# Supplementary Information

## Two-layer circuit cascade-based DNA machine for highly sensitive miRNA imaging in living cells

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**Additional Experimental Section**

**Apparatus.** A F-7000 fluorescence spectrophotometer (Hitachi, Japan) was used to record the fluorescence spectra with a Xenon lamp. Time-dependent fluorescence intensity was measured using a Varioskan Flash microplate reader (Thermo Fisher Scientific, USA). Confocal laser scanning microscopy (CLSM) images were obtained on a Leica TCS SP5 microscope (Germany). The reverse transcription of miRNAs and quantitative real-time PCR (qRT-PCR) experiments were carried out on a PTC-1148 PCR (Bio-Rad, USA) and an ABI Prism 7300 qRT-PCR (ABI, USA), respectively.

**Materials.** All the DNA molecules used in this work were synthesized and HPLC purified by Sangon Biotechnology Co. Ltd. (Shanghai, China). MiRNA sequences were from Genepharma (Shanghai, China). Diethy pyrocarbonate (DEPC) was provided by Sangon Biotechnology Co. Ltd. (Shanghai, China). Tris-HCl solution (50 mM Tris-HCl, 100 mM NaCl, 12.5 mM $MgCl_2$ pH 7.4) was served as the reaction buffer. The 40% acrylamide mix solution, ammonium persulfate (APS), and 1,2-bis(dimethylamino)-ethane (TEMED) were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). In order to create and maintain an RNase-free environment, millipore water (18.2 MΩ·cm at 25 °C) treated with 0.1% DEPC and autoclaved was used throughout the experiments. The tips and tubes are RNase-free and do not require pretreatment to inactivate RNases. All the chemical reagents were of analytical grade and used without further purification. The oligonucleotide sequences used in this work are listed in Table S1.

**Table S1.** Oligonucleotide sequences used in this work

| Name | Sequence (from 5' to 3') |
|---|---|
| A | TTTCTAGTGGTCCTAAACATTTCACTCACTATTT |
| $A_{TAMRA}$ | TAMRA-TTTCTAGTGGTCCTAAACATTTCACTCACTATTT |
| B | TTTGTGAGTGAAATGTTTAGGACC |
| $B_{BHQ2}$ | TTTGTGAGTGAAATGTTTAGGACC-BHQ2 |
| Fuel | TTTGCTCTAGTGAGTGAAATGTTTAGGACCTTT |
| MiR-203 | GUGAAAUGUUUAGGACCACUAG |
| H1 | BHQ2-GTGAGTGAAATGTTTAGGTCAACTACTTCAACTCCTAAACATTTCACTCACTAGAGC |
| H2 | TCAACTACTTCAACTCCTGTGAGTGAAATGTTTAGGAGTTGAAGTAGTTGACCTAAA-TAMRA |

| | |
|---|---|
| MiR-21 | UAGCUUAUCAGACUGAUGUUGA |
| MiR-141 | UAACACUGUCUGGUAAAGAUGG |
| MiR-200b | UAAUACUGCCUGGUAAUGAUGA |
| MiR-429 | UAAUACUGUCUGGUAAAACCGU |

Underlined sequences were toehold regions. The mixture of 10 μL of strand A (100 μM) and 10 μL of strand B (100 μM) was incubated in 200 μL of Tris-HCl buffer at 37 °C for 2 h to obtain A/B duplex. H1 (5 μM) and H2 (5 μM) in Tris-HCl buffer were heated at 90 °C for 10 min and then cooled down gradually to room temperature, followed by maintaining for 2 h to ensure the formation of hairpin structure. The prepared DNA solutions were stored at 4 °C for further use.

**Cell Culture**

Human breast cancer cells (MCF-7) and normal human liver cells (L02) were obtained from the cell bank of type culture collection of Chinese academy of sciences (Shanghai, China) and cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin (100 U/mL) at 37 °C in a humidified 5% $CO_2$ incubator.

**The calculation method of detection limit (LOD)[1]**

The LOD was calculated through the following method. The calibration curve was plotted as:

$$Y = a + b \cdot X$$

Where a and b are obtained from the calibration curve. For the first-layer circuit, X is the logarithm of miR-203 concentration. For the second-layer circuit, X is the logarithm of the concentration of fuel strands. For the two-layer circuit cascade, X is the logarithm of miR-203 concentration. When b > 0, the LOD is calculated as

$$LOD = 10^{\frac{y0 + 3\,SD - a}{b}}$$

Where SD is the standard deviation and $y_0$ is the fluorescence intensity of the blank sample;

Where b < 0, the LOD is calculated as

$$LOD = 10^{\frac{y0 - 3\,SD - a}{b}}$$

SD is calculated according to the following formula:

$$SD = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(X_i - X_{average})^2}$$

## Optimization of experimental conditions

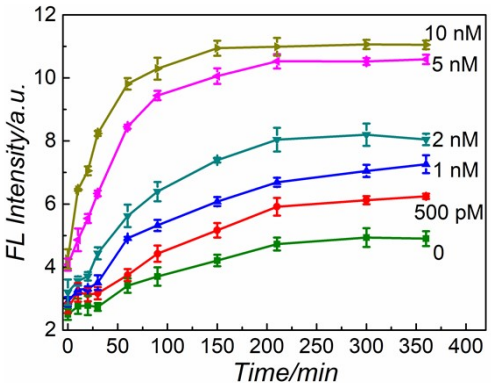


**Fig. S1** Time-dependent fluorescence intensity at 577 nm measured by a Varioskan Flash microplate reader of DNA circuit 1 responded to different concentrations of miR-203.

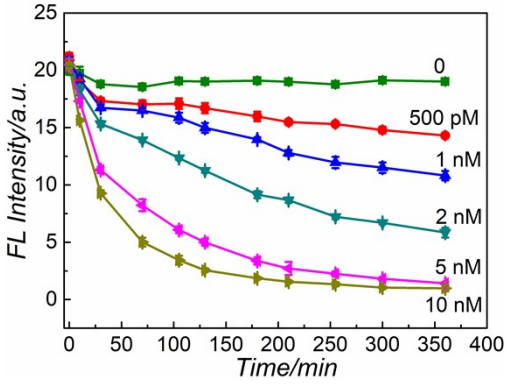


**Fig. S2** Time-dependent fluorescence intensity at 577 nm measured by a Varioskan Flash microplate reader of DNA circuit 2 responded to different concentrations of fuel strands.

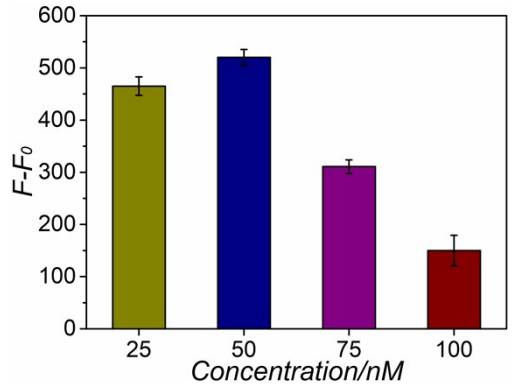


**Fig. S3** Effect of fuel concentration on the two-layer DNA circuit cascade. F-$F_0$ is the difference of fluorescence intensity at 577 nm in the presence and absence of miR-203. The concentration of miR-203 is 20 nM. The concentration of A/B duplex is equal to that of fuel strands. The concentration of H1 and H2 are both 50 nM. Error bars represent the standard deviations from three replicate measurements.

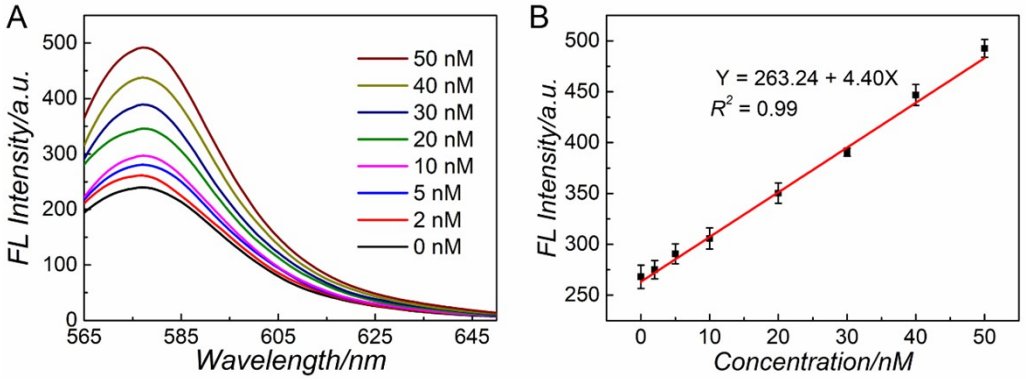


**Fig. S4** (A) Fluorescence spectra of the first-layer circuit in the absence of fuel strands responded to different concentrations of miR-203. (B) The corresponding calibration plot of fluorescence intensity at 577 nm versus miR-203 concentration in the range of 0 nM to 50 nM.

**Table S2.** Comparison of the proposed DNA machine with other reported amplification methods for detection of miRNA

| Methods | Target | Linear range(pM) | Detection limit | Ref. |
|---|---|---|---|---|
| Localized DNA cascade reaction | miR-155 | 100 pM -50 nM | 85.3 pM | 2 |
| DNAzyme walker | miR-21 | 0.05 nM -3 nM | 3.71 pM | 3 |
| Enzyme-powered DNA circuit | miR-21 | 0.2 nM -100 nM | 0.2 nM | 4 |
| DNA tetrahedron-assisted TMSDR | let-7a | 1 nM -150 nM | 22.35 pM | 5 |
| DNAzyme circuit | miR-21 | 0.05 nM -2 nM | 21 pM | 6 |
| Light-controlled HCR | miR-21 | 0.005 nM -100 nM | 3.3 pM | 7 |
| Two-layer circuit cascade based on TMSDR | miR-203 | 10 fM - 20 nM | 4 fM | This work |

## References

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