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## **Electronic Supplementary Information**

# Dual signal amplification for microRNA-21 detection based on duplex-specific nuclease and invertase

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## Supplementary Tables

 Table S1. Oligonucleotides designed in the present study

Name	Sequence(5' to 3')	
miRNA-21	UAGCUUAUCAGACUGAUGUUGA	
Target DNA	TAGCTTATCAGACTGATGTTGA	
DNA strand 1	Biotin-AAAAATCAACATCAGTCTGATAAGCTA-(A)5-SH	
DNA strand 2	Biotin-AAAAATCAACATCAGTCTGATAAGCTA-(A)10-SH	
DNA strand 3	Biotin-AAAAATCAACATCAGTCTGATAAGCTA-(A)15-SH	
DNA strand 4	Biotin-AAAAATCAACATCAGTCTGATAAGCTA-(A)20-SH	
DNA strand 5	Biotin-AAAAATCAACATCAGTCTGATAAGCTA-(A)25-SH	
Mismatch-1	UAGCUUAUCA <u>C</u> ACUGAUGUUGA	
Mismatch-2	UAGCAAAUCAGAC <u>A</u> GAUGUUGA	
Mismatch-3	natch-3 UAGCUUA <u>A</u> CAGACUGAUGAAGA	
hsa-miR-200c	CGTCTTACCCAGCAGTGTTTG	
hsa-miR-4640	UGGGCCAGGGAGCAGCUGGUGGG	
hsa-miR-423	UGAGGGGCAGAGAGCGAGACUUU	
NC	UUGUACUACAAAAAGUACUG	

Limit of detection	Linear range	Strategy	References
1.1 nM	None	Electrochemiluminescence	Shamsi <i>et al</i> . <sup>1</sup>
500 pM	0.5 nM-200 nM	Fluorometric assay	Song <i>et al.</i> <sup>2</sup>
230 pM	0 - 6.25 nM	Fluorometric assay	Lee <i>et al.</i> <sup>3</sup>
80 pM	49.6 pM–50 nM	Chemiluminescence	Wang <i>et al</i> . <sup>4</sup>
8 pM	25 pM-1 nM	Chemiluminescence	Li <i>et al</i> . <sup>5</sup>
2.79 pM	10 pM-100 nM	H <sub>2</sub> O <sub>2</sub> decomposition	Lei <i>et al.</i> <sup>6</sup>
1.8 pM	20 pM–200 pM	DSN-assisted signal amplification	This work

 Table S2. Analytical performance of reported methods and proposed method for

 miRNA detection

## **Supplementary Figures**



Figure S1. Absorption spectra of the thiol-DNA, invertase, and DNA-Inv conjugate.



**Figure S2.** 6% SDS PAGE images of invertase and DNA-Inv conjugate. Lane 1: invertase; Lane 2: DNA-Inv conjugate.



**Figure S3.** The effect of MBs-DNA-Inv probes immobilized with different DNA strand 1-5 with different lengths of adenine domain (5, 10, 15, 20, 25) for the target miRNA-21 detection assay. Data are means  $\pm$  SD (n=3).



**Figure S4.** Linear relationship between the concentration of added miRNA-21 in urine from healthy mice and PGM signal. Data are means  $\pm$  SD (n=3).



**Figure S5.** (A) Real-time qRT-PCR curves of different concentrations of target miRNA-21, which has been converted to cDNA before amplification. (B) The linear relationship between the miRNA-21 concentration and the threshold cycle.

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