

Electronic Supplementary Information

Linear-Hairpin Variable Primer RT-qPCR for MicroRNA

Lin Lan¹, Qiuping Guo^{1,3,4}, Hemin Nie¹, Chang Zhou⁵, Qingyun Cai^{2,3}, Jin Huang^{2,3,4,*},
Xiangxian Meng^{1,*}

¹ College of Biology, Hunan University, Changsha, P. R. China

² College of Chemistry and Chemical Engineering, Hunan University, Changsha, P. R. China

³ State Key Laboratory of Chemo/Biosensing and Chemometrics, P. R. China

⁴ Key Laboratory for Bio-Nanotechnology and Molecular Engineering of Hunan Province, P. R. China

⁵ School of Life Sciences, Hunan Normal University, Changsha, P. R. China

* Email: xxmeng@hnu.edu.cn; jinhuang@hnu.edu.cn

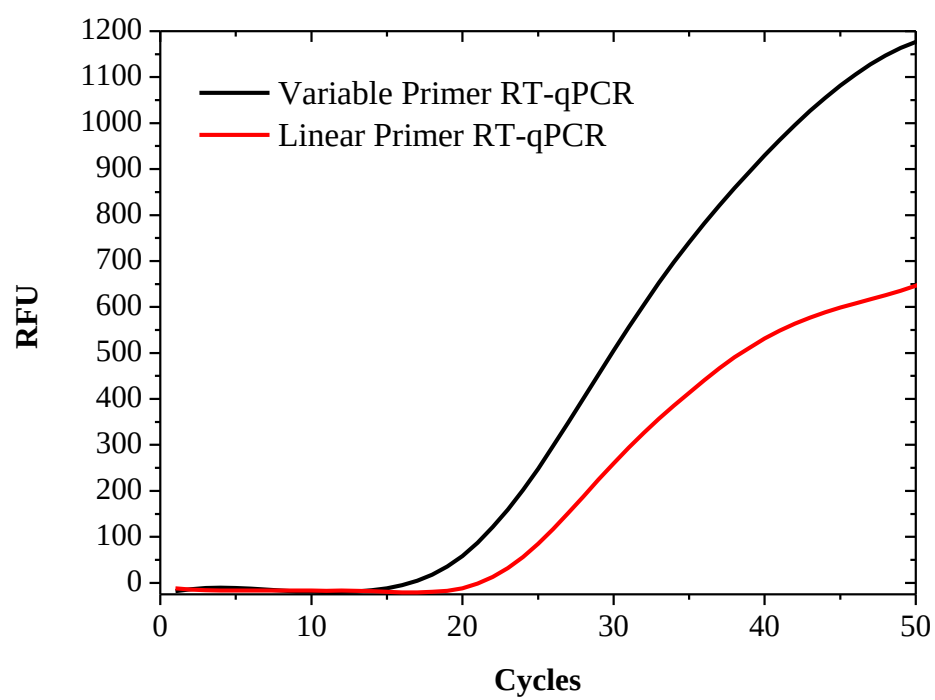


Figure S1. The RT-qPCR amplification curves of the same target let-7d miRNA by using linear-hairpin variable primer with “b=10” and linear primer with “b=0”, respectively.

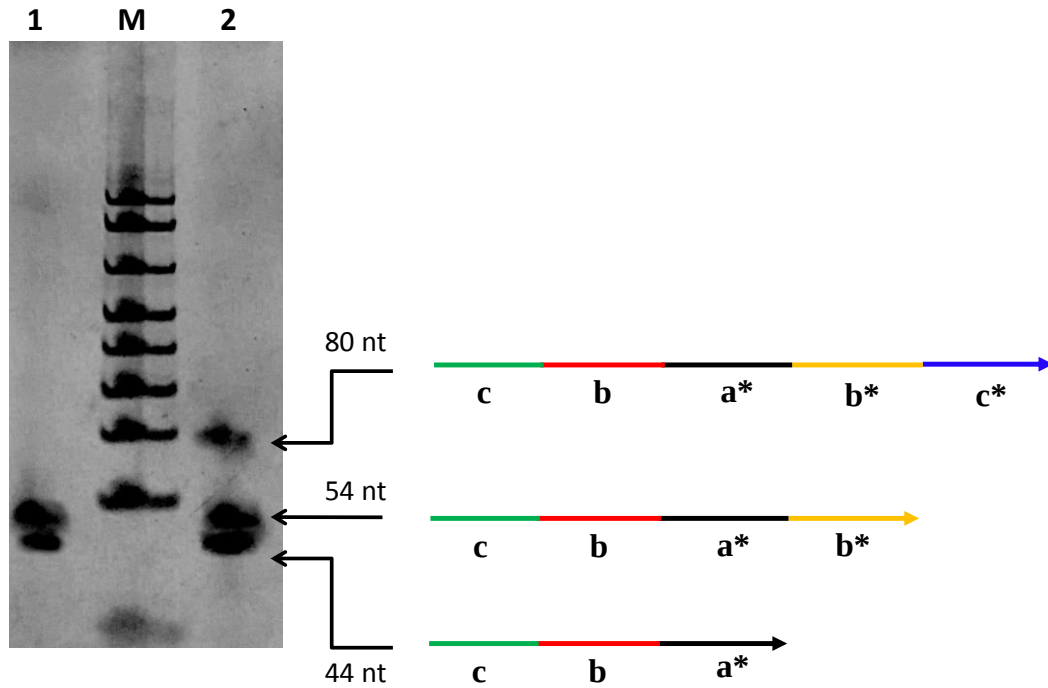


Figure S2. Denaturing PAGE analysis of the RT stage between that using linear primer (lane 1) and variable primer (lane 2), respectively. 'a'='a*' = 8 nt; 'b'='b*' = 10 nt; 'c'='c*' = 26 nt; M is DNA marker. The samples with 50 nM linear primer (lane 1) or variable primer (lane 2) and 1.5 nM let-7d were incubated for 45 min at 42 °C, then inactivated for 5 min at 85 °C.

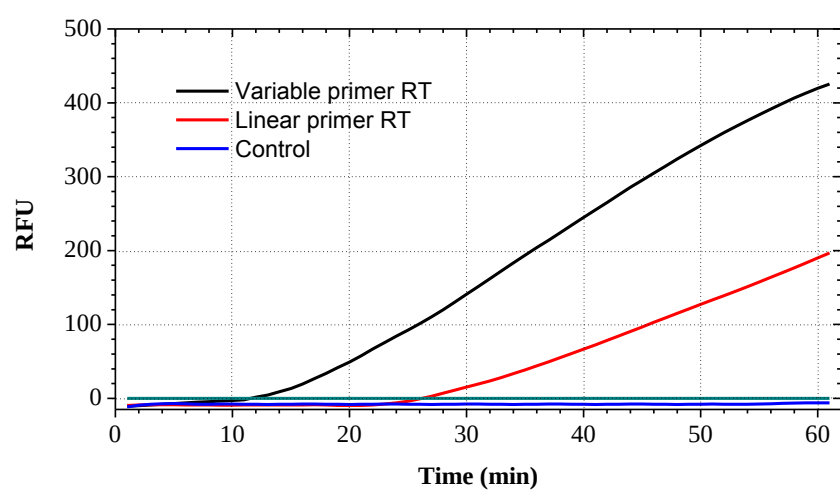


Figure S3. Real-time monitoring the RT process of variable primer and linear primer, respectively. The RT samples with 50 nM variable primer or linear primer and 1.5 nM let-7d were monitoring at 42 °C.

(a) The sequences for optimizing the lengths of 'a*' in the variable primer.

miRNA	miRNA sequence(5'-3')	RT primer(5' - 3')		qPCR primers (5' - 3')
let-7d	AGAGGUAGUAGG UUGCAUAGUU	ACGACATGATGAGTTCTGAAGGCCTT AGAGGTAGT CAACC	fw	ACGACATGATGAGTTCTG
			rv	AGAGGTAGTAGGTTGT
		ACGACATGATGAGTTCTGAAGGCCTT AGAGGTAGT AGCAACC	fw	ACGACATGATGAGTTCTG
			rv	AGAGGTAGTAGGTTGT
		ACGACATGATGAGTTCTGAAGGCCTT AGAGGTAGT ATGCAACC	fw	ACGACATGATGAGTTCTG
			rv	AGAGGTAGTAGGTTGT
		ACGACATGATGAGTTCTGAAGGCCTT AGAGGTAGT AATGCAACC	fw	ACGACATGATGAGTTCTG
			rv	AGAGGTAGTAGGTTGT
		ACGACATGATGAGTTCTGAAGGCCTT AGAGGTAGT ATATGCAACC	fw	ACGACATGATGAGTTCTG
			rv	AGAGGTAGTAGGTTGT

(b) The sequences for optimizing the lengths of 'b' in the variable primer.

miRNA	miRNA sequence(5'-3')	RT primer (5' - 3')		qPCR primers(5' - 3')
let-7d	AGAGGUAGUAGG UUGCAUAGUU	ACGACATGATGAGTTCTGAAGGCCTT AGAGGTA ATGCAACC	Fw	ACGACATGATGAGTTCTG
			Rv	AGAGGTAGTAGGTTGC
		ACGACATGATGAGTTCTGAAGGCCTT AGAGGTAGT ATGCAACC	Fw	ACGACATGATGAGTTCTG
			Rv	AGAGGTAGTAGGTTGC
		ACGACATGATGAGTTCTGAAGGCCTT AGAGGTAGT ATGCAACC	Fw	ACGACATGATGAGTTCTG
			Rv	AGAGGTAGTAGGTTGC
		ACGACATGATGAGTTCTGAAGGCCTT AGAGGTAGT AATGCAACC	Fw	ACGACATGATGAGTTCTG
			Rv	AGAGGTAGTAGGTTGC
		ACGACATGATGAGTTCTGAAGGCCTT AGAGGTAGT AGAACTATGC	Fw	ACGACATGATGAGTTCTG
			Rv	AGAGGTAGTAGCATAG

Table S1. (a) The sequences for optimizing the lengths of 'a*' in the variable primer. (b) The sequences for optimizing the lengths of 'b' in the variable primer.

miRNA	miRNA sequence(5'-3')	RT primer (5' - 3')		qPCR primers (5' - 3')
let-7d	AGAGGUAGUAGG UUGCAUAGUU	ACGACATGATGAGTTCTGAAGGCCTTAGAGGTAGTAATGCAACC	fw	ACGACATGATGAGTTCTG
		Variable primer	rv	AGAGGTAGTAGGTTGC
		ACGACATGATGAGTTCTGAAGGCCTGAATGAAACAATGCAACC	fw	ACGACATGATGAGTTCTG
		Linear primer	rv	AGAGGTAGTAGGTTGC

Table S2. The sequences for RT-qPCR based on variable primer and linear primer, respectively.

miRNA	miRNA sequence (5' - 3')	RT primer (5' - 3')	qPCR primers(5' - 3')	
miR-122-5p	UGGAGUGUGACAAUGGUGUUUG	TGGAGTGTGACTATCTTCTAGTCGTTTGGAGTGTGATCACCATTG	fw	TGGAGTGTGACTATCTTC
			rv	TGGAGTGTGACAATGG
miR-30c-1-3p	CUGGGAGAGGGUUGUUUACUCC	CTGGGAGAGGTACTATGATAGTAACTGGGAGAGGTTTAAACAAC	fw	CTGGGAGAGGTACTATGA
			rv	CTGGGAGAGGGTTGTT
miR-21a-5p	UAGCUUAUCAGACUGAUGUUGA	TAGCTTATCACAGTATGCTAGTCGTTTAGCTTATCATCATCAGTC	fw	TAGCTTATCACAGTATGC
			rv	TAGCTTATCAGACTGATG
miR-24-3p	UGGCUCAGUUCAGCAGGAACAG	TGGCTCAGTTGACATTCTAGTCTTGGCTCAGTTCATCCTGCTG	fw	TGGCTCAGTTGACATTC
			rv	TGGCTCAGTTCAGCAG
miR-let-7a	UGAGGUAGUAGGUUGUAUAGUU	TGAGGTAGTAGAGATTGCTAGTCGTTTGAGGTAGTAATACAACC	fw	TGAGGTAGTAGAGATTGC
			rv	TGAGGTAGTAGGTTGTAT
miR-1a-3p	UGGAAUGUAAAGAAGUAUGUAU	TGGAATGTAAACAGTATGCTAGTCGTTTGGAATGTAAATACTTCT	fw	TGGAATGTAA CAGTATGC
			rv	TGGAATGTAAAGAAGT
miR-199a	ACAGUAGUCUGCACAUUGGUUA	ACAGTAGTCTGAGTAACTAGTGATACAGTAGTCTCAATGTGC	fw	ACAGTAGTCTGAGTAAAC
			rv	ACAGTAGTCTGCACAT
miR-196a	UAGGUAGUUUCAUGUUGUUGGG	TAGGTAGTTTCAGTATCGTAGTCCATTAGGTAGTTTACAACATG	fw	TAGGTAGTTTCAGTATCG
			rv	TAGGTAGTTTCATGTTG

Table S3. Sequences used for miRNAs profiling in mouse tissue by the linear-hairpin variable primer RT-qPCR assay.

miRNA	miRNA sequence (5' - 3')	RT primer (5' - 3')	TaqMan probe		qPCR primers(5' - 3')
miR-122-5p	UGGAGUGUGAC AAUGGUGUUUG	CCTCAAACGAGTGCAGGGTCC GAGGTATTCGCACTCGTTTGA GGTAGTTCAAACAC	(6-AFM)CGTTTGAGGTAGTTCAAACACCA(BHQ1)	fw	CTGGAGTGTGACAATGGTG
				rv	GTGCAGGGTCCGAGGT
miR-30c-1-3p	CUGGAGAGAGG UUGUUUACUCC	CCTCAAACGAGTGCAGGGTCC GAGGTATTCGCACTCGTTTGA GGTAGTCGGAGTA	(6-AFM)GTTTGAGGTAGTCGGAGTAAAC(BHQ1)	fw	CCTGGGAGAGGGTTGTTT
				rv	GTGCAGGGTCCGAGGT
miR-21a-5p	UAGCUUAUCAG ACUGAUGUUGA	CCTCAAACGAGTGCAGGGTCC GAGGTATTCGCACTCGTTTGA GGTAGTTCACAT	(6-AFM)TCGTTTGAGGTAGTTCAACATC(BHQ1)	fw	CGACTAGCTTATCAGACTGATG
				rv	GTGCAGGGTCCGAGGT
miR-24-3p	UGGCUCAGUUC AGCAGGAACAG	CCTCAAACGAGTGCAGGGTCC GAGGTATTCGCACTCGTTTGA GGTCGTCCTGTTCC	(6-AFM)GTTTGAGGTGCTGTTCTCTGC(BHQ1)	fw	TGGCTCAGTTCAGCAGGA
				rv	GTGCAGGGTCCGAGGT
miR-1et-7a	UGAGGUAGUAG GUUGUAUAGUU	CCTCAAACGAGTGCAGGGTCC GAGGTATTCGCACTCGTTTGA GGTAGTAATACAACC	(6-AFM)TCGTTTGAGGTAGTAATACAACC(BHQ1)	fw	GCCGCTGAGGTAGTAGGTTGTA
				rv	GTGCAGGGTCCGAGGT
miR-1a-3p	UGGAAUGUAAA GAAGUAUGUAU	CCTCAAACGAGTGCAGGGTCC GAGGTATTCGCACTCGTTTGA GGTGGTATACATAC	(6-AFM)TCGTTTGAGGTGGTATACATAC(BHQ1)	fw	GGCGTGGAATGTAAAGAAGTAT
				rv	GTGCAGGGTCCGAGGT
miR-199a	ACAGUAGUCUG CACAUUGGUUA	CCTCAAACGAGTGCAGGGTCC GAGGTATTCGCACTCGTTTGA GGTCGTAAACCAA	(6-AFM)CTCGTTTGAGGTCGTAAACCAAT(BHQ1)	fw	GGACAGTAGTCTGCACATTGG
				rv	GTGCAGGGTCCGAGGT
miR-196a	UAGGUAGUUUC AUGUUUUGGG	CCTCAAACGAGTGCAGGGTCC GAGGTATTCGCACTCGTTTGA GGTCGTACCCAACA	(6-AFM)CTCGTTTGAGGTCGTACCCAACAAC(BHQ1)	fw	GCGTTAGGTAGTTTCATGTTGT
				rv	GTGCAGGGTCCGAGGT

Table S4. Sequences used for miRNAs profiling in mouse tissue by the hairpin RT-qPCR assay with EvaGreen assay and TaqMan assay.

(a) The Cq values by the linear-hairpin variable primer RT-qPCR assay.

Sample	1a-3p	30c-1-3p	21a-5p	let-7a	24-3p	122-5p	196a	199a
heart	23.09	23.29	25.17	21.75	20.12	21.75	28.07	28.06
brain	33.41	28.21	26.34	25.21	20.15	27.15	28.04	19.67
kidney	22.04	25.03	21.57	25.56	33.03	33.54	27.85	27.29
lung	27.53	25.21	22.36	22.34	21.07	32.44	25.84	28.32
muscle	19.02	28.02	25.37	24.43	29.14	25.73	27.43	29.15
liver	25.05	27.02	19.54	18.26	25.12	18.26	20.70	26.77
cereb	31.08	27.54	25.44	19.91	21.57	33.58	26.12	24.59
Negative control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

(b) Relative expression by Linear-hairpinvariable primer RT-qPCR.

Sample	1a-3p	30c-1-3p	21a-5p	let-7a	24-3p	122-5p	196a	199a
heart	10.32	4.92	1.17	3.81	12.91	11.83	0	1.09
brain	0	0	0	0.35	12.88	6.43	0.03	9.48
kidney	11.37	3.18	4.77	0	0	0.04	0.22	1.86
lung	5.88	3.00	3.98	3.22	11.96	1.14	2.23	0.83
muscle	14.39	0.19	0.97	1.13	3.89	7.85	0.64	0
liver	8.36	1.19	6.8	7.3	7.91	15.32	7.34	2.38
cereb	2.33	0.67	0.9	5.65	11.46	0	1.92	4.56

Table S5. The Cq Values (a) and relative expression (b) for miRNAs profiling in mouse tissue by using linear-hairpin variable primer RT-qPCR. 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.^[1]

(a) The Cq values by the TaqMan RT-qPCR assay.

Sample	1a-3p	30c-1-3p	21a-5p	let-7a	24-3p	122-5p	196a	199a
heart	24.12	25.31	26.32	22.87	21.02	22.13	30.02	28.54
brain	35.59	30.37	27.47	26.36	20.98	27.01	29.95	20.53
kidney	22.87	28.21	23.26	27.21	34.12	32.59	29.53	28.57
lung	28.02	26.53	24.38	23.56	22.04	33.62	27.72	29.57
muscle	19.53	29.27	26.21	25.31	29.77	26.19	28.21	30.24
liver	25.09	n.d.	18.59	18.96	26.54	18.41	22.53	27.73
cereb	33.45	28.27	26.57	21.13	22.56	34.15	27.02	25.61
Negative control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

(b) Relative expression by TaqMan RT-qPCR.

Sample	1a-3p	30c-1-3p	21a-5p	let-7a	24-3p	122-5p	196a	199a
heart	11.47	5.06	1.15	4.34	13.10	12.02	0	1.70
brain	0	0	0	0.85	13.14	7.14	0.07	9.71
kidney	12.72	2.16	4.21	0	0	1.56	0.49	1.67
lung	7.57	3.84	3.09	3.65	12.08	1.53	2.30	0.67
muscle	16.06	1.10	1.26	1.90	4.35	7.96	1.81	0
liver	10.5	n.d.	8.88	8.25	7.58	15.74	7.49	2.51
cereb	2.37	2.10	0.90	6.09	11.56	0	3.00	4.63

Table S6. The Cq Values (a) and relative expression (b) for miRNAs profiling in mouse tissue by using Taqman RT-qPCR.

(a) The Cq values measured by singleplex.

Sample	1a-3p	30c-1-3p	21a-5p	let-7a	24-3p	122-5p	196a	199a
heart	23.09	23.29	25.17	21.75	20.12	21.75	28.07	28.06
brain	33.41	28.21	26.34	25.21	20.15	27.15	28.04	19.67
kidney	22.04	25.03	21.57	25.56	33.03	33.54	27.85	27.29
lung	27.53	25.21	22.36	22.34	21.07	32.44	25.84	28.32
muscle	19.02	28.02	25.37	24.43	29.14	25.73	27.43	29.15
liver	25.05	27.02	19.54	18.26	25.12	18.26	20.70	26.77
cereb	31.08	27.54	25.44	19.91	21.57	33.58	26.12	24.59
Negative control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

(b) The Cq values measured by mutiplex.

Sample	1a-3p	30c-1-3p	21a-5p	let-7a	24-3p	122-5p	196a	199a
heart	24.13	24.31	25.30	22.02	20.23	22.54	29.04	28.53
brain	34.37	28.35	27.37	26.34	21.57	26.89	27.94	19.72
kidney	22.89	24.98	22.83	26.65	33.53	33.52	28.13	27.32
lung	29.02	25.24	24.01	23.21	21.14	32.21	26.03	28.53
muscle	21.01	28.01	26.24	25.59	28.57	25.72	26.32	29.53
liver	25.99	26.79	20.67	19.02	24.88	18.21	21.84	25.96
cereb	32.21	27.59	26.53	20.21	21.60	33.59	25.89	25.02
Negative control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table S7. The Cq Values measured for miRNAs profiling in mouse tissue using two different methods: singleplex and multiplex. 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.^[1]

[1] P. Androvic, L. Valihrach, J. Elling, R. Sjoback, M. Kubista, Nucleic Acids Res. 2017, 45, e144.