SUPPLEMENTARY FILE

5' hemiprobe	Cq let-7a	Δ Cq (relative to let-7a) let-7e	let-7f	let-7g
10-mer	14.54	5.42	4.61	9.17
8-mer	14.09	5.24	5.30	9.78
7-mer	14.06	5.26	5.89	10.14
6-mer	14.11	5.36	6.06	10.44
5-mer	14.23	5.90	6.93	11.03
4-mer	14.79	5.92	6.48	10.58

Figure S1. Effect of the 5'-hemiprobe length on specificity. Two-tailed RT primers with 6 nt long 3'-hemiprobe and 5'-hemiprobe with variable length designed to target let-7a were assayed with four members of the let-7 family. Same PCR primers were used.

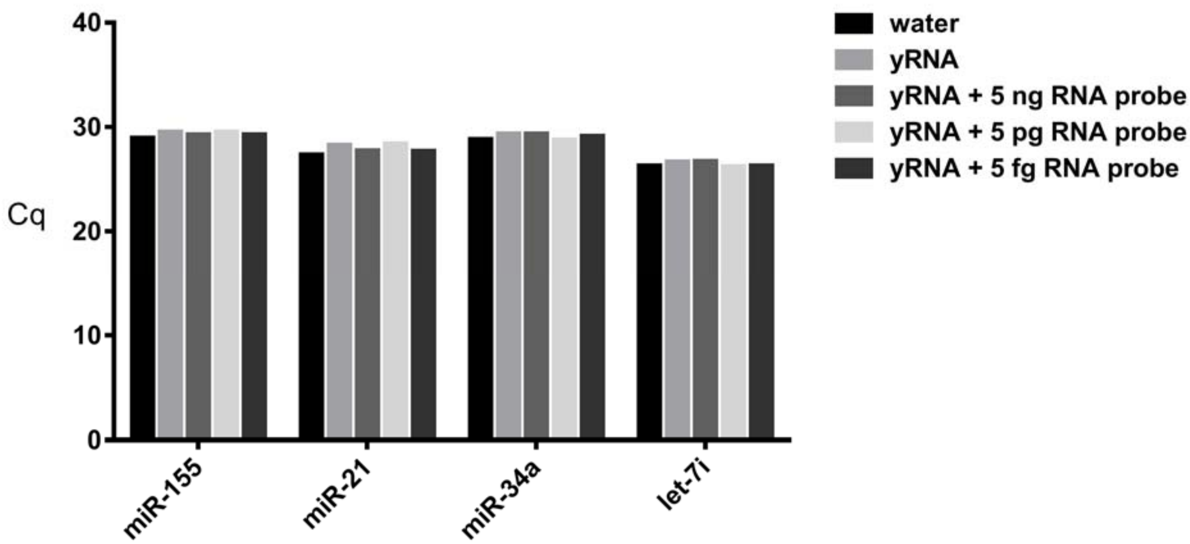

Figure S2. Effect of complementary RNA background on sensitivity. Hsa-miR-155 + hsa-miR-21 and hsa-miR-34a + hsa-let-7i (10^5 copies/rxn) were mixed with either water, 10 ng yeast RNA, and 10 ng yeast RNA containing two mRNA probes at various concentrations. Target miRNAs were partially complementary to the mRNA probes (suppl. file). Mixtures were subjected to one freeze-thaw cycle to simulate sample storage conditions. Single RT reaction per sample was performed, since we have observed that technical variation is negligible (table 1). Presence of partially complementary long RNA in the background had no significant effect on Cq values.

Primers and templates

Legend	
binding hemiprobes:	blue
primer arms:	orange
stem:	green
loop:	purple

Proof of concept experiment

miRNA	miRNA sequence (5' - 3')	RT primers		qPCR primers
let-7a	UGAGGUAGUAGGUUGUAUAGUU	**RT1:** TACTACCTCACTATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTA	fw	ATGCTCTCCAGGTACAGTTG
		ATGATGGAGTCTATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTA		
let-7f	UGAGGUAGUAGAUUGUAUAGUU	CTATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTA	rv	GCCCATGAGGTAGTAGGTTGTATA
		RT2: AACCTACTCTATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTA		

Cross-reactivity: mature isoforms

miRNA	miRNA sequence (5' - 3')	RT primer		qPCR primers
let-7a	UGAGGUAGUAGGUUGUAUAGUU	AACCTACTCTATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTA	fw	CGAACCTACTCTATGCTCTCCAG
			rv	CGGGTGAGGTAGTAGGTTGTA
let-7b	UGAGGUAGUAGGUUGUGUGGUU	ACCTAACATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACCA	fw	GCACCTAACATGCTCTCCAG
			rv	CGGTGAGGTAGTAGGTTGTG
let-7c	UGAGGUAGUAGGUUGUAUGGUU	ACCTAACATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACCA	fw	GCACCTAACATGCTCTCCAG
			rv	CGGGTGAGGTAGTAGGTTGTA
let-7d	AGAGGUAGUAGGUUGCAUAGUU	CCTCTACATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTAT	fw	CGCCTCTACATGCTCTCCAG
			rv	CGCAGAGGTAGTAGGTTGC
let-7e	UGAGGUAGGAGGUUGUAUAGUU	CCTCCACATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTAT	fw	TCCTCCACATGCTCTCCAG
			rv	AGGGTGAGGTAGGAGGTTG
let-7f	UGAGGUAGUAGAUUGUAUAGUU	ATCTACACATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTAT	fw	CCATCTACACATGCTCTCCAG
			rv	GCCGTGAGGTAGTAGATTGTA
let-7g	UGAGGUAGUAGUUUGUACAGUU	AACTACGCATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTGT	fw	AACTACGCATGCTCTCCAG
			rv	GCGGTGAGGTAGTAGTTTGTA
let-7i	UGAGGUAGUAGUUUGUGCUGUU	AACTACGCATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACAGC	fw	AACTACGCATGCTCTCCAG
			rv	GCGGTGAGGTAGTAGTTTGTG

Cross-reactivity: precursors

miRNA	miRNA sequence (5' - 3')	RT primer		qPCR primers
let-7a	UGAGGUAGUAGGUUGUAUAGUU	AACCTACTCTATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTA	fw	CGAACCTACTCTATGCTCTCCAG
			rv	ACCCTGAGGTAGTAGGTTGTA
let-7b	UGAGGUAGUAGGUUGUGUGGUU	ACCTAACATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACCA	fw	GCACCTAACATGCTCTCCAG
			rv	ACCCTGAGGTAGTAGGTTGTG
let-7f	UGAGGUAGUAGAUUGUAUAGUU	ATCTACACATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTAT	fw	CCATCTACACATGCTCTCCAG
			rv	GCACCTGAGGTAGTAGATTGTA

MiRNA profiling in mouse tissues

miRNA	miRNA sequence (5' - 3')	RT primer		qPCR primers
mmu-miR-122-5p	UGGAGUGUGACAAUGGUGUUUG	GTCACACTTCAAGCTCTCCAGGTACAGTTGGTACCTGACTCCACGCCAAACA	fw	GTCACACTTCAAGCTCTCCAG
			rv	GGTGGAGTGTGACAATGGTG
mmu-miR-30c-1-3p	CUGGGAGAGGGUUGUUUACUCC	CCCTGTCCCGATATGTGAGACGTACGTTGAGTACGTCAAGTGAAGTGAGTAA	fw	CCCTGTCCCGATATGTGAGAC
			rv	GCTGGGAGAGGGTTGTTT
mmu-miR-615-5p	GGGGGUCCCCGGUGCUCGGAUC	GACCCCCGCTAGCTATGCAGGTACAGTTGGTACCTGACTCTTGTTGATCCG	fw	GACCCCCGCTAGCTATGC
			rv	GGGGTCCCCGGTGCT
mmu-miR-193a-3p	AACUGGCCUACAAAGUCCCAGU	TTGTAGGCGCTTGAGTCCTCGTAGAGTTGCTACGAGATATGATAAACTGGG	fw	TTGTAGGCGCTTGAGTCC
			rv	GGAACTGGCCTACAAAGTCC
mmu-miR-1a-3p	UGGAAUGUAAAGAAGUAUGUAU	TTTACATTAGGGCACTCGTCCTAGAGTTGCTAGGACTACGGACTTATACAT	fw	GGTTTACATTAGGGCACTCGTC
			rv	CGGGTGGAATGTAAAGAAGTATG
mmu-miR-21a-5p	UAGCUUAUCAGACUGAUGUUGA	ATAAGCTACAACGACCAGAGCTAGAGAACCTAGCTCACCCACTACTCAACA	fw	CGCATAAGCTACAACGACCAGAG
			rv	CGGGTAGCTTATCAGACTGATGT
mmu-miR-24-3p	UGGCUCAGUUCAGCAGGAACAG	CTGAGCCACGACGAATACTGCTAGAGTTGCTAGCAGAGCCCTTAACTGTTC	fw	CCTGAGCCACGACGAATAC
			rv	GTGGCTCAGTTCAGCAGG

Sensitivity to isomiRs

miRNA	miRNA sequence (5' - 3')	RT primer		qPCR primers
miR-21_minus 2	UAGCUUAUCAGACUGAUGUU	ATAAGCTACAACGACCAGAGCTAGAGAACCTAGCTCACCCACTATAACATC	fw	CGCATAAGCTACAACGACCAGAG
miR-21_minus 1	UAGCUUAUCAGACUGAUGUUG			
miR-21	UAGCUUAUCAGACUGAUGUUGA		rv	CGGGTAGCTTATCAGACTGATGT
miR-21_plus 1	UAGCUUAUCAGACUGAUGUUGAC			
miR-21_plus 2	UAGCUUAUCAGACUGAUGUUGACA			

Crossreactivity optimization (primers used in figure S1)

miRNA	miRNA sequence (5' - 3')	RT primer		qPCR primers
let-7a	UGAGGUAGUAGGUUGUAUAGUU	AACCTACTACTAATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTAT	fw	ATGCTCTCCAGGTACAGTTG
		AACCTACTCTATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTAT		
		AACCTACACATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTAT		
		ACCTACACATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTAT	rv	CGGGTGAGGTAGTAGGTTGTA
		ACCTAACATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTAT		
		CCTAACATGCTCTCCAGGTACAGTTGGTACCTGTCTCCACTTAACTAT		

Pre-miRNAs synthesis

pre-miRNA name	Sequence (5' - 3')	Oligos used to create template for in vitro transcription	
hsa-let-7a-1	UGGGAUGAGGUAGUAGGUUGUAUAGUUUUAGGGUCACACCCACCACUGGGAGAUAACUAUACAAUC UACUGUCUUUCCUA	template	GACAGTAGATTGTATAGTTATCTCCCAGTGGTGGGTGTGACCCTAAAACTATACAACCTACTACCTCATCCC
		fw	TAATACGACTCACTATAGGGATGAGGTAGTAGGT
		rv	TAGGAAAGACAGTAGATTGTATAGTTATCTC
hsa-let-7b	CGGGGUGAGGUAGUAGGUUGUGUGGUUUCAGGGCAGUGAUGUUGCCCCUCGGAAGAUAACUAUAC AACCUACUGCCUUCCCUG	template	CAGTAGGTTGTATAGTTATCTTCCGAGGGGCAACATCACTGCCCTGAAACCACACAACCTACTACCTCACCCC
		fw	TAATACGACTCACTATAGGGGTGAGGTAGTAGG
		rv	CAGGGAAGGCAGTAGGTTGTATAGTTATCTTC
hsa-let-7f-1	UCAGAGUGAGGUAGUAGAUUGUAUAGUUGUGGGGUAGUGAUUUUACCCUGUUCAGGAGAUAACUA UACAAUCUAUUGCCUUCCCUGA	template	AGGCAATAGATTGTATAGTTATCTCCTGAACAGGGTAAAATCACTACCCCACAACTATACAATCTACTACCTCACTCTGAC
		fw	TAATACGACTCACTATAGGGTCAGAGTGAGGTAGTAG
		rv	GTCAGGGAAGGCAATAGATTGTATAGTTATC

Quanta qScript microRNA system

miRNA	miRNA-specific primer:
let-7a	CCGAGCTGAGGTAGTAGGTTGTATA
let-7b	CGTTCTGAGGTAGTAGGTTGTGTG
let-7c	CCGAGCTGAGGTAGTAGGTTGTATG
let-7d	GGGACGAGAGGTAGTAGGTTGC
let-7e	CGGTGGTGGTGAGGTAGGAG
let-7f	CGGTGGTGGTGAGGTAGTAGA
let-7g	CCGAGCTGAGGTAGTAGTTTGTAC
let-7i	CGTTCTGAGGTAGTAGTTTGTGCT
miR-21	GCTAGCTTATCAGACTGATGTTGAAA

Note: Primer sequences were obtained from the manufacturer´s website (http://www.quantabio.com/products/microrna-profiling)

miQPCR

miRNA	miRNA-specific primer:
miLINKER	pp(r)A.GGCCGAACTACGACCTGCATAAACGG.ddC
mQ-RT	CCCAGTTATGGCCGTTTATGCAGGT
Upm2A	CCCAGTTATGGCCGTTTA
let-7a	~~AGTAAGGTTGTATAGTTG~~
	~~TAGTAGGTTGTATAGTTG~~
	TGAGGTAGTAGGTTGTATAGTTGGC
let-7b	~~TAGTAGGTTGTGTGGTTG~~

	TGAGGTAGTAGGTTGTGTGGTTG
let-7c	~~TAGTAGGTTGTATGGTTG~~
	TGAGGTAGTAGGTTGTATGGTTGG
let-7d	AGAGGTAGTAGGTTGCATAGTTG
let-7e	~~TAGGAGGTTGTATAGTTG~~
	TGAGGTAGGAGGTTGTATAGTTGG
let-7f	~~AGTAGATTGTATAGTTGG~~
	TGAGGTAGTAGATTGTATAGTTGGC
let-7g	TGAGGTAGTAGTTTGTACAGTTG
let-7i	TGAGGTAGTAGTTTGTGCTGTTG
miR-21	TAGCTTATCAGACTGATGTTGAGG

Note: Primers striken-through were taken from supplementary file 1 from Benes et al., 2015, *Scientific reports*, 5, 11590, but failed to amplify. They were then replaced with primers from supplemenary file 2 from the same publication, which contained primers with optimized melting temperature.

Limit of Detection: Cq values

miRNA target input in RT reaction	1000	500	250	125	62.5	31.25	15.63	7.81	3.91	no target control
Cq	31.53	32.92	33.61	34.73	35.11	35.81	n.d.	35.99	n.d.	n.d.
	31.17	32.72	33.45	34.52	34.90	n.d.	n.d.	n.d.	n.d.	n.d.
	31.11	32.34	33.82	34.06	33.62	35.04	n.d.	36.53	n.d.	n.d.
	31.29	32.13	33.29	33.60	34.17	36.58	36.66	n.d.	36.58	n.d.
	31.42	32.56	32.87	34.19	35.43	34.37	n.d.	n.d.	n.d.	n.d.
	31.11	32.50	32.72	35.62	35.39	35.44	36.41	35.59	n.d.	n.d.

Cq values of a two-fold dilution series of a let-7d synthetic miRNA. cDNA was diluted 10 x in qPCR reactions.

MiRNA profiling in mouse tissues: Cq values and relative quantities

Two-tailed RT-qPCR: singleplex

Cq values

Sample	122-5p	193a-3p	1a-3p	21a-5p	24-3p	30c-1-3p	let-7a
brain	26.52	30.83	30.66	26.66	21.13	30.54	22.08
cereb.	28.02	30.57	29.67	25.85	20.81	29.00	20.33
heart	28.79	30.67	21.23	25.01	20.18	29.22	21.97
kidney	32.15	27.26	32.86	24.38	21.49	29.76	22.76
liver	18.23	27.89	35.81	23.41	23.46	32.91	23.67
lung	28.36	28.24	28.21	23.60	19.79	30.26	21.36
muscle	23.33	27.47	20.19	25.95	21.75	31.62	23.27
negative control	n.d.	n.d.	n.d.	n.d.	36.91	n.d.	n.d.

Relative expression

Sample	122-5p	193a-3p	1a-3p	21a-5p	24-3p	30c-1-3p	let-7a
brain	5.63	0	5.15	0	2.33	2.37	1.59
cereb.	4.13	0.26	6.14	0.81	2.65	3.91	3.34
heart	3.36	0.16	14.58	1.65	3.28	3.69	1.7
kidney	0	3.57	2.95	2.28	1.97	3.15	0.91
liver	13.92	2.94	0	3.25	0	0	0
lung	3.79	2.59	7.6	3.06	3.67	2.65	2.31
muscle	8.82	3.36	15.62	0.71	1.71	1.29	0.4

Two-tailed RT-qPCR: multiplex

Cq values

Sample	122-5p	193a-3p	1a-3p	21a-5p	24-3p	30c-1-3p	let-7a
brain	26.40	31.77	31.92	29.07	21.24	30.46	22.81
cereb.	28.11	31.55	31.33	28.02	21.01	29.28	21.18
heart	28.58	31.34	22.60	27.06	19.84	29.09	22.47
kidney	32.48	28.21	34.76	26.63	21.35	29.83	23.02
liver	18.03	28.74	37.54	25.91	23.18	32.72	24.11
lung	28.38	29.20	29.68	25.95	19.83	30.70	22.12
muscle	23.21	28.34	21.89	28.28	21.57	31.39	24.51
negative control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Relative expression

Sample	122-5p	193a-3p	1a-3p	21a-5p	24-3p	30c-1-3p	let-7a
brain	6.08	0.00	5.62	0.00	1.94	2.26	1.70
cereb.	4.37	0.22	6.21	1.05	2.17	3.44	3.33
heart	3.90	0.43	14.94	2.01	3.34	3.63	2.04
kidney	0.00	3.56	2.78	2.44	1.83	2.89	1.49
liver	14.45	3.03	0.00	3.16	0.00	0.00	0.40
lung	4.10	2.57	7.86	3.12	3.35	2.02	2.39
muscle	9.27	3.43	15.65	0.79	1.61	1.33	0.00

TaqMan miRNA assays

Cq values

Sample	122-5p	193a-3p	1a-3p	21a-5p	24-3p	30c-1-3p	let-7a
brain	27.25	31.29	30.56	25.07	22.51	34.60	22.46
cereb.	28.97	30.77	30.12	24.29	22.24	33.85	21.16
heart	29.86	30.82	21.30	23.51	21.55	34.71	22.87
kidney	34.07	27.52	32.89	22.90	22.98	34.61	23.58
liver	19.14	28.08	35.75	21.82	24.87	n.d.	24.58
lung	29.27	28.31	28.20	21.91	21.22	33.34	22.48
muscle	24.28	27.91	20.07	24.47	23.09	36.33	24.05
negative control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Relative expression

Sample	122-5p	193a-3p	1a-3p	21a-5p	24-3p	30c-1-3p	let-7a
brain	6.82	0	5.19	0	2.36	5.4	2.12
cereb.	5.1	0.52	5.63	0.78	2.63	6.15	3.42
heart	4.21	0.47	14.45	1.56	3.32	5.29	1.71
kidney	0	3.77	2.86	2.17	1.89	5.39	1
liver	14.93	3.21	0	3.25	0	0	0
lung	4.8	2.98	7.55	3.16	3.65	6.66	2.1
muscle	9.79	3.38	15.68	0.6	1.78	3.67	0.53

Cq Values are presented as average of 2 qPCR replicates. Fold-changes were calculated relative to the sample with the lowest expression for a given miRNA. For the calculation, missing data were replaced with the max. Cq + 4, or with Cq 40 if a result was over 40.

Hybridization experiment:

Sequences of mRNA probes with highlighted complementary regions to spiked miRNAs are shown. Nucleotides in red are perfectly complementary between mRNA probe and miRNA target. Only sites with at least seven consecutive complementary nucleotides are shown.

>nNOS cds + miR-34a, let-7i
ATGGAAGAATATGAGTTCAGCGTTAAACAACTGCAGCCGAATGTTATATCTGTGCGCCTCTTTAAGCGCA
AAGTAGGAGGGCTGGGATTCCTGGCTAAGCAAAGGAGGAACAAACCACCTGTAATTATTTCTGATTTAAT
TCGGGGAGGAGCAGCAGAACAGAGTGGGTTGGTGCAAGTAGGGGATATTATCTTAGCTGTGAATGATAGG
CCCCTTGTAGATGCCAGCTATGAAAGTGCCCTGGAGATACTGCGTAGCATTTCTTCAGAGACTTTTGTTG
TACTGATCCTTAGAGGTCCAGAAGGATTCACAACCCACTTGGAGACCACCTTTTCTGGAGATGGAACACC
CAAGACCATTCGAGTCACCAGACCTCTTTGTTCTAAGTCCAAGTCAGCTGAATTGACAAGTCAGAGCCTT
TATAGCAAAGATCACATGGTGGACAGTGCCACAAGTTCAGCTGGCTCTTCTTGGCAGGAGGTTCATCAGC
TTCTTAACTTGAATGGACTTGATGGTGGTAAAGGTGATGACATCAGCATCAGCCCTAGCCTTAACAGAGG
AGAAAAGGCTAATGACATATTGAAAGAAATCGAGCCfoxaAATCGTTAGCCTTCTACAGAATGCAGGAAA
CTAAATGGGGATGAACATCGAAATGTGGAGCAAAAGGATGCGGAAGTTCAGGTTGAAAGCAACTCGCAGA
CACAGCCGAGTATGCAGAAGGACCAAGTCAATGGTATCTGGAAAAACAACAACAAGATGCCCGTGGTTTT
AAACAATCCATATCTGGAGTGTGAGCAGGTCACATTAACTGGTAGACATTCCCCTGCAAAGAGCCAAGTG
AATGGAAGTCCATCCAAATGTCCTCGTTATTTGAAAATTCGTAATTGGGATTCAAACACTACACTTAATG
ACACCCTGCACAGCAAGGCTTCCATGCCAACACCCTGCACGCAACAGATATGCATGGGAGCCGTAATGAC
ACCCCCACATTATGTTCGCAAACCAGAGGACATTCGGACCAAAGAAGAGCTTCTTCCCCTTGCTAAGGAA
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TGACAAAGGAGATTGAAGCCACAGATACCTATCAGCTACGGGATACAGAGCTTATTTACGGAGCCAAGCA
TGCCTGGAGGAATGCATCCAGATGTGTGGGGAGAATCCAGTGGTCAAAATTGCAGTTATTTGATGCTCGA
GACTGCACCACTGCTCATGGGATGTTCAATTACATTTGCAACCACATTAAATATGCCACCAACAAAGGCA
ACCTCAGATCAGCAATCACTATCTTCCCCCAAAGAACGGATATGAAGCACGACTTTAGAATCTGGAATGC
TCAGCTTATTCGCTATGCTGGATACAAACAGCCTGATGGTTCGGTGCTGGGAGACCCTGCAAATGTAGAA
TTGACAGAGATCTGCATTCAACAGGGCTGGAAGGCCCCTCATGGACGCTTTGATGTACTACCTCTAATGC
TCCAAGCAGATGGCAATGATCCTGAATTGTTTGAGATCCCTCCTGAACTGATAATGGAAGTCAACATAAG
GCATCCAAGACTAGAGTGGTTTAAGGATCTGGGATTAAAGTGGTATGGACTGCCTGCTGTATCCAACTTG
CTGCTGGAAATCGGAGGCTTGGAATTCAGTGCCTGTCCCTTCAGCGGATGGTACATGGGGACAGAAATTG
GTGTTCGAGATTACTGTGACAACTCCCGCTACAACATACTGGAGGAAGTGGCCTTAAAAATGAATCTGGA
CACAAGGAAAACCTCCTCTCTGTGGAAGGATCAAGCTCTGGTGGAGATAAACATTGCTGTGTTGTACAGT
TTCCAGAGCGATAAAGTAACAATCGTGGACCATCACTCTGCTACTGAGTCTTTCATCAAACATATGGAGA
ATGAATACCGGTGCCGAGGAGGCTGCCCAGCAGACTGGGTTTGGATAGTGCCGCCGATGTCTGGAAGCAT
TACACCTGTATTTCACCAGGAAATGTTAAACTACCGTCTTACACCTTCATTTGAGTACCAGCCGGATCCA
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TCTGCTGATGCCCAGAAATCATCTGCTAATGAGACGGGACCCAGAGACAACTTTGGAAGTGCAAGTCCCC
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AGCGGTTGACACGTTGTTGGAGGAGCTTGGAGGAGAAAGAATAATGAAAATGGGGGAAGGGGATGAACTG
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CAAGTACCGAATCAGCTTTGTGGCCGAGGCACCAGAACTTACACAAGCTTTATACAGTATTCACAAGAAG
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TCCTCAAACTTCACTCTAATGGACATAAAGAACTGTGCTATAAACCTGGAGACCATTTAGGAGTCTTTCC
TGGAAACCATGAAGATCTAGTGAATGCCTTGATAGAGCGATTAGACGATGCTCCTCCTGTGAACCAAATG
GTGAGGGTGGAGATGCTGGAAGAAAGAAATACCGCTTTAGGTGTGATCAGTAACTGGACAGAGGAGGAAC
GAATTCCACCCTGCACCATTTTCCAAGCCTTTAAGTATTTCCTAGATATTACAACGCCCCCTACCCCTCT
CCTCATGCAGCAAATTGCACTCCTGGCAACTGATGAGAAGGAAAAGAAACGACTGGAGATCCTAAGCAAG
GGTTTGCAGGAATATGAGCAGTGGAAGTGGTACAAGAATCCAACTATTGTTGAGGTTCTAGAGGAGTTCC
CTTCAATTCAGATGCCTTCATCCCTGCTGCTTACACAACTACCCCTGCTACAACCACGATACTACTCCAT
CAGCTCTTCACCTGAGATGTATCCAGAAGAAGTCCACCTTACTGTTGCTGTTGTGTCCTACCGCACAAGA
GATGGAGATGGGCCTATACACCATGGTGTCTGCTCCTCATGGCTCAACAGAATACACCCAGATGAAGTTG
TGCCCTGCTTTGTCAGAGGAGCTCCAAGTTTCCAAATGCCCGAAGACCCACAGGTCCCCTGCATTCTGAT
AGGACCAGGCACTGGTATTGCTCCATTTAGGAGTTTTTGGCAGCAGCGTCTCTACGATATGCAGCACAGA
GGACTGAAACCTTGTCCCATGATCTTGGTCTTTGGGTGTAGGGAATCCAAAATTGACCACATCTACAAAG
AGGAAACAATGCATGCAAAGAACAAAGGAGTGTTTAAAGAACTCTTCACAGCATATTCTCGGGAACCTAA
TAAACCAAAGAAATATGTTCAAGATGTACTGAAGGAACAGTTATCTGAAGTCACATACAAAGCTCTGAAG
GATCAAGGGGGTCACATATATATTTGTGGTGATGTCAACATGGCTGGGGATGTCCTGAAGAGTCTTCAAC
ATGTTGTGAAAGAGAGCGGAAATCTGACGATAGAAGAAGCTGGGGCCTTCATCAGCAAGCTAAGGGATGA
CAATCGGTACCATGAAGATATTTTTGGAGTCACTCTTAGGACATATGAAGTTACAAATCGACTCAGATCT
GAATCTATTGCATTCATTGAGGAAAGCAAAAAGGACTCAGATGAGGTTTTCTGCTCATAA

>fos cds + miR-34a, let-7i
ATGTATCACGCCTTCTCCAGCAACACTGACTACGATGCAGCTTCTTCCCGTTGCAGCAGTGCATCTCCAG
CCGGGGACAGCCTGACCTACTACCCGTCCTCTGCAGCCTCCTTCTCTAGTATGGGGTCTCCTGTTTCACC
ACAGGACTTTGGTGATTCAAGTAGCAGCTTTGTACCCACAGTCACTGCCATTTCCACCTCTGCAGATCTT
CAGTGGCTTGTGCAGCCAGCCCTTATTTCTTCTGTAGCCCCATCACAGTCTCGGGCACACCCATATGCGT
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>foxi1e + miR-155, miR-21
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>tuba4b + miR-155, miR-21
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AAAAAAAAAAA

Cost estimate:

		Two-tailed RT-qPCR	TaqMan	Quanta	miQPCR
Cost per: (USD)	**Assay**	25.85	254.27	11.97	11.97
	RT reagents/rxn	2.44	0.74	4.82	7.25
	qPCR reagents/rxn	0.43	0.91	0.43	0.43
	Assay + 20 RT and 60 qPCR reactions	100.62	323.81	134.21*	182.83**

Cost was estimated based on reaction volumes and reagents used as described in Materials and methods. Prices were taken from vendor/distributor websites as of June 2017, without VAT and special discounts. Prices for kits with comparable size were taken (usually 50 rxn/ RT kit, 500 rxn/PCR mastermix). Assay cost was calculated based on prices for custom oligonucleotides from Integrated DNA Technologies, except for TaqMan, where the sequences are proprietary and assays are sold commercially.

* Cost of another widely used commercial method based on poly(A) tailing - miRCURY LNA™ microRNA qPCR system (Exiqon) is 354.7 USD (assay 152.1 USD, RT reagents/rxn 7.2 USD, qPCR reagents/rxn 1.0 USD)

** miQPCR method requires additional initial purchase of pre-adenylated linker (~ 850 USD)