Supplementary Information

Polymerization and isomerization cyclic amplification for nucleic acid detection with attomolar sensitivity

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A B C

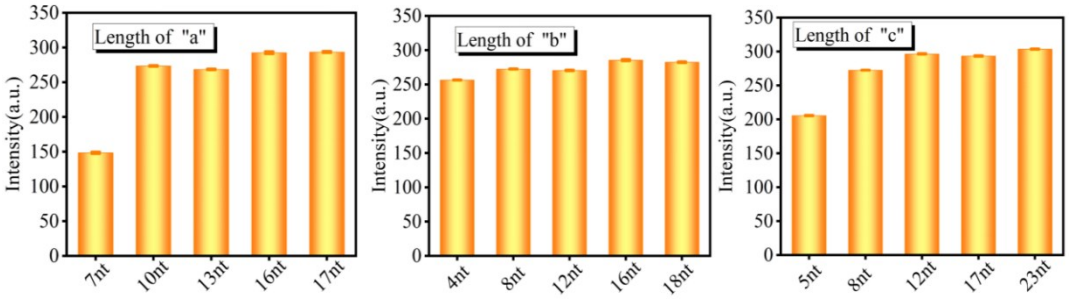


Fig S1：optimization of lengths of domain a, b, c of the hairpin probe. (A) Optimization of domain a (7nt、10nt、13nt、16nt、17nt）of the hairpin probe. The base number is 7nt, the fluorescence signal is relatively weak, but at 8-17 bases, the fluorescence signal is at a relatively strong level. (B) Optimization of domain b (4nt、8nt、12nt、16nt、18nt）of the hairpin probe. It could be seen that the fluorescence intensity of b was almost the same from 4-18 nt, and even when B is o, similar results were obtained (the results are not shown). (C) Optimization of domain c (5nt、8nt、12nt、17nt、23nt）of the hairpin probe. It could be seen that the all of lengths from 10nt to 23nt had similar and strong signals. Domain a (16nt), domain b (4nt) and domain c (17nt) were chosen as the length of the hairpin probe in the following PICA mechanism experiments except for special indication.

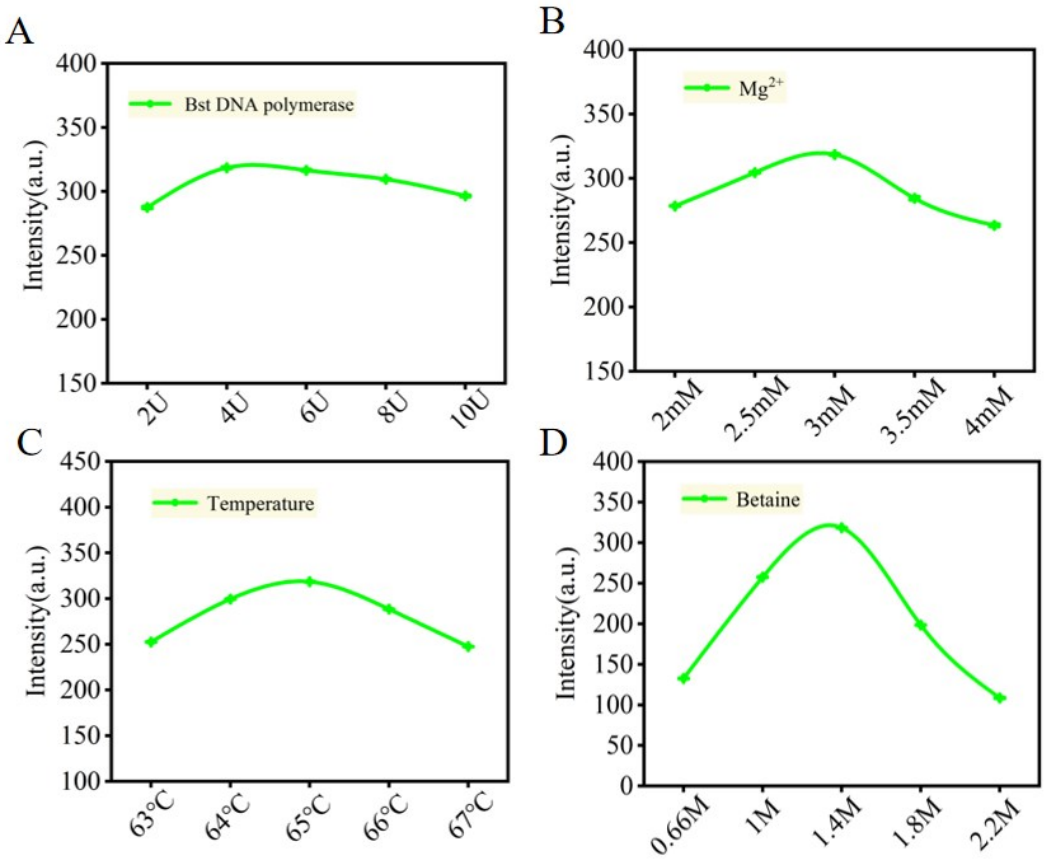


Fig S2. Condition optimization of the PICA reaction. (A) Optimization of Bst DNA polymerase, (B) Optimization of Mg^{2+}, (C) Optimization of temperature and (D) Optimization of betaine. 4U Bst DNA polymerase, 3mM Mg^{2+}, 65 ^{o}C and 1.4M betaine were chosen in the PICA reaction.

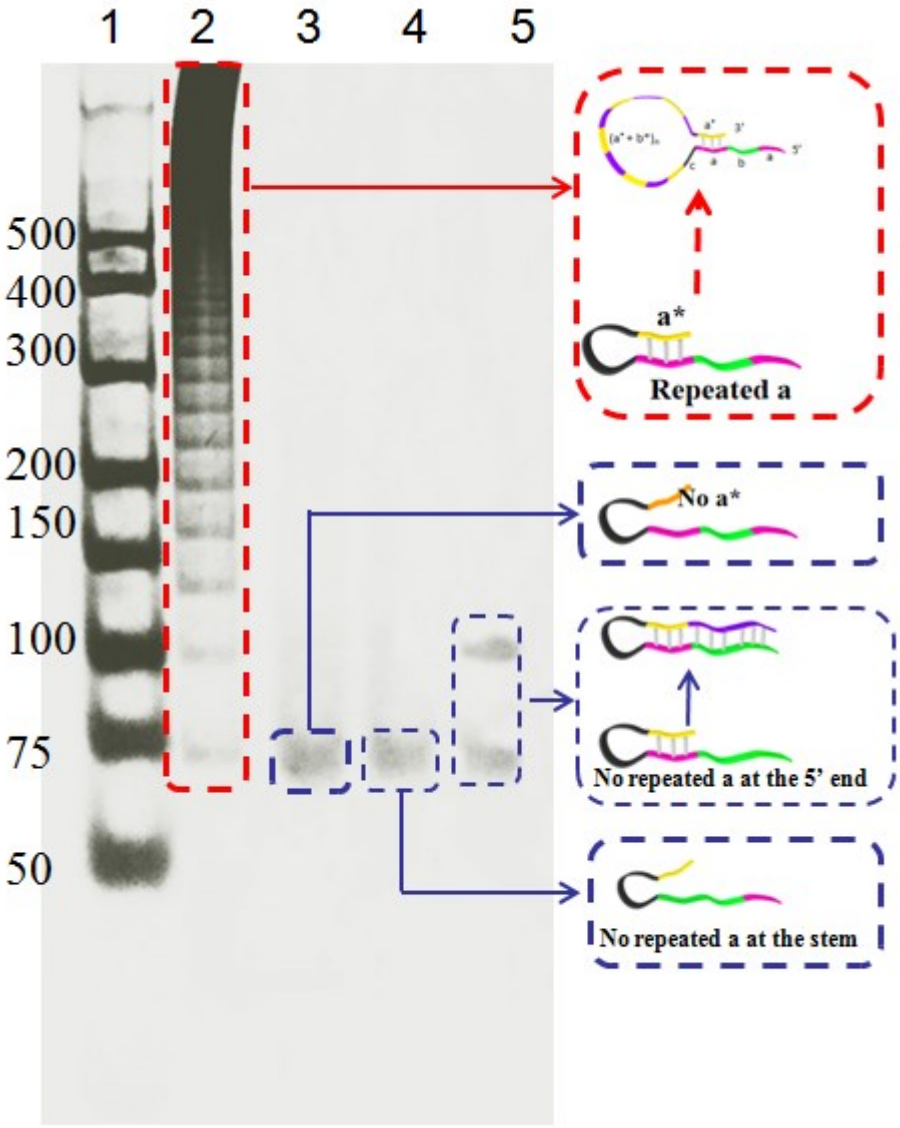
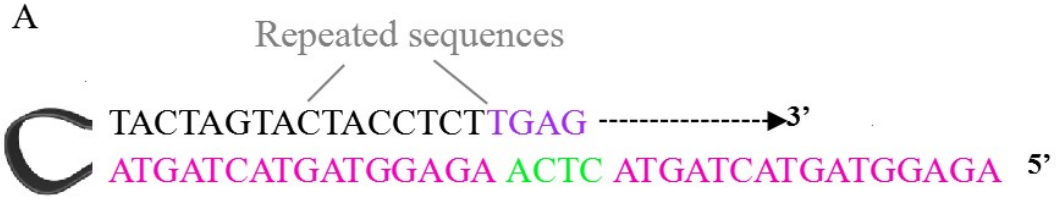


Figure S3. Necessity analysis of the repeated a and a* in PICA. From the 8% PAGE gel analysis, it was known that only when the hairpin probe had a* at the 3' end and repeated a at the 5' end simultaneously, the PICA reaction could happen. (lane 1: DNA marker; lane 2: PICA product from the hairpin probe with a* at 3' the end; lane 3: PICA product from the hairpin probe without a* at the 3' end; lane 4: PICA product from the hairpin probe without repeated a at the stem; lane 5: PICA product from the hairpin probe without repeated a at the 5' end).

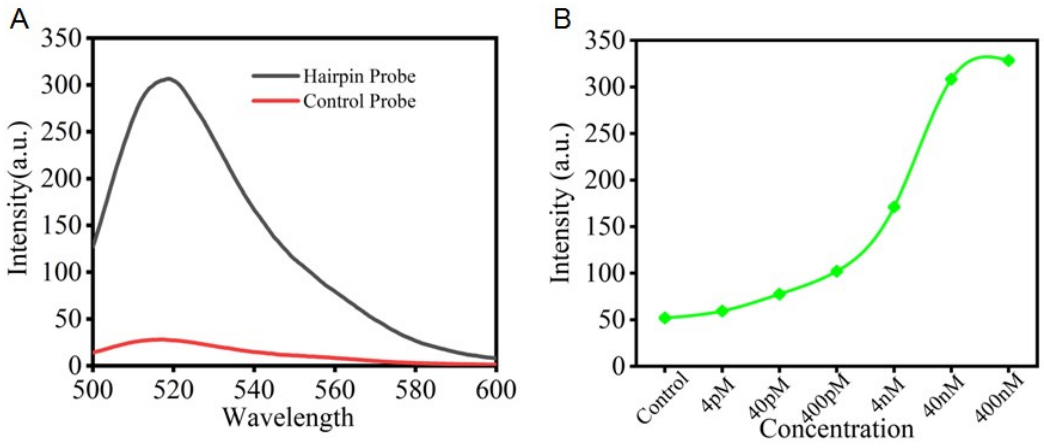
A



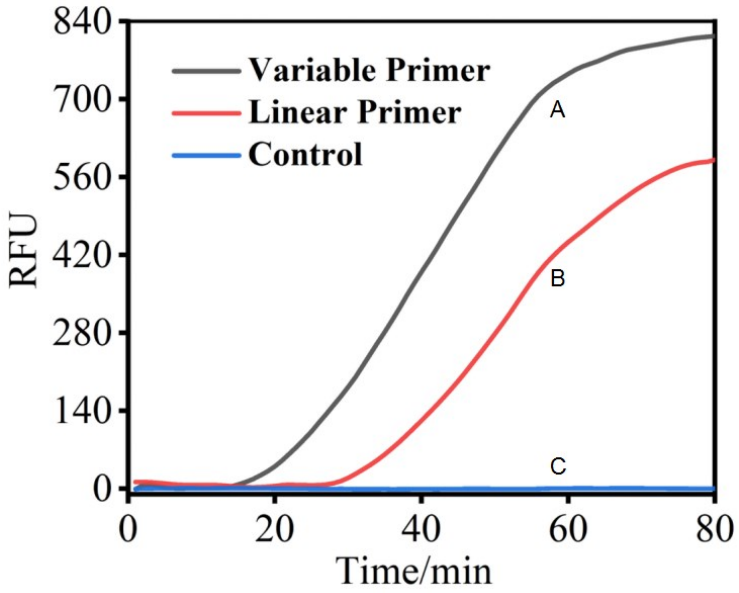
B

Nucleic acids		Sequences (5'-3')
20-bp tandem repeats with variable numbers	227bp	TACTAGTACTACCTCTTGAGTACTACTA CTA**GGA**TCTTGCGTACTAGTAC**GC**CCT CTTGAG**AC**CTAGT**T**C**A**A**A**T**GT**GCTG AGTACTAG**AG**CTACC**GG**TCTTTGCGTA CTT**G**C**A**A**T**C**A**TCTTGGAGTACTAGTA CTACCTCTTCGGCTACTAGTA**GT**ACCT **GA**TAAGTACTAGT**TG**TACTCTAGAGTT GAGTACTAGTACTACTCTA**T**GGGACTA GTA**G**TACCC

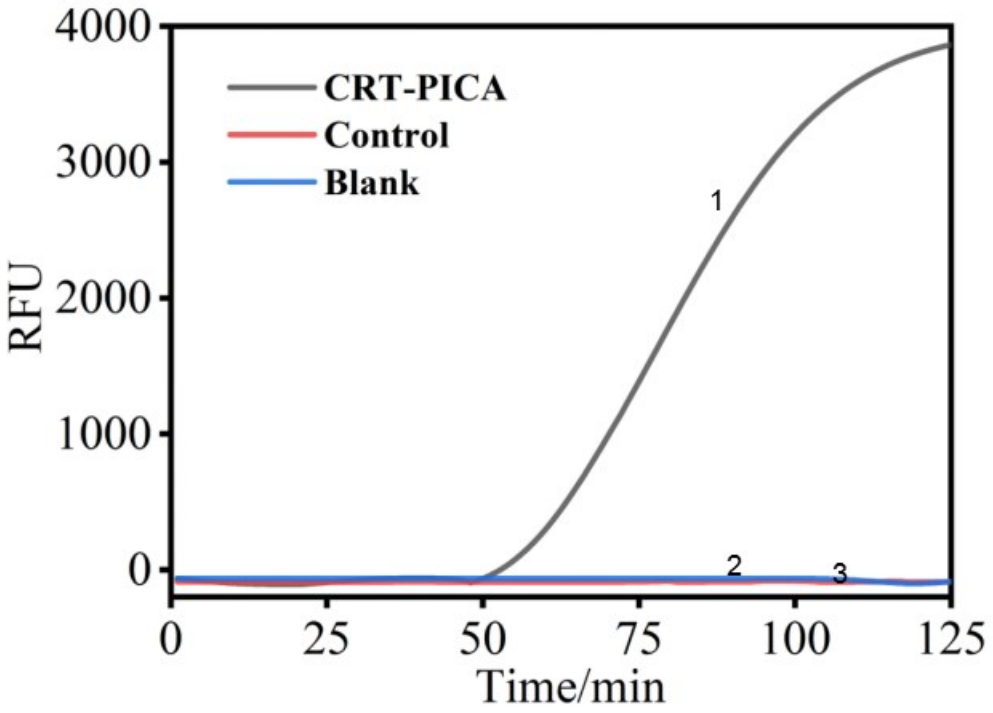
Fig S4. the PICA products were subcloned into a TA cloning vector, and sequenced. (A) The predicted repeated sequence of the hairpin probe by PICA. The hairpin probe with domain a, b and c. The red part represents domain a, the green part represents domain b, the black part represents domain c, and the orange part represents domain a*. The hairpin probes are formed by ligation corresponding fragments (a+b+a and c+a*). (B) The sequencing data of 20-bp amplification products. The dotted line represented the insertion of unexpected bases. the bold indicates the mutated base.



FigS5. Fluorescence analysis of PICA. (A) Fluorescence signal of PICA products between the hairpin probe and control probe. Here, hairpin Probe without a*(Table S2) was chosen the control probe. (B) Relationship between fluorescent intensities and the concentrations of hairpin probes. It could be seen that the sensitivity of the assay could be as low as 4pM.



FigS6. Fluorescence analysis of CRT. Real-time monitoring of the fluorescence of cyclic reverse transcription with curve A (variable primer + let-7d +reverse transcriptase), with curve B (linear primer + let-7d +reverse transcriptase), with curve C (variable primer + H_2O +reverse transcriptase).



FigS7.Fluorescence analysis of CRT-PICA. curve 1: variable primer + let-7d + two enzymes (reverse transcriptase + Bst polymerase); curve 2: variable primer + miR122 + two enzymes; curve 3: variable primer + H_2O +two enzymes.

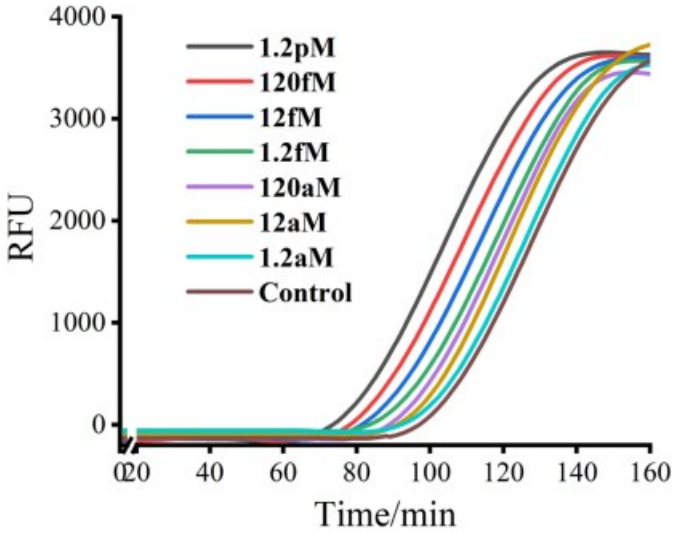
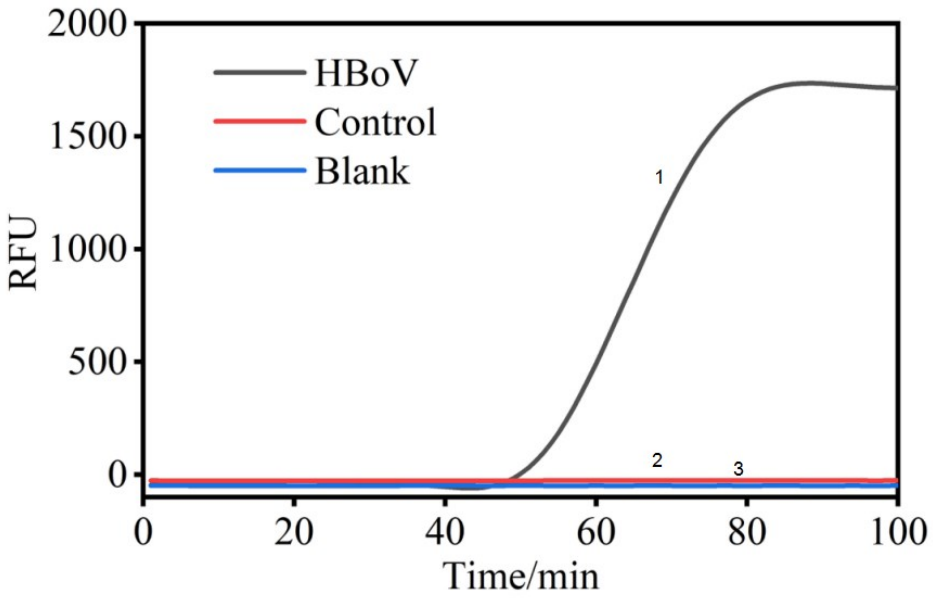
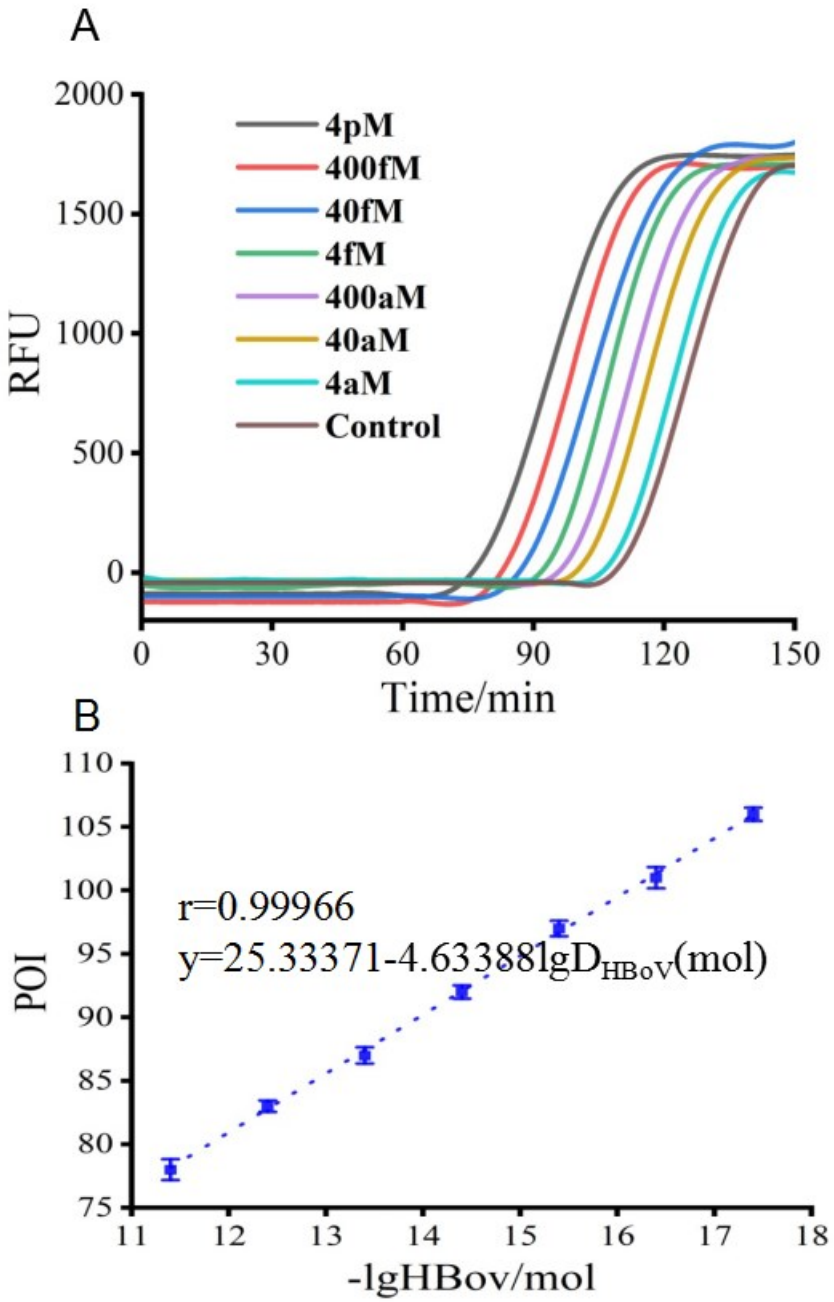


Fig S8. Fluorescence analysis of CRT-PICA. the target miRNA let7d in the range from 1.2 aM to 1.2 pM by real-time measurement of the fluorescence intensity.



FigS9. Real-time monitoring of the fluorescence of CR-PICA from HBoV. (A) curve 1: variable primer + HBoV + Bst polymerase; curve 2: variable primer + Cpn+ Bst polymerase; curve 3: variable primer + H_2O+ Bst polymerase.



FigS10. Fluorescence analysis of CR-PICA. (A) The real-time fluorescence curve for the CR-PICA detection platform triggered by HBoV. (B) Relationship between the POI value and the logarithm of the amount of HBoV. the HBoV could be detected quantitatively in the range from 4aM to 4pM by real-time measurement of the fluorescence intensity of the CR-PICA products with the help of SYBR Green II.

	Length	Sequence(5'-3')
Length of " a "	7nt	AGAGGTACTCATTTCCTTTTCTCTATTTAAGAGGTAAGTTGCCTTCAGAACTCTACCTCT
	10nt	AGAGGTAGTACTCATTTCCTTTTCTCAGAGGTAGTAAGTTGCCTTCAGAACTCTACTACCTCT
	13nt	AGAGGTAGTACTACTCATTTCCTAGAGGTAGTACTAAGTTGCCTTCAGAACTCTAGTACTACCTCT
	16nt	AGAGGTAGTACTAGTACTCAAGAGGTAGTACTAGTAAGTTGCCTTCAGAACTCTACTAGTACTACCTCT
	17nt	AGAGGTAGTACTAGTATCTAGAGGTAGTACTAGTATAGTTGCCTTCAGAACTCATACTAGTACTACCTCT
Length of " b "	4nt	AGAGGTAGTACTAGTACTCAAGAGGTAGTACTAGTAAGTTGCCTTCAGAACTCTACTAGTACTACCTCT
	8nt	AGAGGTAGTACTAGCTCATTTCAGAGGTAGTACTAGAGTTGCCTTCAGAACTCCTAGTACTACCTCT
	12nt	AGAGGTAGTACTCTCATTTCCTTTAGAGGTAGTACTAGTTGCCTTCAGAACTCAGTACTACCTCT
	16nt	AGAGGTAGTACTCATTTCCTTTTCTCAGAGGTAGTAAGTTGCCTTCAGAACTCTACTACCTCT
	18nt	AGAGGTAGTCTCATTTCCTTTTCTCTAAGAGGTAGTAGTTGCCTTCAGAACTCACTACCTCT
Length of " c "	5nt	AGAGGTAGTACTAGTACTCAAGAGGTAGTACTAGTAAGTTGTACTAGTACTACCTCT
	8nt	AGAGGTAGTACTAGTACTCAAGAGGTAGTACTAGTAAGTTGCCTTACTAGTACTACCTCT
	12nt	AGAGGTAGTACTAGTACTCAAGAGGTAGTACTAGTAAGTTGCCTTCAGTACTAGTACTACCTCT
	17nt	AGAGGTAGTACTAGTACTCAAGAGGTAGTACTAGTAAGTTGCCTTCAGAACTCTACTAGTACTACCTCT
	23nt	AGAGGTAGTACTAGTACTCAAGAGGTAGTACTAGTAAGTTGCCTTCAGAACTCTACTACTACTAGTACTACCTCT

TableS1. The hairpin probes with different length of domain a, b and c. The red part represents domain a, the green part represents domain b, the black part represents domain c, and the orange part represents domain a*. The hairpin probes are formed by ligation corresponding fragments (a+b+a and c+a*).

Species	Sequence (5'-3')
Hairpin Probe	AGAGGTAGTACTAGTACTCAAGAGGTAGTACTAGTAAGTTGCCTTCAGAACTCTACTAGT ACTACCTCT
Hairpin Probe without a*	AGAGGTAGTACTAGTACTCAAGAGGTAGTACTAGTAAGTTGCCTTCAGAACTCTTACCGA ATACTCTAA
Hairpin Probe without repeat a at the stem	AGAGGTAGTACTAGTACTCAAGAGGTAGTACTAGTAAGTTGCCTTCAGAACTCTACTAGT ACTACCTCT
Hairpin Probe without repeat a at the 5' end	AGAGGTAGTACTAGTACTCAAGAGGTAGTACTAGTAAGTTGCCTTCAGAACTCTACTAGT ACTACCTCT

TableS2. The sequence of the hairpin probe was equipped with a* and repeated a for the PICA reaction. The red part represents domain a of the hairpin probe, the green part represents domain b, the black part represents domain c, and the orange part represents domain a*. The blue part is different from the hairpin probe. The hairpin probes are formed by ligation corresponding fragments (a+b+a and c+a*).

Species	Sequence (5'-3')
Hairpin	AGAGGTAGTACT GAGTTCTGAAGGCCTT AGAGGTAGTACT AGTTGCCTTCAGAACTC
Probe	AGTACTACCTCT
Primer	GTAGTACTGAGTTCTGAAG

TableS3. The sequences of hairpin probe with a restriction site (underlined base) in the b domain and the corresponding primer. The red part represents domain a of the hairpin probe, the green part represents domain b, the black part represents domain c, and the orange part represents domain a*. The hairpin probe is formed by ligation corresponding fragment (a+b+a and c+a*).

Species	Sequence (5’-3’)
P1-Primer	AGTAAGTTGCCTTCAGAACTCTAC
P2-Primer	GGGGGAGAGGTAGTACTAGTACTCA

TableS4. The sequences of primers used in the sequencing for PICA products.

Species	Sequence (5'-3')
Variable Primer	AGAGGTAGTAGAGTTCTGAAGGCCTTAGAGGTAGTAATGCAACC
Linear Primer	AGAGGTAGTAGAGTTCTGAAGGCCTTGAATGAAACAATGCAACC
Let7d	AGAGGUAGUAGGUUGCAUAGUU
miR122	UGGAGUGUGACAAUGGUGUUUG

TableS5. The sequences of the Variable Primer and Linear Primer and their related targets in the CRT-PICA reaction system for miRNA detection.

		Sequence (5'-3')
Length of " a "	**7nt**	AGAGGTAGAGTTCTGAAGGCCTTAGAGGTAATGCAACC
	8nt	AGAGGTAGGAGTTCTGAAGGCCTTAGAGGTAGATGCAACC
	9nt	AGAGGTAGTGAGTTCTGAAGGCCTTAGAGGTAGTATGCAACC
	10nt	AGAGGTAGTAGAGTTCTGAAGGCCTTAGAGGTAGTAATGCAACC
	11nt	AGAGGTAGTATGAGTTCTGAAGGCCTTAGAGGTAGTATATGCAACC
Length of " c "	**5nt**	AGAGGTAGTAGAGTTCTGAAGGCCTTAGAGGTAGTACAACC
	6nt	AGAGGTAGTAGAGTTCTGAAGGCCTTAGAGGTAGTAGCAACC
	7nt	AGAGGTAGTAGAGTTCTGAAGGCCTTAGAGGTAGTATGCAACC
	8nt	AGAGGTAGTAGAGTTCTGAAGGCCTTAGAGGTAGTAATGCAACC
	9nt	AGAGGTAGTAGAGTTCTGAAGGCCTTAGAGGTAGTAAATGCAACC

TableS6. The sequences of the "a" and "c" length sequences in the variable primer for let 7d. The different length of "a" (7,8,9,10,11nt) and The different length of "c" (5,6,7,8,9nt) were tested. The red bases represented "a". The green bases represented "b". The black bases represented "c".

Length of “a”	POI
7	78
8	73.6
9	70.5
10	68.8
11	68.9

Length of “c”	POI
5	80
6	76
7	71
8	68.8
9	69

Table S7. Optimization of the "a" and "c" length sequences in the variable primer of CRT-PICA for miRNA Let 7d. From the corresponding POI value, the 10 nt length of "a" and the 8 nt length of "c" were chosen in the following experiments, respectively.

Variable Primer (5’-3’)	target	Sequence (5’-3’)
TGAGGTAGTAGAGATTGCTAGTCGTTTGAGGTA GTAATACAACC	let7a	UGAGGUAGUAGGUUGUAUAGUU
	let7e	UGAGGUAGGAGGUUGUAUAGUU
	let7f	UGAGGUAGUAGAUUGUAUAGUU

TableS8. Sequences of the variable primer of let7a and targets of let7a, let7e and let7f. red base indicate mismatched bases to let7a.

miRNA	miRNA sequence (5'-3')	Variable Primer (5'-3')
miR-122-5p	UGGAGUGUGACAAUGGUGUUUG	TGGAGTGTGACTATCTTCTAGTCGTTTGGAGTGTGATCACCATTG
miR-30c-1-3p	CUGGGAGAGGGUUGUUUACUCC	CTGGGAGAGGTACTATGATAGTAACTGGGAGAGGTTTAAACAAC
miR-21a-5p	UAGCUUAUCAGACUGAUGUUGA	TAGCTTATCACAGTATGCTAGTCGTTTAGCTTATCATCATCAGTC
let-7a	UGAGGUAGUAGGUUGUAUAGUU	TGAGGTAGTAGAGATTGCTAGTCGTTTGAGGTAGTAATACAACC
miR-1a-3p	UGGAAUGUAAAGAAGUAUGUAU	TGGAATGTAACAGTATGCTAGTCGTTTGGAATGTAAATACTTCT
miR-199a	ACAGUAGUCUGCACAUUGGUUA	ACAGTAGTCTGAGTAAACTAGTGTATACAGTAGTCTCAATGTGC
miR-196a	UAGGUAGUUUCAUGUUGUUGGG	TAGGTAGTTTCAGTATCGTAGTCCATTAGGTAGTTTACAACATG

TableS9. Sequences of 7 miRNAs and related variable primers for CRT-PICA in mouse tissues.

miRNA	miRNA sequence(5'-3')	RT primers(5'-3')	Taqman Probe(5'-3')		Primers (5'-3')
miR- 122- 5p	UGGAGUGUGACAAUGGUGUUUG	CCTCAAACGAGTGCAGGGTCCGAGGTATTCGCACTCGTTTGAGGTAGTTCAAACAC	(6-FAM)CGTTTGAGGTAGTTCAAACACCA(BHQ1)	fw	CTGGAGTGTGACAATGGTG
				rv	GTGCAGGGTCCGAGGT
miR- 30c- 1- 3p	CUGGGAGAGGGUUGUUUACUCC	CCTCAAACGAGTGCAGGGTCCGAGGTATTCGCACTCGTTTGAGGTAGTCGGAGTA	(6-FAM)GTTTGAGGTAGTCGGAGTAAAC(BHQ1)	fw	CCTGGGAGAGGGTTGTTT
				rv	GTGCAGGGTCCGAGGT
miR-24-3p	UGGCUCAGUUCAGCAGGAACAG	CCTCAAACGAGTGCAGGGTCCGAGGTATTCGCACTCGTTTGAGGTCGTCTGTTGC	(6-FAM)GTTTGAGGTCGTCTGTTCCTGC(BHQ1)	fw	TGGCTCAGTTCAGCAGGA
				rv	GTGCAGGGTCCGAGGT
let-7a	UGAGGUAGUAGGUUGUAUAGUU	CCTCAAACGAGTGCAGGGTCCGAGGTATTCGCACTCGTTTGAGGTAGTAATACAACC	(6-FAM)TCGTTTGAGGTAGTAATACAACC(BHQ1)	fw	GCCGCTGAGGTAGTAGGTTGTA
				rv	GTGCAGGGTCCGAGGT
miR-1a-3p	UGGAAUGUAAAGAAGUAUGUAU	CCTCAAACGAGTGCAGGGTCCGAGGTATTCGCACTCGTTTGAGGTGGTATACATAC	(6-FAM)TCGTTTGAGGTGGTATACATAC(BHQ1)	fw	GGCGTGGAATGTAAAGAAGTAT
				rv	GTGCAGGGTCCGAGGT
miR-199a	ACAGUAGUCUGCACAUUGGUUA	CCTCAAACGAGTGCAGGGTCCGAGGTATTCGCACTCGTTTGAGGTTCGTTAACCAA	(6-FAM)CTCGTTTGAGGTCGTTAACCAAT(BHQ1)	fw	GGACAGTAGTCTGCACATTGG
				rv	GTGCAGGGTCCGAGGT
miR-196a	UAGGUAGUUUCAUGUUGUUGGG	CCTCAAACGAGTGCAGGGTCCGAGGTATTCGCACTCGTTTGAGGTCGTACCCAACA	(6-FAM)CTCGTTTGAGGTCGTACCCAACAAC(BHQ1)	fw	GCGTTAGGTAGTTTCATGTTGT
				rv	GTGCAGGGTCCGAGGT

TableS10. Sequences of the TaqMan RT-qPCR assay in 7 miRNAs detection of mouse tissues.

Sample	1a-3p	30c-1-3p	let-7a	24-3p	122-5p	196a	199a
heart	93.35	93.36	91.56	90.27	91.30	106.23	99.85
brain	111.2	105.56	101.84	89.07	101.96	105	89.01
kidney	86.86	97.16	103.56	110.37	105.23	104.65	103.93
lung	98.95	96.1	94.11	93.04	106.14	100.52	104.2
muscle	82.56	103.24	98.26	104.34	98.96	102.1	106.61
liver	94.64	n.d.	88.36	96.67	87.66	92.65	99.64
cereb	102.8	98.06	90.58	95.28	107.56	97.98	96.74
Negative control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Sample	1a-3p	30c-1-3p	let-7a	24-3p	122-5p	196a	199a
heart	17.85	12.2	12	20.1	16.26	0	6.76
brain	0	0	1.72	21.3	5.6	1.23	17.6
kidney	24.34	8.4	0	0	2.33	1.58	2.68
lung	12.25	9.46	9.45	17.33	1.42	5.71	2.41
muscle	28.64	2.32	5.3	6.03	8.6	4.13	0
liver	16.56	n.d.	15.2	13.7	19.9	13.58	6.97
cereb	8.4	7.5	12.98	15.09	0	8.25	9.87

TableS11. The POI value (a) and relative expression (b) of 7 miRNAs were analyzed in mouse 7 tissues by using the variable primer CRT-PICA method. The POI value was expressed as the average of 2 repeated reactions. For a given miRNA, the fold change was calculated relative to the sample with the lowest expression.

Sample	1a-3p	30c-1-3p	let-7a	24-3p	122-5p	196a	199a
heart	25.56	25.88	22.41	21.53	21.96	30.4	28.01
brain	38.01	31.26	26.03	20.99	26.88	30.1	20.04
kidney	23.10	28.27	27.10	34.61	31.98	29.81	28.11
lung	29.11	27.01	23.45	22.21	33.02	28.22	29.23
muscle	19.02	29.96	25.01	30.17	25.79	28.63	30.15
liver	25.98	n.d.	18.76	26.64	18.20	22.97	27.66
cereb	34.14	28.54	21.01	23.01	33.89	27.81	24.94
Negative control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Sample	1a-3p	30c-1-3p	let-7a	24-3p	122-5p	196a	199a
heart	12.45	5.38	4.69	13.08	11.93	0	2.14
brain	0	0	1.07	13.62	7.01	0.3	10.11
kidney	14.91	2.99	0	0	1.91	0.59	2.04
lung	8.90	4.25	3.65	12.4	0.87	2.18	0.92
muscle	18.98	1.30	2.09	4.44	8.10	1.77	0
liver	12.03	n.d.	8.34	7.97	15.69	7.43	2.49
cereb	3.87	2.72	6.09	11.6	0	2.59	5.21

TableS12. Taqman RT-qPCR method analyzes the Ct value (a) and relative expression (b) of 7 miRNAs in mouse 7 tissues (Ct value was expressed as the average of 2 replicates, for a given miRNA, the fold change was calculated relative to the sample with the lowest expression).

Sample	1a-3p	30c-1-3p	let-7a	24-3p	122-5p	196a	199a
heart	93.24	93.28	91.58	90.25	91.28	106.28	99.88
brain	110.18	105.58	101.86	89.03	102.8	104.88	89.25
kidney	86.89	97.14	103.55	110.33	105.27	104.43	103.95
lung	98.83	95.93	94.01	93.02	106.4	100.39	104.35
muscle	82.43	103.26	98.23	104.24	98.93	102.13	106.49
liver	94.53	n.d.	88.38	96.63	87.63	92.57	99.72
cereb	101.9	98.08	90.54	95.13	107.6	97.99	96.76
Negative control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Sample	1a-3p	30c-1-3p	let-7a	24-3p	122-5p	196a	199a
heart	16.94	12.3	11.97	20.08	16.25	0	6.75
brain	0	0	1.69	21.3	4.73	1.24	17.55
kidney	23.29	8.44	0	0	2.26	1.84	2.68
lung	11.35	9.65	9.54	17.31	1.03	35.7	2.48
muscle	27.75	2.32	5.32	6.09	8.6	4.2	0
liver	15.65	n.d.	15.17	13.7	19.9	13.65	6.89
cereb	8.28	7.5	13.01	15.2	0	8.26	9.74

TableS13.The POI value (a) and relative expression (b) of 7 miRNA profiles in mouse 7 tissues measured using multiple methods. The POI value was expressed as the average of 2 repeated reactions. The fold change was calculated relative to the sample with the lowest expression for a given miRNA.

ΔPOI (relative to singleplex protocol)							
Sample	1a-3p	30c-1-3p	let-7a	24-3p	122-5p	196a	199a
heart	-0.11	-0.08	0.02	-0.02	-0.02	0.05	0.03
brain	-1.02	0.02	0.02	-0.04	0.84	-0.12	0.24
kidney	0.03	-0.02	-0.01	-0.04	0.04	-0.22	0.02
lung	-0.12	-0.17	-0.1	-0.02	0.26	-0.13	0.15
muscle	-0.13	0.02	-0.03	-0.1	-0.03	0.03	-0.12
liver	-0.11	n.d.	0.02	-0.04	-0.03	-0.08	0.07
cereb	-0.9	0.02	-0.04	-0.15	0.07	0.01	0.02
average	-0.34	-0.03	-0.02	-0.06	0.16	-0.06	0.058
St.dev.	0.43	0.07	0.05	0.048	0.316	0.099	0.113

TableS14. Comparison of singleplex and multiplex variable primer CRT-PICA analysis.$\Delta POI = POI_{multiplex} - POI_{singleplex}$.

Species	Sequence(5'-3')
Variable Probe	GCCGGCAGACGAGTTCTGAAGGCCTTGCCGGCAGACTCCAATAT
HBoV	GCCGGCAGACATATTGGATTCCAAGATGGCGTCTGTACAACCACGTCACATATAAAATAATAAATATTCACAAG
Control	AGAGGTAGTACTAGTAGAGTTCTGAAGGCCTTATTAAGTTGCCTTCAGAACTCTACTACTACTAGTACTACCTCT

TableS15. Sequences of HBoV detection for CR-PICA.

A

Bst DNA Polymerase	POI
2U	72
4U	63
6U	64
8U	65
10U	68

B

Mg^{2+}	POI
2mM	71
2.5mM	65
3mM	63
3.5mM	66
4mM	77

C

Temperature	POI
63℃	68
64℃	64
65℃	63
66℃	65
67℃	73

D

Betaine	POI
0.66M	81
1M	67
1.4M	63
1.8M	74
2.2M	83

E

Length of “a”	POI
7	76
8	70
9	67
10	63
11	63.8

F

Length of “c”	POI
5	73
6	71
7	68
8	63
9	63.3

Table S16. Condition optimization of the CR-PICA reaction. (A) Optimization of Bst DNA polymerase, (B) Optimization of Mg^{2+}, (C) Optimization of temperature and (D) Optimization of betaine. 4U Bst DNA polymerase, 3mM Mg^{2+}, 65 ^{o}C and 1.4M betaine were chosen in the PICA reaction. Optimization of the "a" (E) and "c" (F) length sequences in the variable primer of CR-PICA for HBoV. From the corresponding POI value, the 10nt length of "a" and the 8nt length of "c" were chosen in the following experiments, respectively.

	Length	Sequence (5'-3')
Length of " a "	**7nt**	GCCGGCAGAGTTCTGAAGGCCTTGCCGGCATCCAATAT
	8nt	GCCGGCAGGAGTTCTGAAGGCCTTGCCGGCAGTCCAATAT
	9nt	GCCGGCAGAGAGTTCTGAAGGCCTTGCCGGCAGATCCAATAT
	10nt	GCCGGCAGACGAGTTCTGAAGGCCTTGCCGGCAGACTCCAATAT
	11nt	GCCGGCAGACTGAGTTCTGAAGGCCTTGCCGGCAGACTTCCAATAT
Length of " c "	**5nt**	GCCGGCAGACGAGTTCTGAAGGCCTTGCCGGCAGACAATAT
	6nt	GCCGGCAGACGAGTTCTGAAGGCCTTGCCGGCAGACCAATAT
	7nt	GCCGGCAGACGAGTTCTGAAGGCCTTGCCGGCAGACCCAATAT
	8nt	GCCGGCAGACGAGTTCTGAAGGCCTTGCCGGCAGACTCCAATAT
	9nt	GCCGGCAGACGAGTTCTGAAGGCCTTGCCGGCAGACATCCAATAT

TableS17. The sequences of the "a" and "c" length sequences in the variable primer for HBoV. The different length of "a" (7,8,9,10,11nt) and The different length of "c" (5,6,7,8,9nt) were tested. The red bases represented "a". The green bases represented "b". The black bases represented "c".