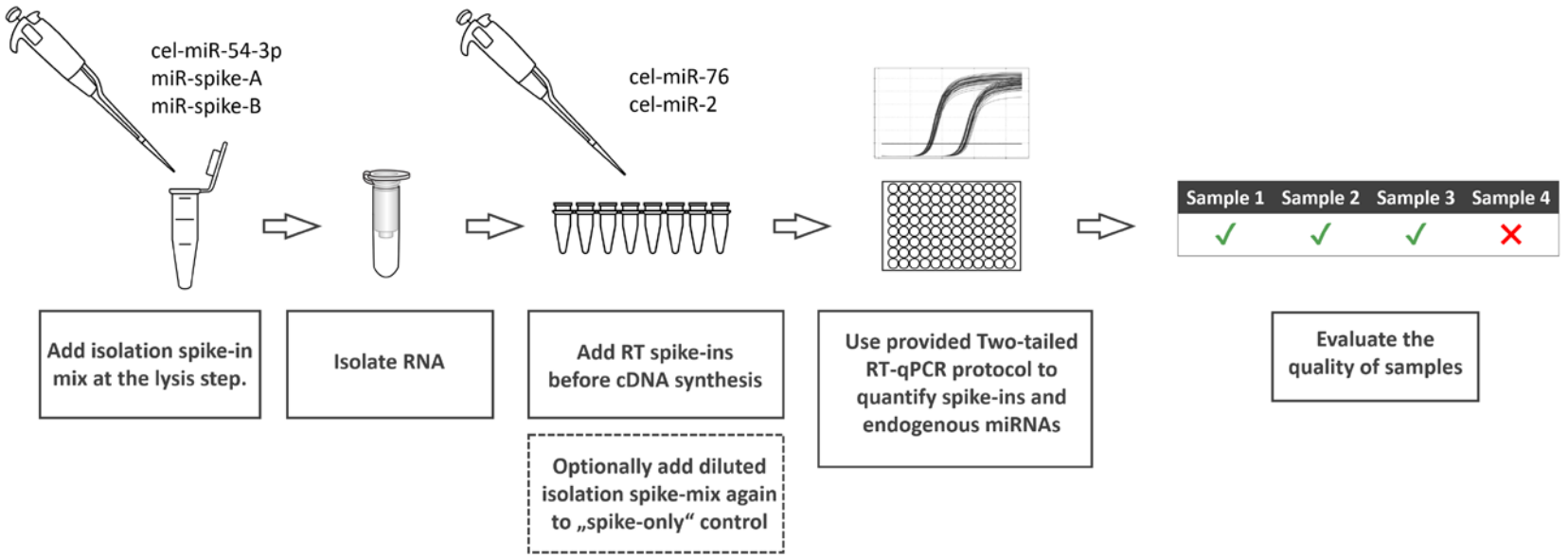
Test system for optimization

- 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)

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	

ibt

tataabiocenter

Factors tested/optimized

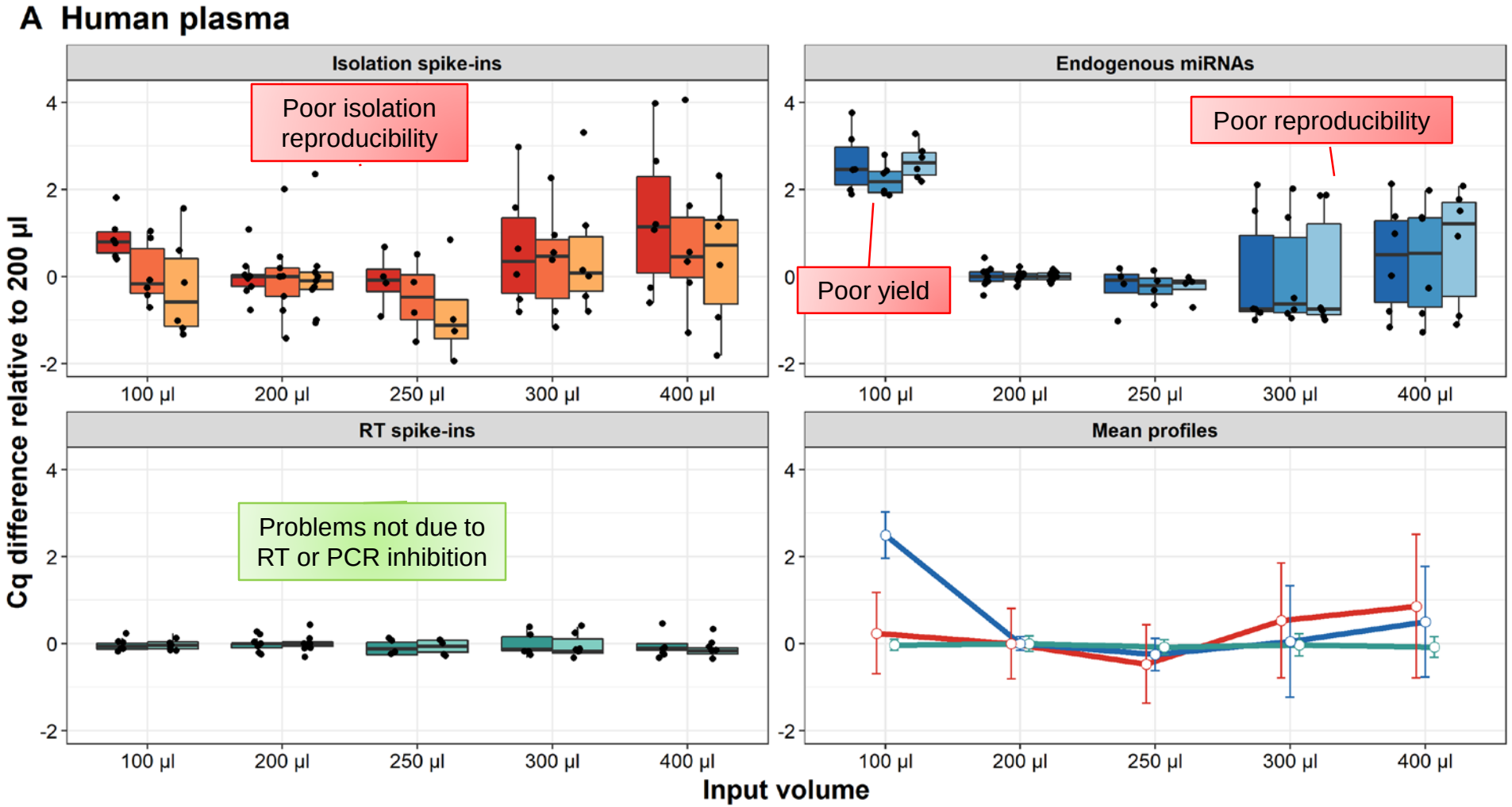
- Initial **input volume** used for RNA isolation. Risk for carry over of contaminants. Saturation of column. Most vendors recommend: 200 µl. However, optimum volume seem to depend on:
  - isolation protocol
  - sample type
  - organism.

- **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

Human plasma

miRNeasy Serum/Plasma Advanced kit (Qiagen)

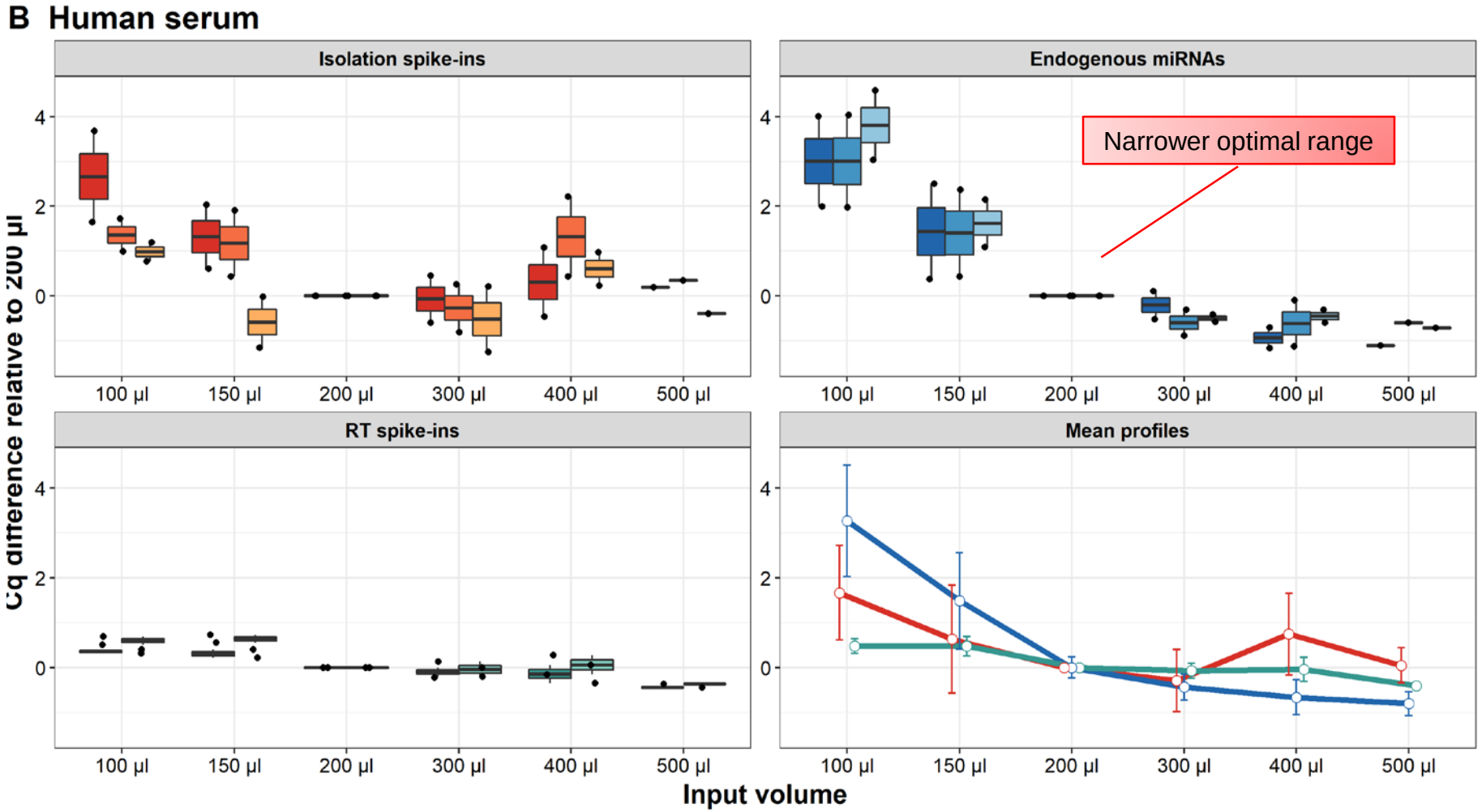


ibt

tataabiocenter

Human serum

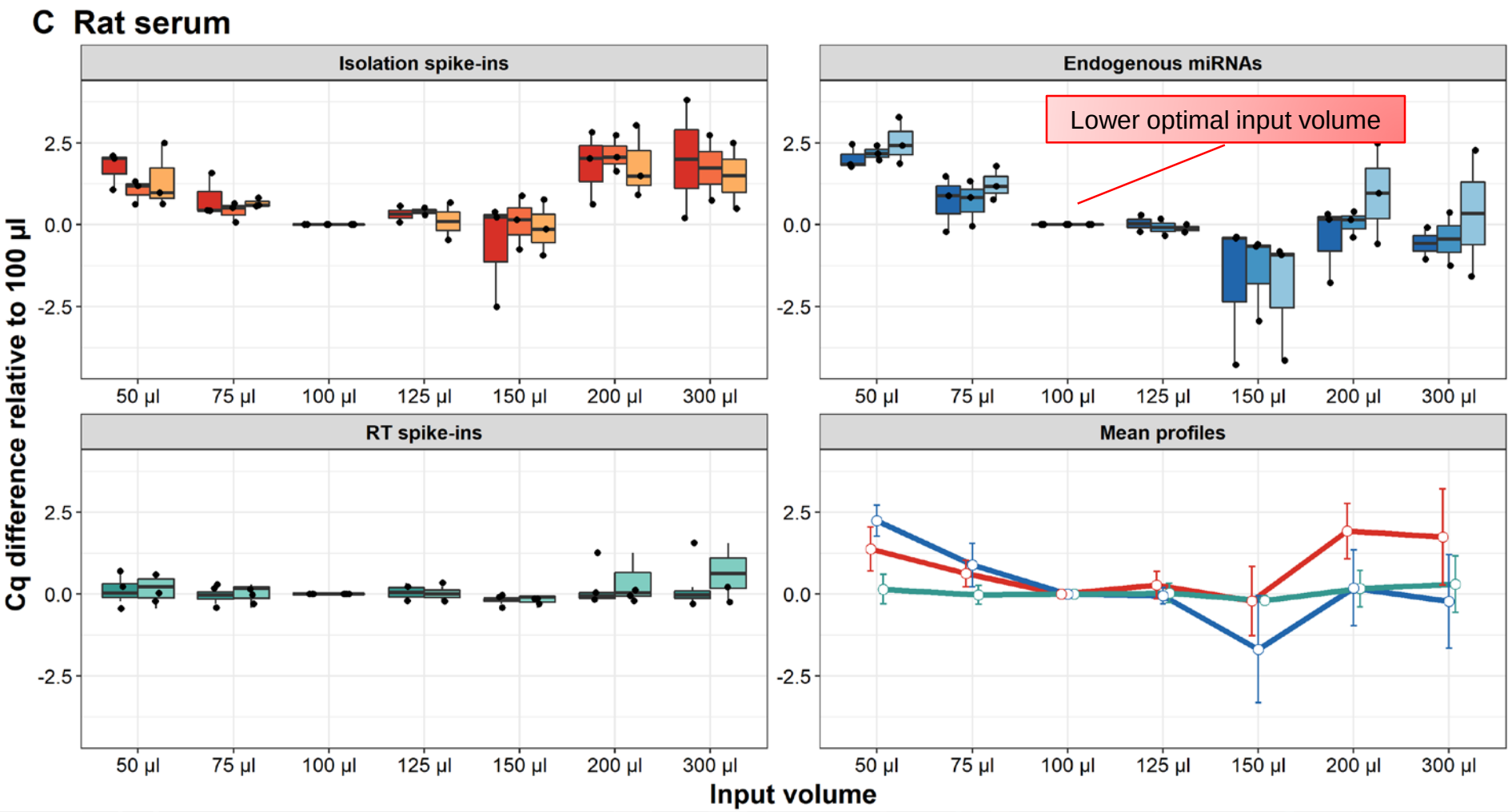
miRNeasy Serum/Plasma Advanced kit (Qiagen)



tataabiocenter

Rat serum

miRNeasy Serum/Plasma Advanced kit (Qiagen)



ibt

tataabiocenter

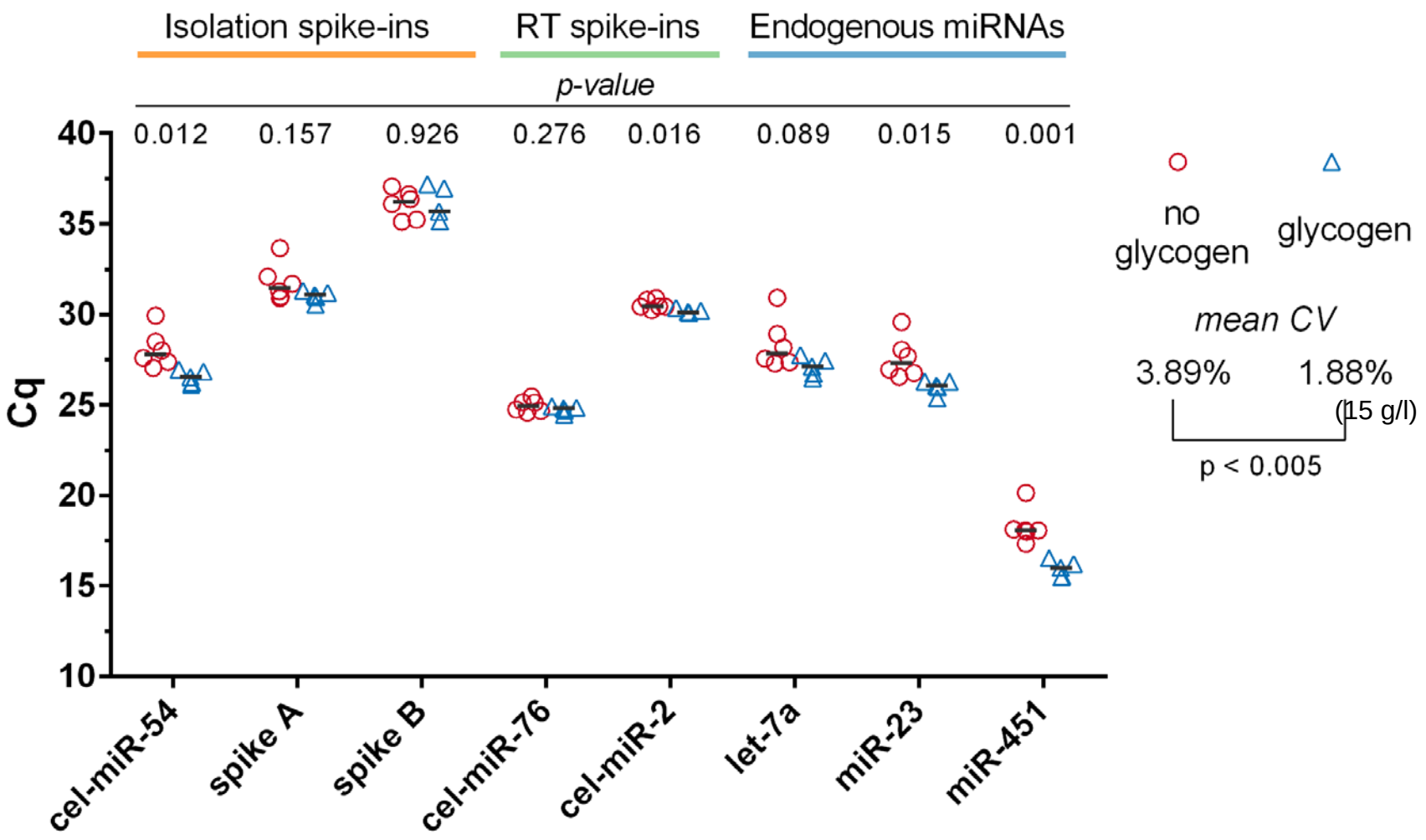
Conclusions

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

Effect of glycogen (human plasma)

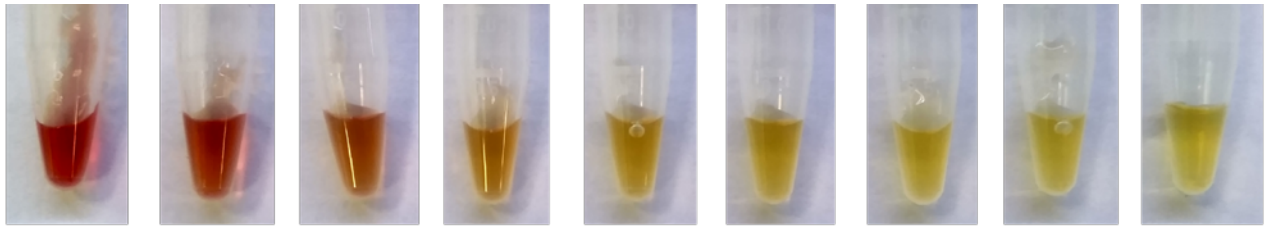


Higher reproducibility: F-test, p<0.001)
Higher yield: ΔCq=1.25, paired t-test, p<0.011)

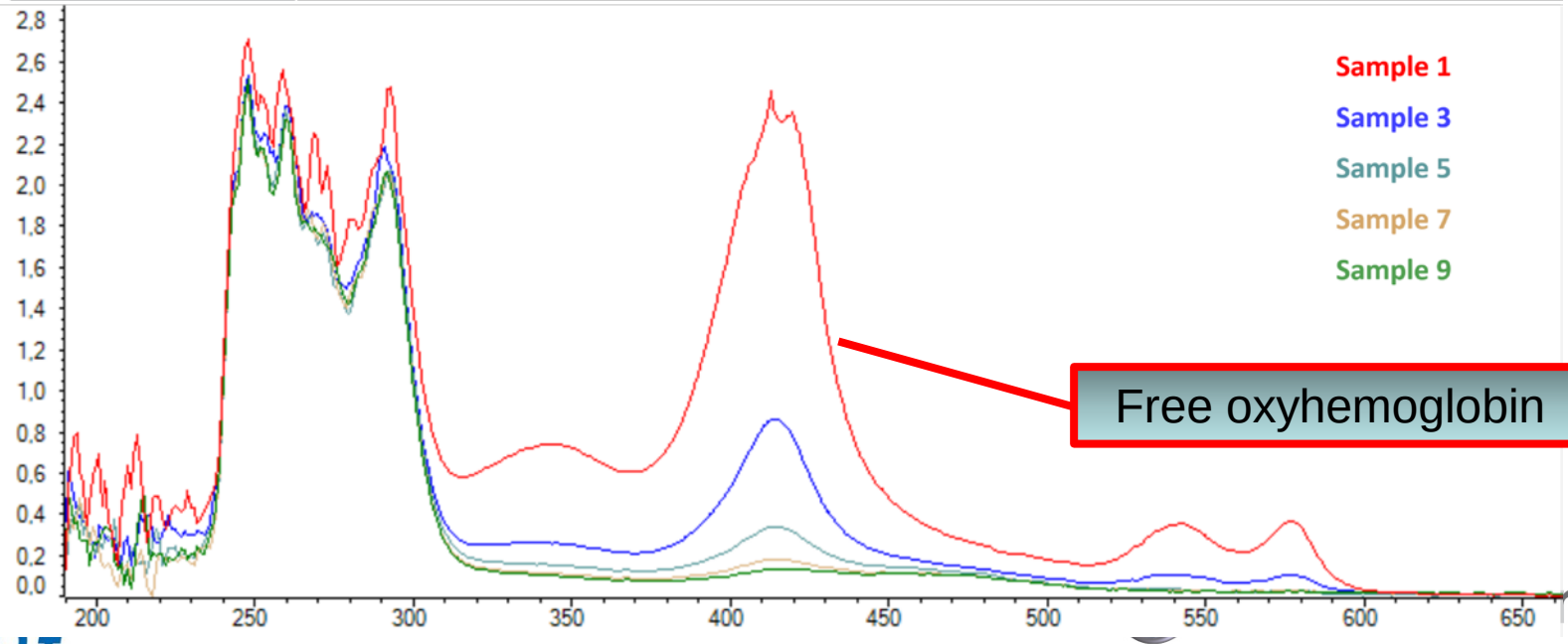
ibt

tataabiocenter

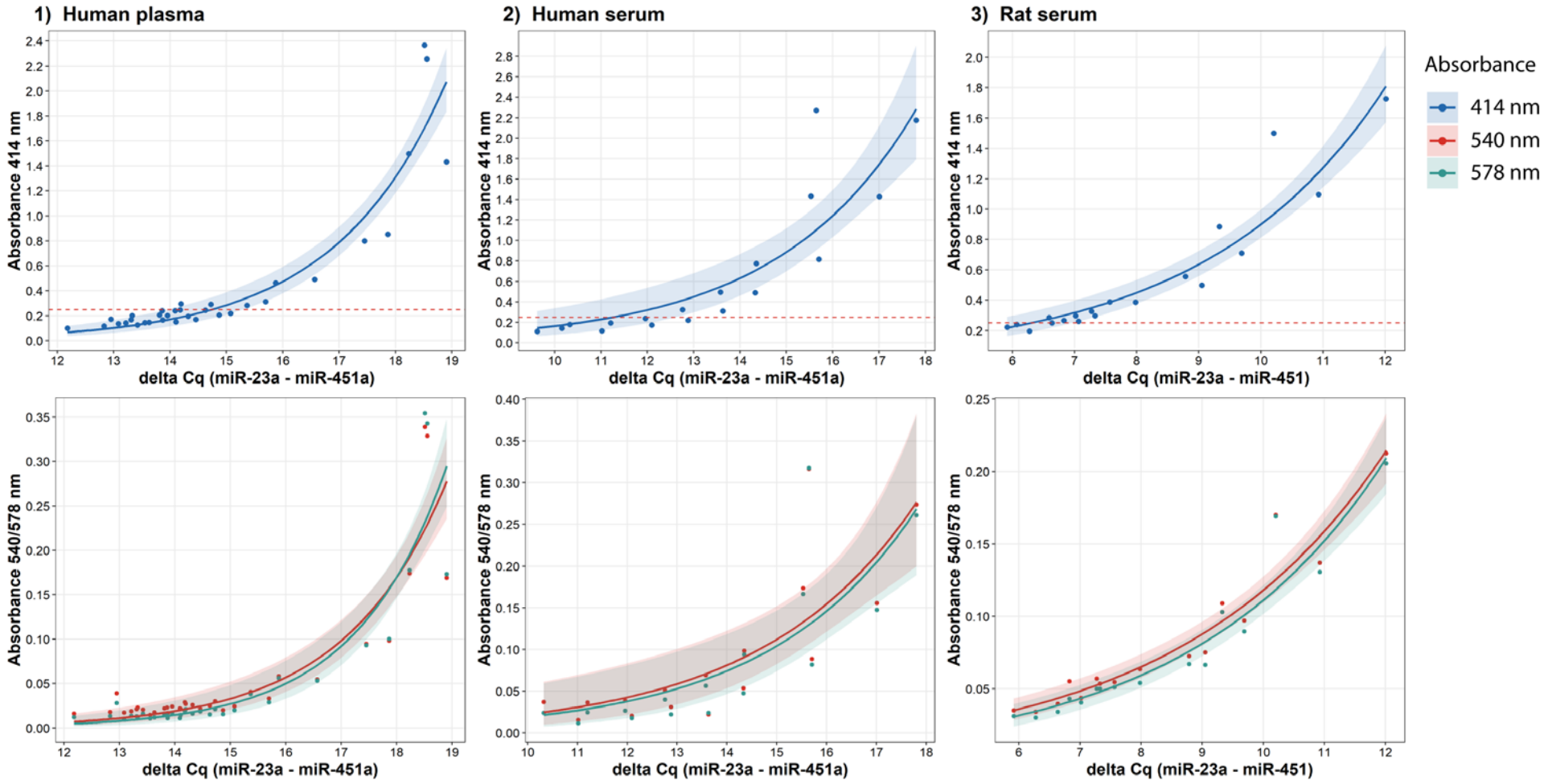
Hemolysis



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



ibt

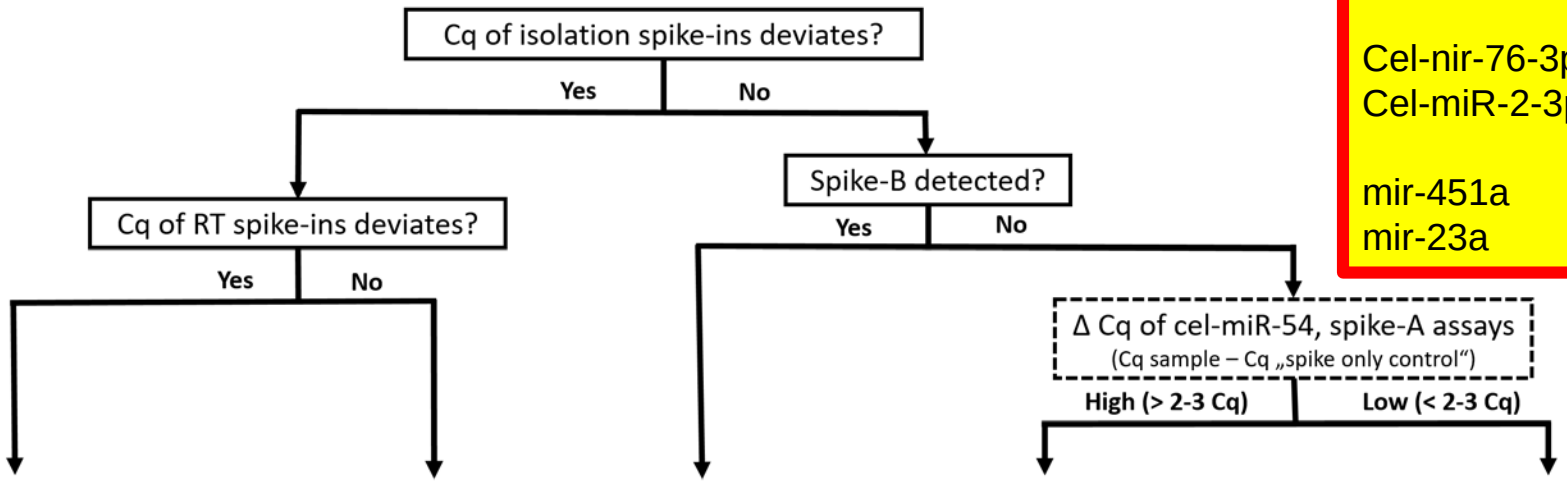
tataabiocenter

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

ibt

tataabiocenter

MENU

SCIENTIFIC REPORTS

Article | OPEN | Published: 12 March 2019

Two-tailed RT-qPCR panel for quality control of circulating microRNA studies

Peter Androvic, Nataliya Romanyuk, Lucia Urdzikova-Machova, Eva Rohlova, Mikael Kubista & Lukas Valihrach

New Advances in Two-Tailed RT-qPCR

Mansi Maheta[1,2], Eva Rohlova[1,2] Peter Androvic[1,3], Lukas Valihrach[1], Mikael Kubista[1,4]

[1]Laboratory of Gene Expression, Institute of Biotechnology CAS, BIOCEV, Vestec, Czech Republic; [2]Faculty of Science, Charles University, Prague, Czech Republic; [3]Faculty of Science, Palacký University, Olomouc, Czech Republic; [4]TATAA Biocenter, Gothenburg, Sweden

Re-isolate the samples/purify or dilute the template RNA and check again for inhibition. Alternatively exclude affected samples from the study

Re-isola
or excl
from t

luates are of good quality.
time increase the amount
lation spike-in added per
tion / prepare fresh stock solution

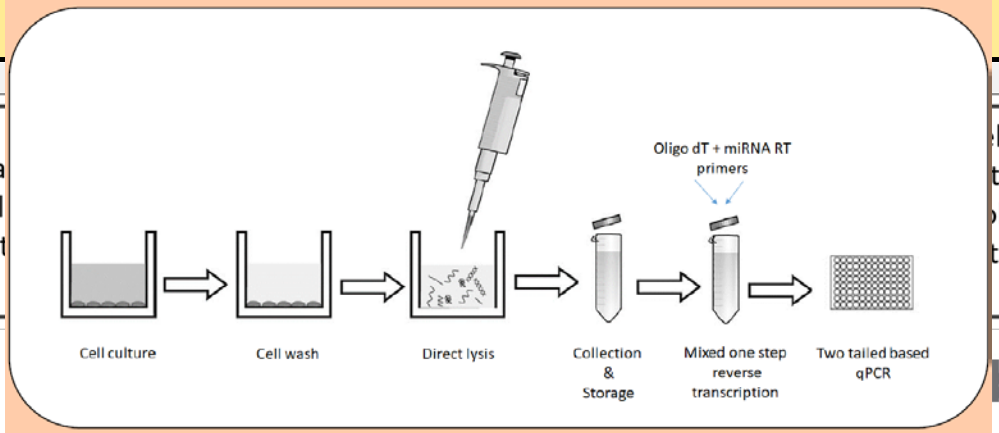


Figure 6. Schematic overview of direct lysis followed by RT-qPCR.

biocenter

Products and Services

- miRia - miRNA Immunoassays
- miRNA – RT-qPCR
- miRNA Isolation kits
- miRNA Diagnostics
- Immunoassays
- Proteins
- Antibodies
- Planar Arrays
- Immunohistochemistry
- Endotoxin Detection & Removal Products
- Cell-Based Assays
- Extracellular Matrix Assays

You are here: Home › Products and Services › miRNA – RT-qPCR › Two-Tailed qPCR › Two-Tailed qPCR

Two-Tailed qPCR

Patent-pending miRNA Two-Tailed RT-qPCR technology shows exceeding performance compared to other techniques.

The challenge detecting small microRNAs is that two conventional PCR primers do not fit the target as their combined length is almost twice the length of the microRNA. Older techniques have solved this by extending the microRNA using e.g., a hairpin primer, adding a poly A-tail, or adding a fragment by ligation. This, however, compromises the assay sensitivity and specificity, as only one of the PCR primers sense the actual microRNA sequence; the other senses the added extension. Further, these methods fail to detect microRNAs modified in the 3'-end as it interferes with the extension process.

The technology has been developed in TATAABiocenter and bought by BioVendor. The miRNA two-Tailed RT-qPCR assays offers a superior solution. Instead of using a single binding probe, Two-tailed PCR uses two hemiprobes, which bind to different stretches of the microRNA, that are connected by a folded tether. While each hemiprobe is too short to bind the microRNA, when both hemiprobes are complementary they bind cooperatively. Binding is exceeding specific, as a mismatch is much more profound in a short hemiprobe. The cDNA formed can then be PCR amplified using two sequence specific primers. SYBR used for detection. High melting resolution analysis can be used for non-specific products detection.

Two-Tailed qPCR: Advantages | Two-Tailed qPCR: Assays available | Two-Tailed qPCR: Customized assays

Two-Tailed qPCR: Assays available

https://www.biovendor.com/

Standard Material for absolute calibration

Material Details

SRM 2372a - Human DNA Quantitation Standard

C - Certificate M - MSDS T - Table

Add Material to Cart

- C Certificate
- M Material Safety Data Sheet (MSDS)
- T Related Materials: 105.8 - DNA Profiling and Nucleic Acid Materials (solid forms)

Details	
Description:	Human DNA Quantitation Standard
Lot:	N/A
Expiration Date:	2/13/2023
Unit Price * :	$794.00
Unit of Issue:	3 vials x 55 µL
Status:	Now Selling See 'Additional Information' for details.
Certificate Date:	3/13/2018
MSDS Date:	2/27/2017
Technical Contact:	Erica Romsos
Additional Information:	Full details on the production, analysis, and statistical evaluation of SRM 2372a are provided in: NIST Specia Publication 260-189, Certification of Standard Reference Material® 2372a Human DNA Quantitation Standar This publication is available free of charge at https://doi.org/10.6028/NIST.SP.260-189.

NIST Special Publication 260-189

Certification of Standard Reference Material® 2372a

Human DNA Quantitation Standard

Erica L. Romsos
Margaret C. Kline
David L. Duewer
Blaza Toman
Natalia Farkas

This publication is available free of charge from:
https://doi.org/10.6028/NIST.SP.260-189

Calibrated Human Genomic DNA (Secondary Standard)

TATAA Biocenter

★★★★★

(4.9/5) Based on 28 rating.

Volume (ul): -Choose-

Information | Specifications | Opinions (0) | Share

Quantify the absolute amount of human genomic DNA

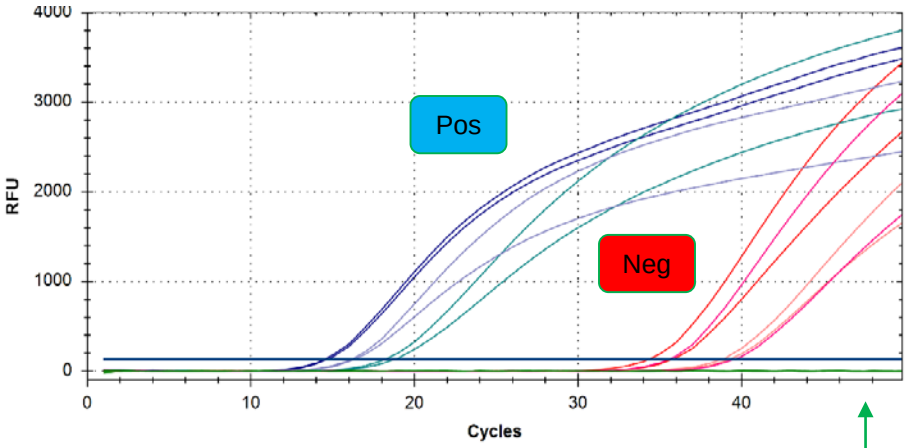
ataabiocenter

https://webshop.tataa.com/product.html/validprime?category_id=27

Alu control assay for DNA contamination

- The Alu element is the most abundant sequence in the human genome being present in over 1 million copies (11 %).
- TATAA Alu assays are supersensitive for human genomic DNA.

Mastermixes from three suppliers showing significant contamination of human gDNA when tested with Alu-assays.



Heat&Run gDNA removal kit

Clean

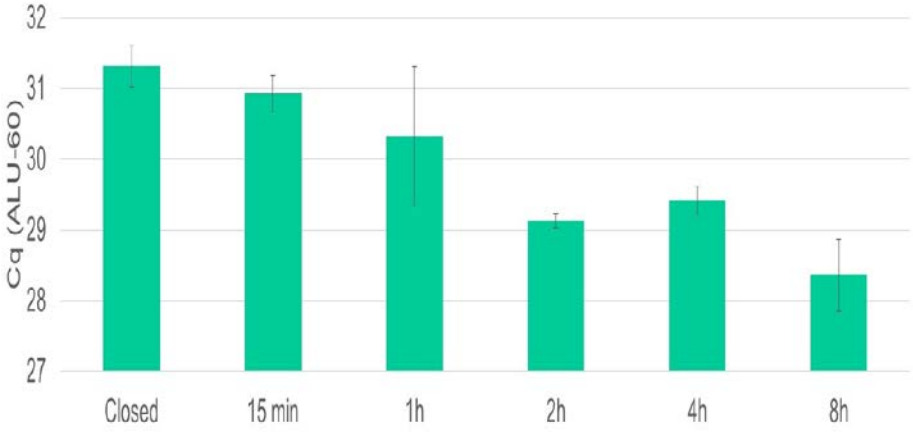
ArcticZymes

★★★★★

(4.9/5) Based on 21 rating.

Heat-labile dsDNA specific nuclease

Eppendorf tube left open in laboratory, being contaminated by DNA in the air



Particularly relevant for rare mutation detection

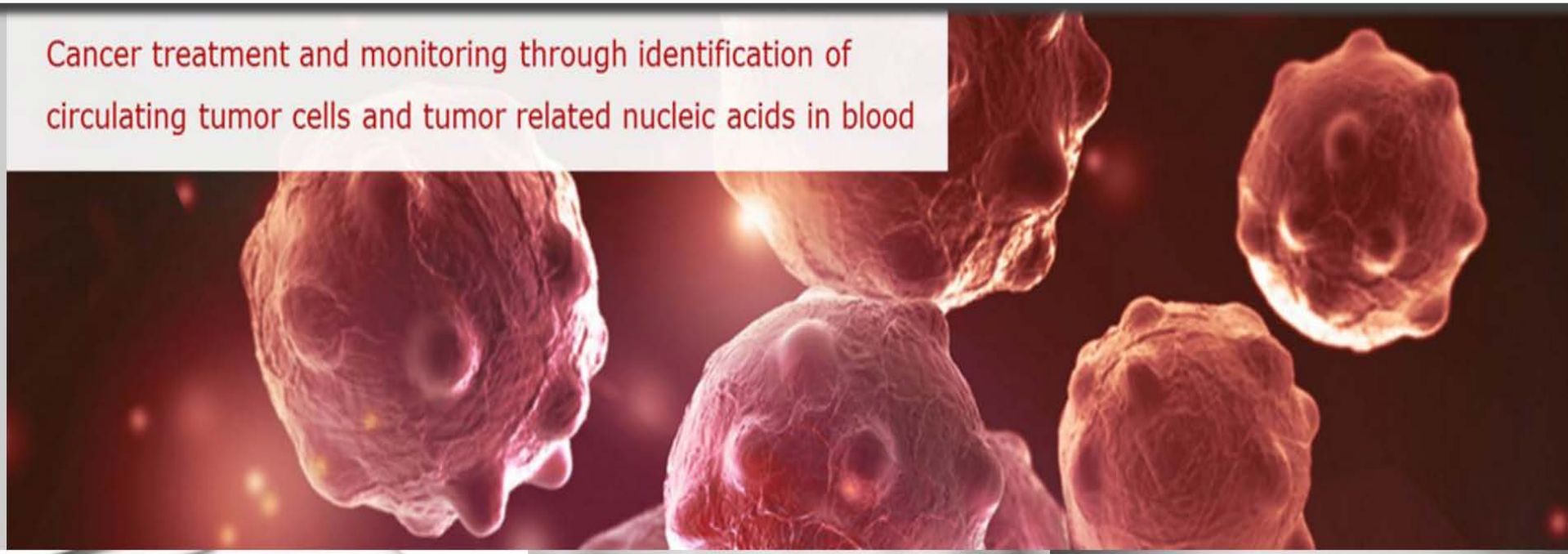
https://webshop.tataa.com/product.html/tataa-alu-assay?category_id=66

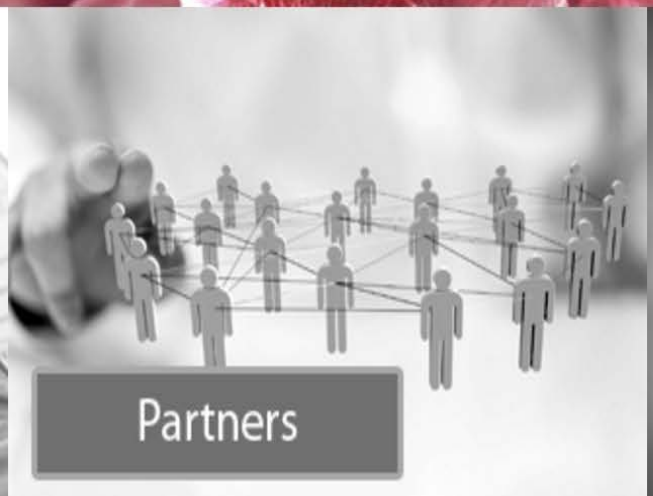
tataabiocenter

CANCER ID

www.cancer-id.eu

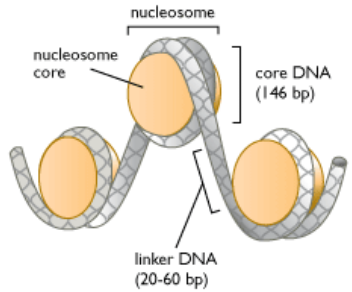
Cancer treatment and monitoring through identification of circulating tumor cells and tumor related nucleic acids in blood

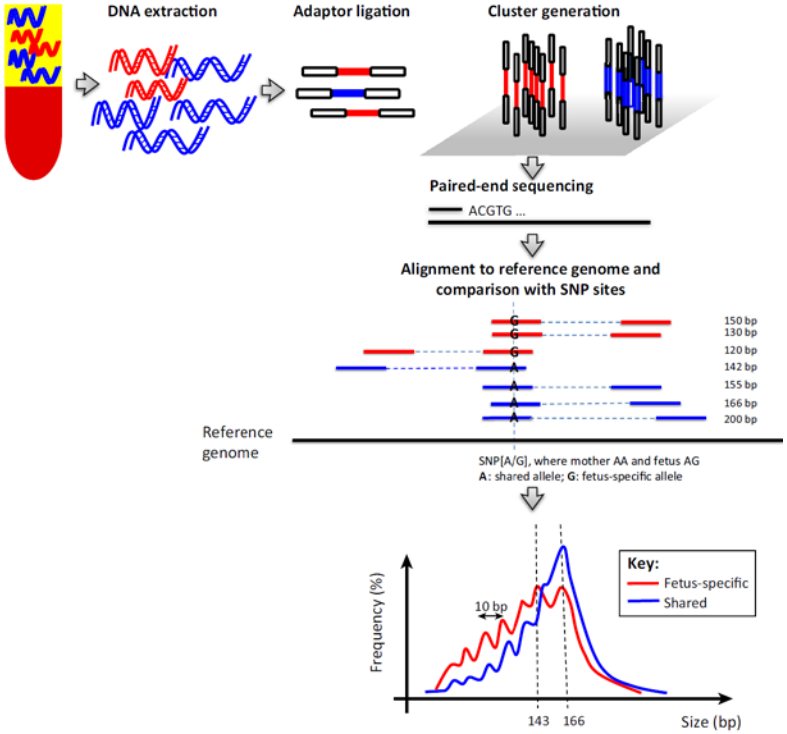




ΔAmp Alu control assays for cellular DNA contamination

cfDNA is fragmented





$$\Delta Cq_{L/S} = Cq_L - Cq_S = 0$$

gDNA

ALuJB2 (S)

60 bp

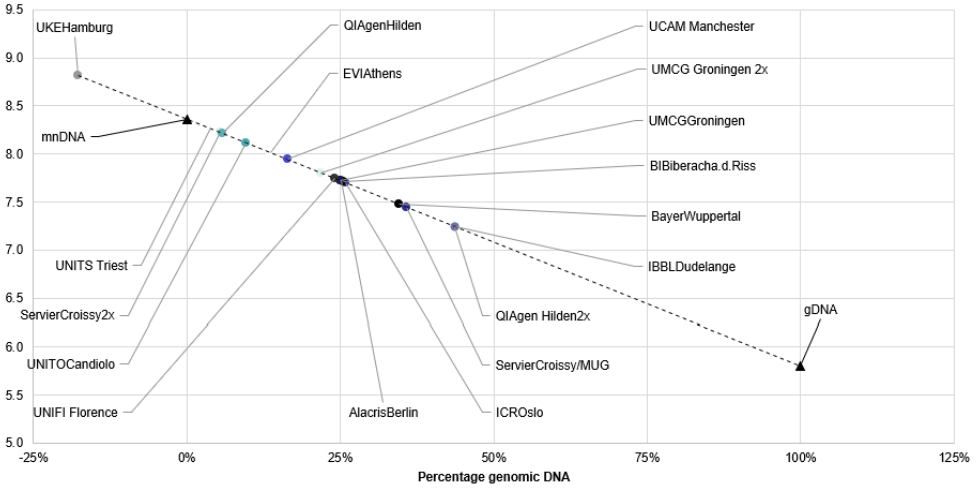
ALuYc1 (L)

187 bp

cfDNA

160 bp

$$\Delta Cq_{L/S} = Cq_L - Cq_S >> 0$$



Trends in Genetics, June 2016, Vol. 32, No. 6

PRECISION DIAGNOSTICS EUROPE 2020

May 26 – 29, 2020

BIOCEV Vestec
Prague
Czech Republic

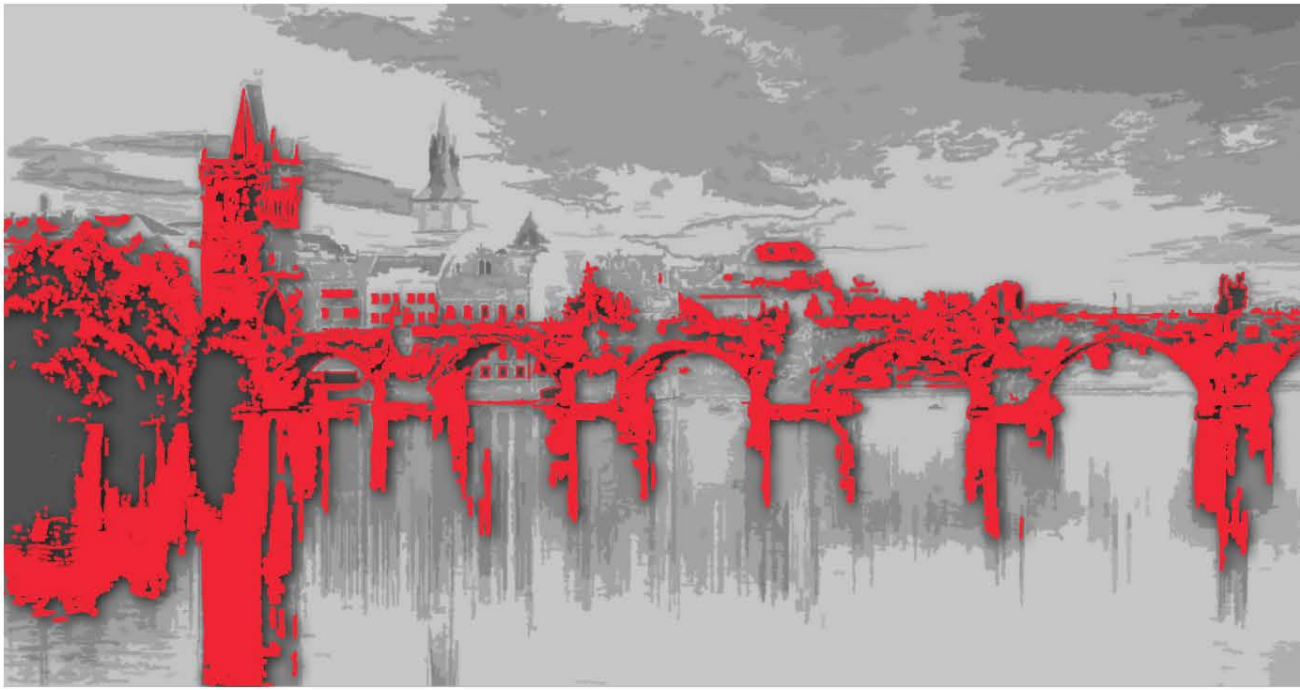
PROGRAM SPEAKERS REGISTRATION PRACTICAL INFO SPONSORSHIP ABSTRACT

Deadlines

Early registration:	24.1.2020
Oral pr. submission:	7.2.2020
Poster submission:	6.3.2020
Registration deadline:	31.3.2020

REGISTRATION

ABSTRACT



http://precisiondiagnostics.eu/

A SPIDIA Conference

ibt

tataabiocenter