Supporting Information

A catalytic hairpin assembly system with sliding replication for the detection of piRNAs

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Name	Oligonucleotide sequence (5' to 3')
AT6	ATATAT
AT8	ΑΤΑΤΑΤΑΤ
AT10	ΑΤΑΤΑΤΑΤΑΤ
AT12	ΑΤΑΤΑΤΑΤΑΤΑΤ
AT14	ΑΤΑΤΑΤΑΤΑΤΑΤΑΤ
piR-hsa-823	ACCTGCTAGTTGCAGATGTTTGCCACTGGA
H1	TCCAGTGGCAAACATCTGCAACTAGCAGGTAAAAATG CCACTGGAACCTGCTAGTTGCAGATGTTTGC
H2	GCAACTAGCAGGTTCCAGTGGCAAACATTTTTACCTGC TTGCAGATGTTTGCCACTGGAACCTGC
t-H1	TCCAGTGGCAAACATCTGCAACTAGCAGGTCCACTGGA GCGCCATATATATACCTGCTAGTTGCAGATGTTTGC
t-H2	TAGCAGGTATATATATGGCGCCGCTCCAGTGGACCTGC TTGCAGATGTTTGCCACTGGAGCGGCGCCATATATATA
Mismatch-1	ACCTGCTAGTTGCATATGTTTGCCACTGGA
Mismatch-2	ACCTGCTAGTTGCCTATGTTTGCCACTGGA
piR-has-651	ACCAAACCCATTCTCCCATCAGTGCTGC
miR-21-3p	CAACACCAGTCGATGGGCTGT
3'-mis1	ACCTGCTAGTTGCAGATGTTTGCCACTGGC
3'-mis2	ACCTGCTAGTTGCAGATGTTTGCCACTTGA
3'-mis3	ACCTGCTAGTTGCAGATGTTTGCTACTGGA
3'-mis4	ACCTGCTAGTTGCAGATGTTTGCTAATGGA
3'-mis5	ACCTGCTAGTTGCAGATGTTTGCTACTAGA
piR-has-823-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGA
	GACTCCAGT

Table S1.	The seo	uences (of (oligonuel	leotides	used in	this	work
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piR-hsa-823 F	TGCTAGTTGCAGATGTTTGCC
piR-hsa-823 R	AGTGCAGGGTCCGAGGTATT
U6 F	CTCGCTTCGGCAGCACA
U6 R	AACGCTTCACGAATTTGCGT

The sequence marked in red represents the embedded primer; The green bases represent mismatches compared with target sequences. The purple bases represent mismatches at 3' -end compared with target sequences.



Fig. S1 Comparing the amplification effects of Bst and Klenow Fragment (KF exo-) DNA polymerase on CTA through PAGE (A) and fluorescence measurement (B). M stands for 500 bp DNA Marker. (A) Lane1:t-H1+t-H2+Bst; Lane2: t-H1+ H2+ piR-823+Bst; Lane3:t-H1+t-H2+ KF exo-; Lane4: t-H1+ t-H2+ piR-823+ KF exo-. The concentration of t-H1, t-H2 and piR-823 is 150 nM, 150 nM and 10 nM, respectively. CTA with Bst and KF exo- reacts at 37 °C. The concentration of Bst is 2.4 U/µL, and the concentration of KF exo- is 2 U/µL recommended by the manufacturer's instructions.



Fig. S2 Selectivity assay of CTA. (A) Fluorescence spectra in the presence of different species of RNA. (B) The change values of fluorescence intensity in the presence of different species of RNA. F_T : fluorescence intensity of the different target group, F_0 : fluorescence intensity of the background group. Error bars represent the standard deviation of three repeated measurements for each concentration.



Fig. S3 The computed tomography (CT) and clinical pathology results of colorectal cancer patients. The numbers in the graphs all correspond to the numbers in Table S3.



Fig. S4 (A) The Calibration plot and corresponding linear equation of RT-qPCR for detection of piRNA-823. (B) Comparison of RT-qPCR and CTA for detection of piRNA-823 in colorectal tumor cells. Error bars represent the standard deviation of three repeated measurements for each concentration.

NO.	Sex	Clinical diagnosis	Fluorescence intensities	Consistency
1	female	positive	2652.03	yes
2	female	positive	3234.13	yes
3	male	positive	3701.23	yes
4	female	positive	810.83	yes
5	male	positive	1291	yes
6	male	positive	613.90	no
7	male	positive	604.10	no
8	female	positive	575.66	no
9	male	positive	509.33	no
10	male	positive	1312.96	yes
11	female	positive	1480.23	yes
12	female	positive	2589.00	yes
13	male	positive	1217.43	yes
14	male	positive	1248.73	yes
15	female	positive	1346.30	yes
16	male	positive	1253.90	yes
17	male	positive	2982.66	yes
18	female	positive	652.06	yes
19	male	positive	1076.93	yes
20	female	positive	845.63	yes

Table S2. Diagnostic information of clinical patients and CTA detection of piRNA-823

results

0.1	<u> </u>		100.13	
21	temale	negative	188.43	yes
22	male	negative	606.79	yes
23	male	negative	262.33	yes
24	female	negative	370.50	yes
25	male	negative	371.16	yes
26	male	negative	485.33	yes
27	female	negative	529.70	yes
28	female	negative	574.70	yes
29	female	negative	575.93	yes
30	male	negative	1711.03	no
31	male	negative	151.16	yes
32	male	negative	985.83	no
33	male	negative	573.43	yes
34	male	negative	172.29	yes
35	male	negative	754.90	no
36	female	negative	417.366	yes
37	female	negative	860.93	no
38	male	negative	493.40	yes
39	female	negative	344.76	yes
40	male	negative	261.43	yes