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Supporting Information

G-triplex based molecular beacon with duplex-specific nuclease amplification for specific detection of microRNA

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1. Experimental Section

Table S1. Sequences of miRNA and DNA probes used in this work

Name	Sequence (5'-3')
MBG3-141	TTGGGTAGG**GCGGG** TTA TCT TTA CCA GAC AGT GTT **CCCGC**
MBG3-141-7	TTGGGTAGG**GCGGGTT** TTA TCT TTA CCA GAC AGT GTT **AACCCGC**
MBG3-141-9	TTGGGTAGG**GCGGGTTCA** TTA TCT TTA CCA GAC AGT GTT **TGAACCCGC**
MBG4-141	TGGGTAGG**GCGGG** TTGGG TTATCTTTACCAGACAGTGTT **CCCGC**
MBG4-141-7	TGGGTA**GGGCGGG** TT GGG TTA TCT TTA CCA GAC AGT GTT **AACCCGC**
MBG4-141-9	TGGG**TAGGGCGGG** TT GGG TTATCTTTACCAGACAGTGTT **CCAACCCGC**
MBG3-141-F	FAM-TTGGGTAGG**GCGGG** TTA TCT TTA CCA GAC AGT GTT **CCCGC**-Dabcyl
Taqman probe	FAM-**AGGGACGGG** CCA TCT TTA CCA GAC AGT GTT A -BHQ1
miRNA-141	U AAC ACU GUC UGG UAA AGA UGG
SmiRNA-141	U AAC ACU GUC UAG UAA AGA UGG
SmiRNA2-141	U AAC ACU AUC UGG UAA AGA UGG
miRNA-200a	U AAC ACU GUC UGG UAA CGA UGU
miRNA-200b	U AAU ACU GCC UGG UAA UGA UGA
miRNA-429	U AAU ACU GUC UGG UAA AAC CGU
miRNA-21	U AGC UUA UCA GAC UGA UGU UGA
miDNA	T AAC ACT GTC TGG TAA AGA TGG

The underlined fragments of MBG3 and MBG4 can self-hybridize into hairpin structure, and their bold fragment can self-assemble into G-quadruplex and G-triplex. SmiRNA-141 and SmiRNA2-141 carry a single-base mismatch from miRNA-141, and the mismatched position is marked in red.

2. Supplementary results

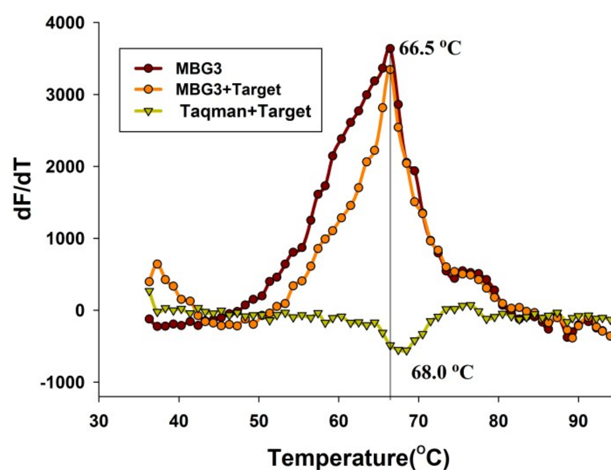

Fig. S1 Melting curves of MBG3, double-strand MBG3/target and Taqman probe/target duplexes. Melting curves study was carried out on an Agilent StrataGene Mx3005P Realtime PCR instrument. Herein, MBG3 and target were MBG3-141-F and miRAN-141, respectively. All the melting curves are around 67 °C which are higher than the working temperature of DSN (60 °C).

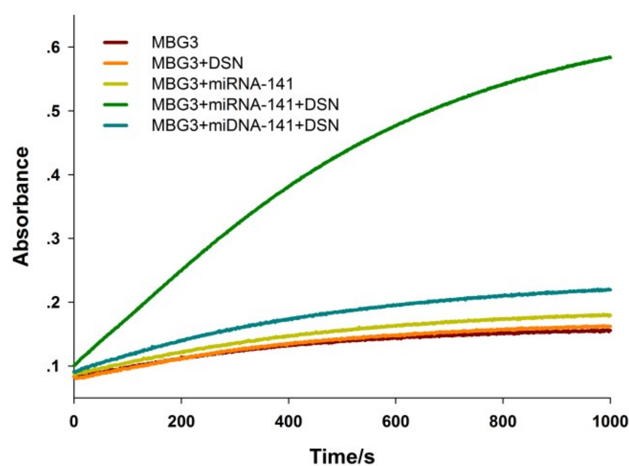

Fig. S2 Peroxidase activity of MBG3 DSNSA assay. The reaction sample (20 µL) containing MBG3, target miRNA-141 and DSN was incubated in 1×DSN master buffer at 60 °C for 40 min, and then 60 µM hemin was added and incubated for 2 h in buffer (20 mM HEPES, pH = 7.4, 20 mM KCl, 200 mM NaCl, 150 mM NH_4Cl and 0.01% TritonX-100). After adding of ABTS and H_2O_2, the detection was then performed by a Shimadzu UV-2700 UV-vis spectrophotometer. The final concentration of MBG3, miRNA, DSN, hemin, ABTS and H_2O_2 were 100 nM, 25 nM, 0.2 U, 500 nM, 2 mM and 2 mM, respectively.

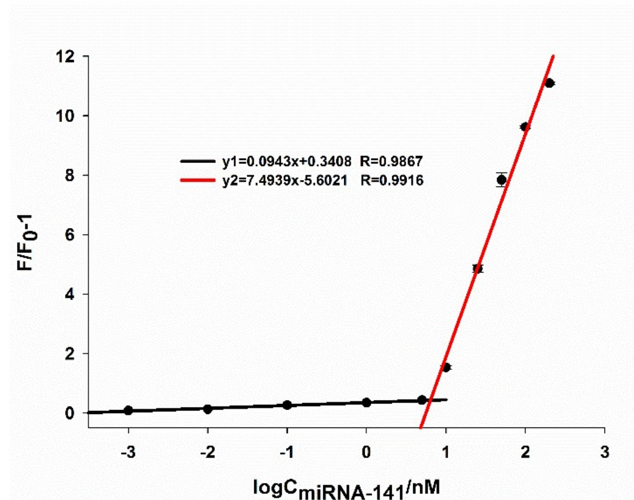

Fig.S3 The relationship between the fluorescence response and the logarithm of miRNA-141 concentration. Error bars are standard deviation of more than three repetitive experiments. A linear curve fitted a regression equation of y1=0.0943x (nM) +0.3408 in the range from 1pM to 5 nM. The point at which this line crossed the ordinate axis was taken as the detection limit and equaled approximately 0.25 pM miRNA-141.

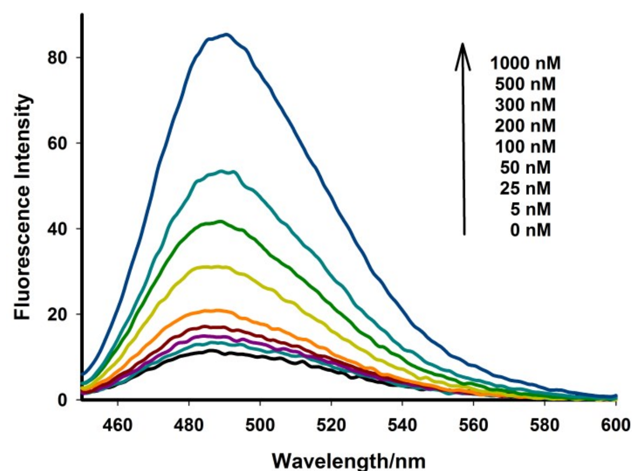

Fig. S4 Fluorescence emission spectra of MBG3 for different concentrations of miRNA-141.

Tab. S2 Comparison of analytical performance of various methods for determination of miRNA.

Method		Sensitivity	Selectivity	Simplicity	ref
EXPAR		16 fM	It is difficult to distinguish mutated base located in the middle.	label free	s1
Northern blotting with LNA probe		0.5 fM	two-base differences	multi-step	s2
Exonuclease III-aided target recycling strategy		10 pM	three -base differences	fluorescence modified	s3
DSN	Taqman probe	0.1 pM	four -base differences	fluorescence modified	s4
	ssDNA probe	1 pM	four -base differences	label free	s5
	G4 based MB probe(colorimetric)	20 pM	-[a]	label free, high background	s6
	G4 based MB probe(fluorescence)	1 pM	one-base difference	fluorescence modified, low background	s7
	2-OMe-RNA MB probe	0.5 pM	one-base difference	2-OMe-RNA and fluorescence modified, low background	s8
	MB probe with short stem	5 pM	one-base difference	fluorescence modified, low background	s9
	This work	1 pM	one-base difference	label free, low background	

[a] "-"represents the data are not available.

Table S3. Recovery experiments of miRNA-141 spiked in human urine and Hela cell lysates[a].

Sample	Added (nM)	Assayed (nM)	Recovery (%)	RSD (%)
Human urine	5.00	4.65	93	5.2
	25.00	25.20	102	2.7
	50.00	49.50	99	0.41
HeLa	5.00	4.87	97	2.2
	25.00	22.59	90	1.4
	50.00	49.62	99	1.3

[a] The Hela (human cervical cancer cell lines with low level expression of miRNA-141) cells were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). We cultured them in DMEM medium supplemented with 10% fetal bovine serum (FBS) at 37 °C under a humidified 5% CO_2 atmosphere. Hela cells (~3×10^7 cells) were collected and washed twice with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4). Then, 500 µL RIPA lysis buffer (Beyotime, China, cat no P0013C) was added and incubated for 40 min on ice, and then centrifuged at 12000 rpm for 20 min at 4 °C. The Healthy human urine was obtained from Dongguan Tungwah Hospital. We spiked different concentrations of miR-141 into 10-fold diluted blood serum and cell lysate, and monitored them in MBG3 DSNSA assay. All values were obtained as the average of 3~5 repetitive determinations plus standard deviation.

2. References

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