Electronic Supplementary Information (ESI)

Ultrasensitive Detection of MicroRNAs Using Catalytic Hairpin Assembly Coupled with Enzymatic Repairing Amplification

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Experimental Section

Reagents and Apparatus. HPLC-purified miRNAs were purchased from Takara Biotechnology Co., Ltd. (Dalian, China).The DNA oligonucleotides were synthesized and purified through HPLC by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). The sequences of these miRNAs and DNA are given in Table S1. RNase inhibitor, DEPC treated water, Ultra Low MW DNA Marker (10-300 bp) and 5× TBE buffer (225 mM Tris-Boric Acid, 50 mM EDTA, pH 8.0) were obtained from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). The Endonuclease IV and 10 × NEBuffer 3 (1000 mM NaCl, 500 mM Tris-HCl (pH 7.9), 100 mM MgCl2 and 10 mM DTT) were obtained from New England Biolabs (Ipswich, MA). DMEM high glucose medium, RPMI 1640 medium, streptomycin, penicillin, and 15% heat-inactivated fetal bovine serum were purchased from Thermo Scientific HyClone (MA, USA). Other chemicals used in this work were of analytical grade and directly used without further purification. All solutions used in the experiments were prepared using RNase-free water.

The fluorescence spectra were recorded at room temperature in a quartz cuvette on a FluoroMax-4 spectrofluorometer (HORIBA, NJ, USA). The excitation wavelength was 490 nm with a recording emission range from 505 to 600 nm and the excitation and emission slits were set at 5 nm.

Detection of miRNA using CHA-ERA reaction. The miRNA detection was performed in a 30 μL reaction mixture containing 50 nM H1, 50 nM H2, 0.0003U/ml endo IV, 1μM probe, 20 U RNase inhibitor and different concentrations of target

miRNA or extracted RNA in 1 × NEBuffer 3 at 37 ºC for 2.5 hours. Then DEPC treated water were added into the reaction with final reaction volume of 100 μL.

Gel electrophoresis analysis. Gel electrophoresis analysis was carried out on 3% (w/w) agarose gels containing 0.5 μg/mL GoldView and 0.5 μg/mL ethidium bromide running in 0.5× TBE buffer at room temperature. The electrophoresis was performed at a constant potential of 101 V for 2 h after loading 10 μL of each sample into the lanes. After electrophoresis, the gel was visualized via a Tanon 4200SF gel imaging system (Tanon Science & Technology Co., Ltd., China).

Cell culture and total RNA extraction. MCF-7 (human breast cancer cell lines), MCF-10A(human mammary epithelial cell line), and Hela (cervical cancer cell lines) was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO2. Cells (1×10^6) were dispensed in an RNase-free 1.5 mL centrifuge tube, washed twice with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4), centrifuged at 2000 rpm for 3min, and then suspended in 100 μL lysis buffer (10 mM Tris-HCl with pH 8.0, 150 mM NaCl, 1%(w/v) NP-40, 0.25 mM sodium deoxycholate, 1% glycerol and 0.1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride). The lysates were incubated for 30 min on ice, and then centrifuged at 12000 rpm for 20 min at 4 °C. The extract was used immediately for CHA-ERA assay or stored at -80 °C.

Quantitative PCR (qPCR) analysis of miRNA in cells. The cDNA samples were prepared by using AMV First Strand cDNA Synthesis Kit (BBI, Toronto, Canada). Briefly ,a total volume of 20 μL containing 4 μL 5× Reaction Buffer, 2 μL total RNA, 2 μL dNTPs, 1 μL RNase inhibitor, 1 μL reverse transcription primer (5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGA GTCAACATCA-3′), and 2 μL AMV reverse transcriptase was incubated at 37 °C for 5 min, 42 °C for 60 min, 70 °C for 10 min. qPCR analysis of miRNA was performed with SybrGreen PCR Master Mix (ABI, CA, USA) according to the indicated protocol on an ABI Stepone plus qPCR instrument (CA, USA). A 20 μL reaction solution contained 1 μL reverse transcription products, 10 μL SybrGreen qPCR Master Mix, 1 μL 10 μM primer F (5′-ACACTCCAGCTGGGTAGCTTATCAGACTG-3′), 1 μL 10 μM primer R (TGGTGTCGTGGAGTCG), and 7 μL RNase-free water. The qPCR reaction was incubated at 95 °C for 2 min followed by 40 cycles of 95 °C for 10 s, 60 °C for 40 s. The endogenous U6 small RNA was used as control to confirmed the integrity of the miRNA and the efficiency of qPCR in each sample. All the data was evaluated with respect to the miRNA expression by normalizing to the expression of U6 and using the $2^{-\Delta\Delta Ct}$ method.[S1]

References:

(S1) Livak, K. J.; Schmittgen, T. D. *Methods* **2001**, *25*, 402-408.

Table S1. Sequences of miRNAs and DNA probes used in this work[a]

Name	Sequence (5′-3′)
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-143	UGAGAUGAAGCACUGUAGCUCA
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-21-1	UAGCUUAUCAGACUGAUCUUGA
miR-21-2	UAGCUUAUCAGACUAAUGUUGA
miR-21-3	UAGCUUAUCATACUGAUGUUGA
H1（1-2-3-4-3*-2*）	TCAACAT-CAGTCTGA-TAAGCTA-CATTGGATGCTC-TAGCTTA-TCAGACTG
H2（3-4*-3*-2*-4）	TAAGCTA-GAGCATCCAATG-TAGCTTA-TCAGACTG-CATTGGATGCTC
Probe（4*-1*）	GAGCAT(FAM)CC**X**(AP)AT(TAMRA)G-ATGTTGA

[a] The underlined bases in miR-21s are mismatched bases. X represents abasic sites.

Table S2. Comparison of the proposed method with other assay

Methods	Sensitivity (limit of detection)	Specificity	Complexity	Ref.
CHA only	5 pM	High specific with single-base distinguish	Low complexity with simple probe design	1
Mimic enzyme amplification	2.2 fM	High specificity	Complex electrode pretreatment and multiple reaction with washing steps	2
RCA	0.68 fM	High specificity	Complex multiple reaction steps non-isothermal reaction	3
Exonuclease amplification	92 fM	High specificity	Complex electrode preparation and multiple reaction	4
Electrochemical Biosensing Strategy Based on Hybridization Chain Reaction	1 pM	High specificity	Complex electrode pretreatment	5
Duplex-Specific Nuclease Signal Amplification Using Taqman probe	100 fM	High specificity with discrimination between similar sequences	Low complexity	6
DNA-Gold Nanoparticle Probes	5 - 8 pM	Not defined	Complex preparation of oligonucleotide-functionalized AuNPs	7
WS2 based DSN Signal Amplification	300 fM	High specificity	Complex preparation of WS2 nanosheet	8
SERS Based on Silver Nanorod Array	28 nM	Not defined	Complex synthesis and treatment of silver nanorod	9
CHA-ERA	50 fM	High specific	Simple one-step isothermality reaction	This method

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Fig. S1. The fluorescence signal ratio of the assay at different concentration of H1 and H2. F0 and F are the fluorescence signals in the absence and the presence of target miRNA, respectively. Error bars are standard deviations of three repetitive experiments.

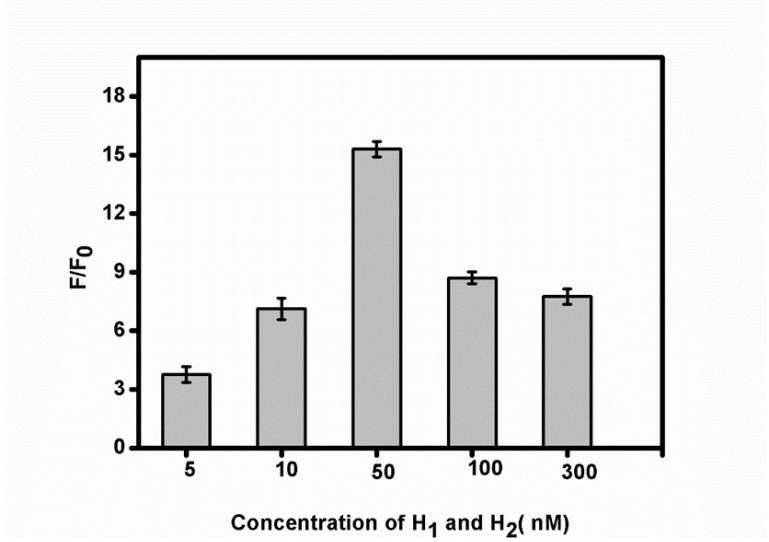

Fig. S2. The variation in fluorescence signal ratio with different reaction time. F0 and F are the fluorescence signals in the absence and the presence of target miRNA, respectively. Error bars are standard deviations of three repetitive experiments.

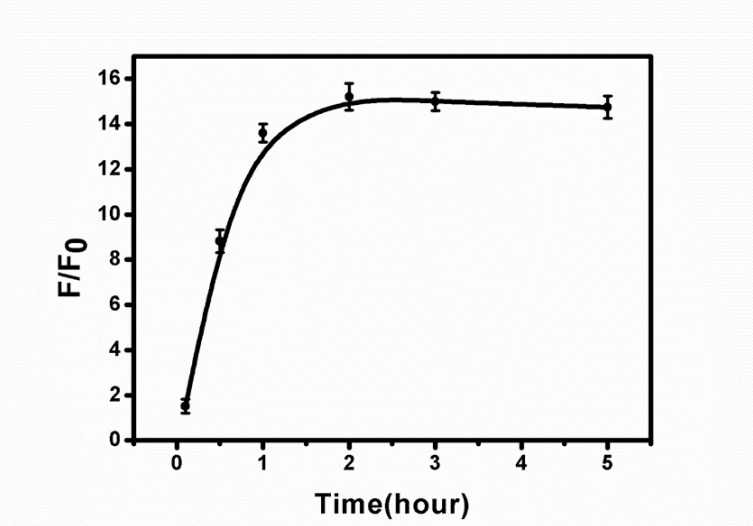

Fig. S3. qPCR curves for U6 small RNA. Each sample was detected in three repetitive assays. The pink horizontal line represents the threshold line, and the number on the line indicates the threshold value.

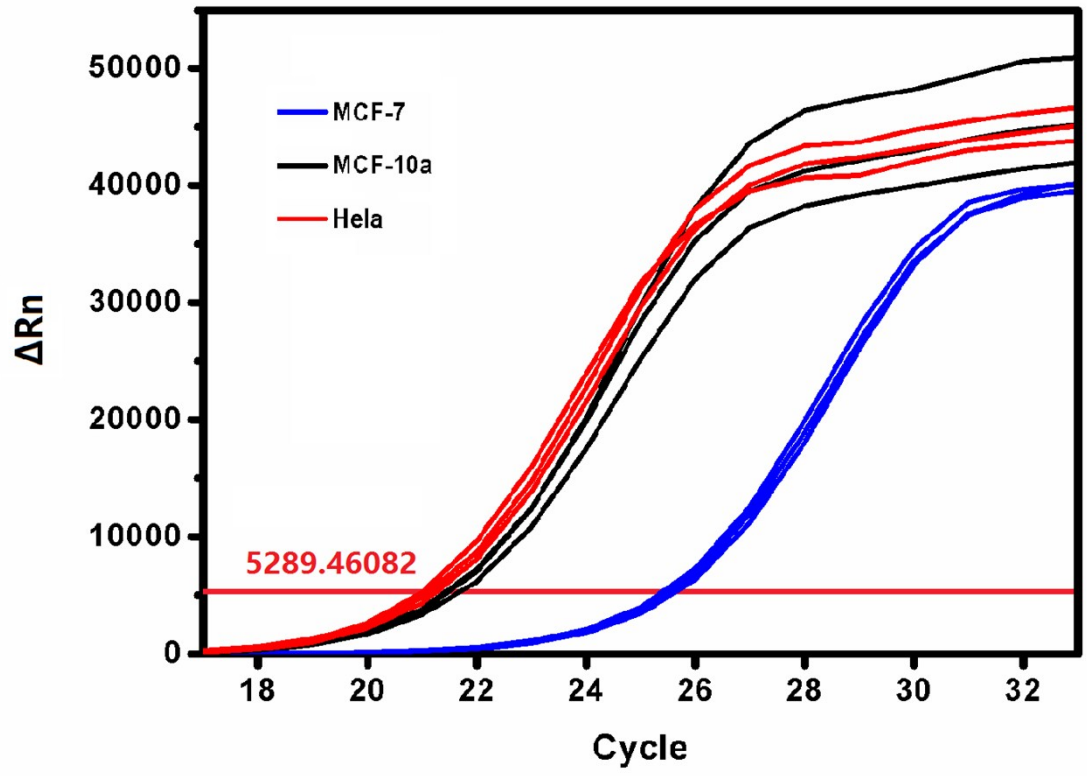

Fig. S4. qPCR curves for miR-21. Each sample was detected in three repetitive assays. The pink horizontal line represents the threshold line, and the number on the line indicates the threshold value.

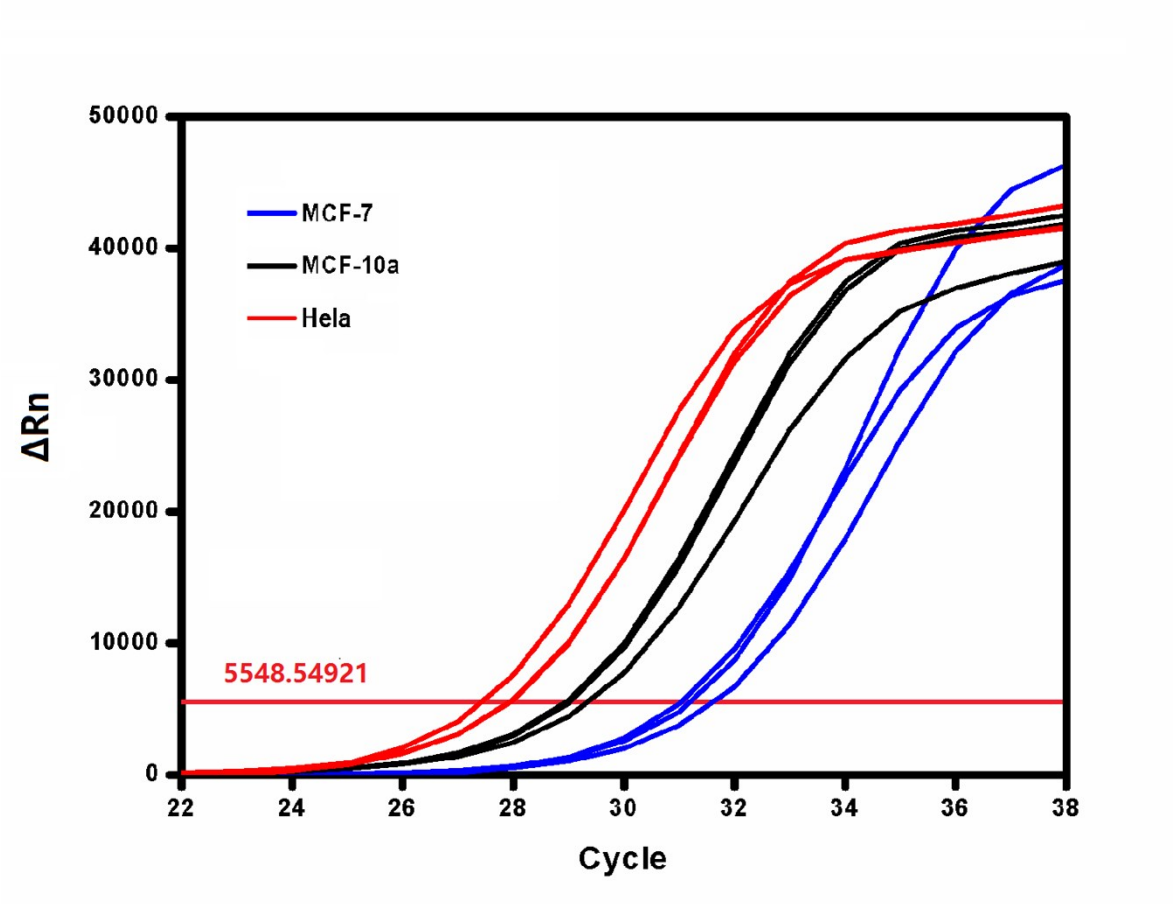

Table S3. Average Ct values in qPCR assay of miR-21.[a]

Cell line	miR-21	U6	ΔCt	ΔΔCt	$2^{-(\Delta\Delta Ct)}$
MCF-7	31.19954	25.53296	5.666583	0	1
MCF-10A	29.00857	21.56584	7.44273	1.776147	0.291962
Hela	27.68337	21.11314	6.570223	0.90364	0.534536

a. The relative expression level can be estimated by the values of $2^{-(\Delta\Delta Ct)}$. From the data, the relative expression level of miR-21 in MCF-10A and Hela cell line were estimated to be 0.291962 and 0.534536 fold of that in MCF-7 cell line, illustrating the up-regulation of miR-21 in MCF-7 cell lines as compared with MCF-10A and Hela cell lines.

Fig. S5. Relative expression levels of miR-21 in different cell lysates, measured by using CHA-ERA method and qRT-PCR method. Error bars are standard deviation of three repetitive experiments.

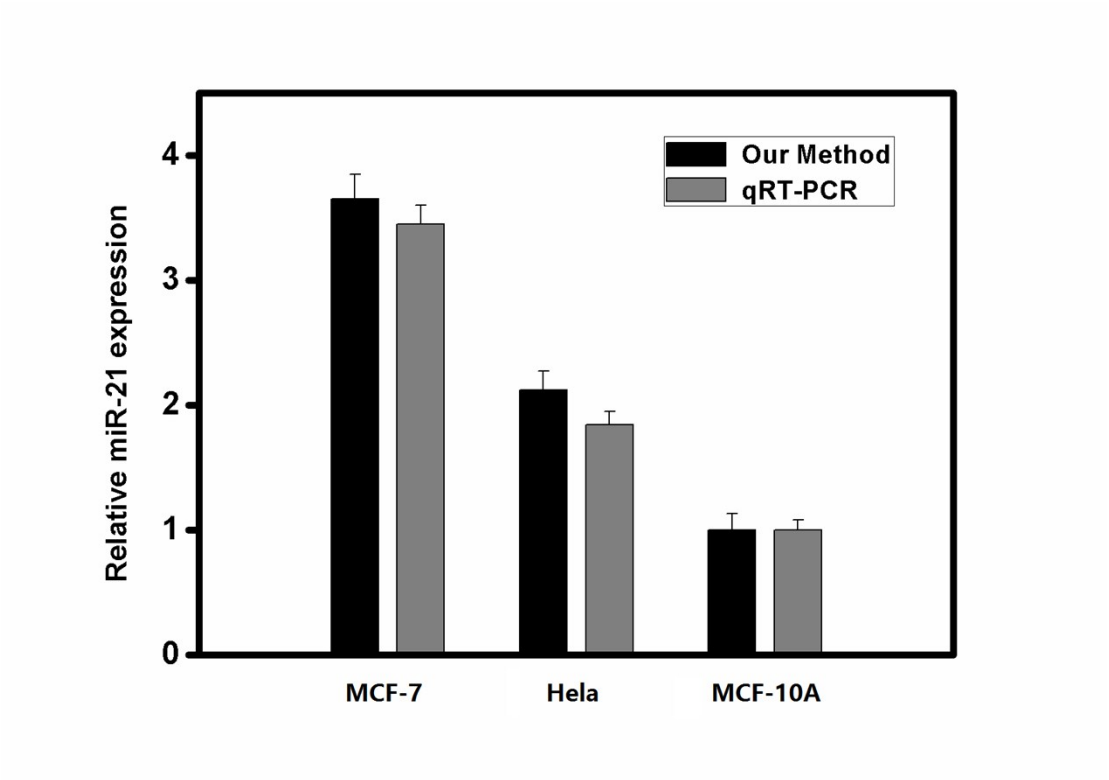

Table S4. Recovery experiments of miR-21 spiked in cell lysates of MCF-10A.

Cell lines	Detected (pM)	Added (pM)	Found (pM)a	Recovery (%)	CV (%)
MCF-10A	186	100	292 ±8	102	2.7
		200	372 ±15	96	4.0
		500	751 ±47	109	6.3