Supporting Information

A facile biosynthesis strategy of plasmid DNA-derived nanowire for readable microRNA logic operations

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S1. Supplementary text

S1.1 The length selection principle of nanowires

In this work, the nanowires of various lengths were derived from plasmid DNA, and the gel electrophoresis was used to identify the nanowire substrates and their dimer conjugate according to the molecular sizes. So that the length of the nanowires could affect the experimental results. Based on these, the length selection principles of nanowires are stated as follows:

First, the candidate plasmid should contain a pair of restriction enzyme recognition sites at a suitable distance. After dual digestion, the 4-nt sticky ends are composed of different bases at both ends of the interested dsDNA fragment.

Second, the interested dsDNA fragment generated by dual digestion of plasmid should have enough length difference with the other piece of the uninterested fragment, so that they can be distinguished by gel electrophoresis during the purification procedure.

Third, as for multiple miRNA identification in Fig 4, each kind of substrate nanowire and nanowire conjugate complex generated by particular miRNA hybridization should have enough difference in length. So that the distribution of each nanowire or conjugate complex can be clearly distinguished by the position of the gel band.

S2. Supplementary Figures

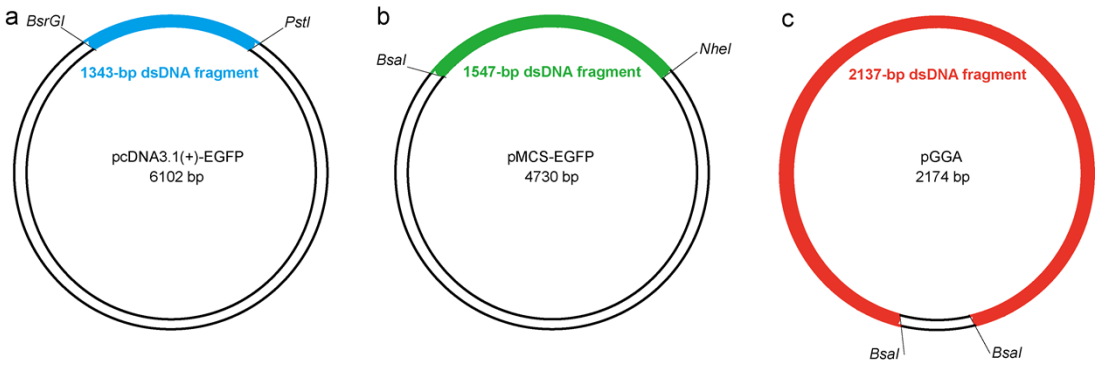

Fig. S1. Maps of plasmids. (a) pcDNA3.1(+)-EGFP can be digested by PstI and BsrGI, producing the 1343-bp dsDNA fragment A. (b) pCMV-EGFP can be digested by BsaI and NheI, producing the 1547-bp dsDNA fragment B. (c) pGGA can be digested by BsaI, producing 2137-bp dsDNA fragment C.

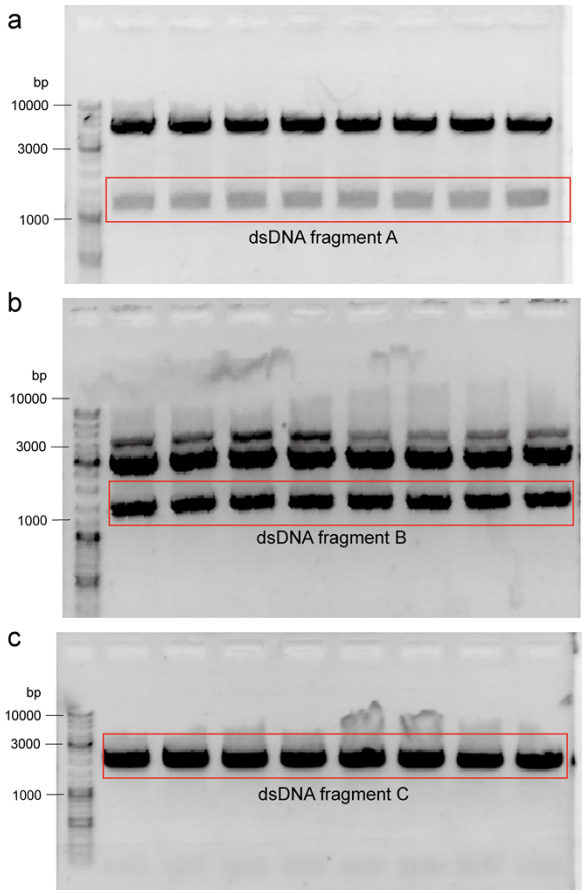

Fig. S2. Plasmid digested by corresponding restriction enzymes. (a) pcDNA3.1(+)-EGFP digested by *PstI* and *BsrGI*, the 1343-bp dsDNA fragment A was marked within the red rectangle. (b) pCMV-EGFP digested by *BsaI* and *NheI*, the 1547-bp dsDNA fragment B marked in the red rectangle. (c) pGGA digested by *BsaI*, the 2137-bp dsDNA fragment C marked within the red rectangle. DNA marker is the l kb ladder.

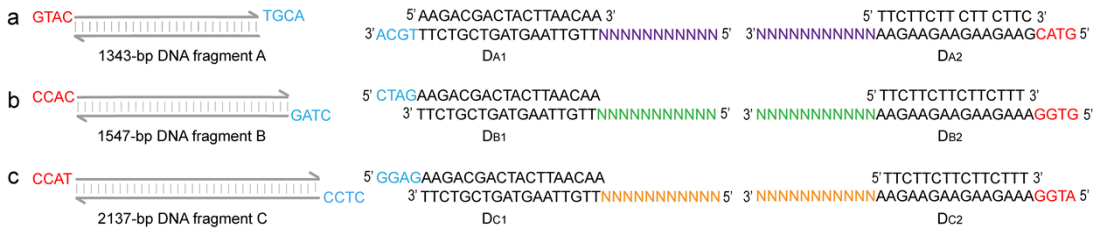

Fig. S3. Sequences of dsDNA fragment with different lengths and pairs of oligo duplexes of (a) $D_{A1,}$ and D_{A2}; (b) $D_{B1,}$ and D_{B2}; and (c) $D_{C1,}$ and D_{C2}.

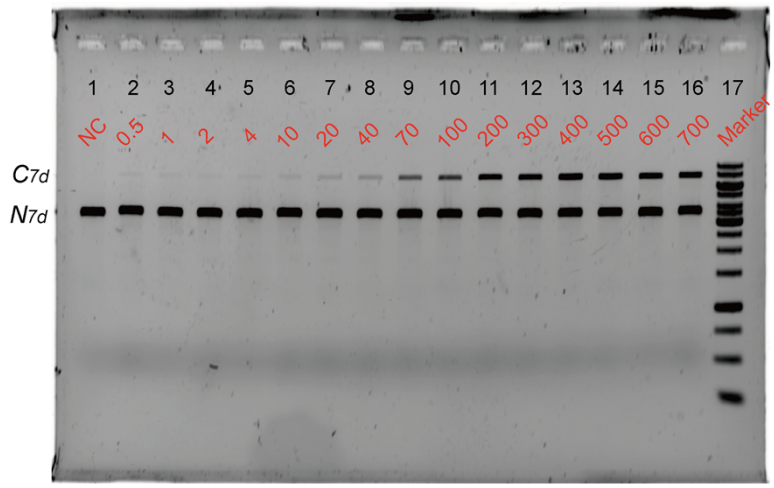

Fig. S4. Intact agarose gel imaging for various concentrations of let-7d ranges from 0.5 to 700 pM. Lane 1 was nanowire pairs without any target. Lane 17 was a 1 kb DNA ladder. Other lanes were target presences at various concentrations ranging from 0.5 to 700 pM (lane 2 to 16: 0.5, 1, 2, 4, 10, 20, 40, 70, 100, 200, 300, 400, 500, 600, and 700 pM).

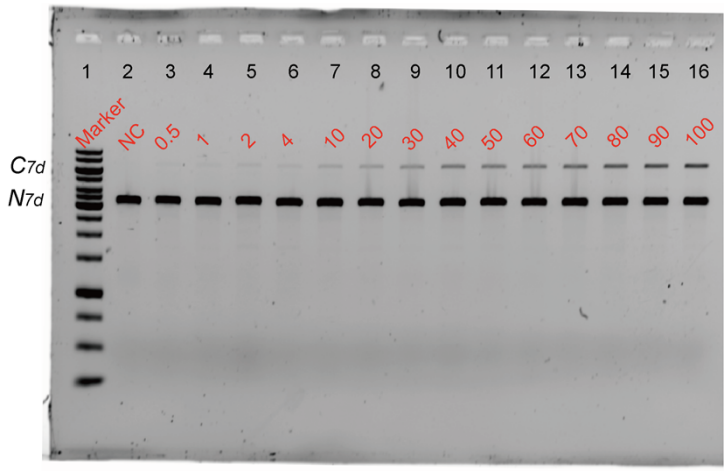

Fig. S5. Intact agarose gel imaging for various concentrations of let-7d range from 0 to 100 pM. Lane 1 was a 1 kb DNA ladder. Lane 2 was nanowire pairs without any target, other lanes were target presences at various concentrations ranging from 0.5 to 100 pM (lane 3 to 16: 0.5, 1, 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 pM).

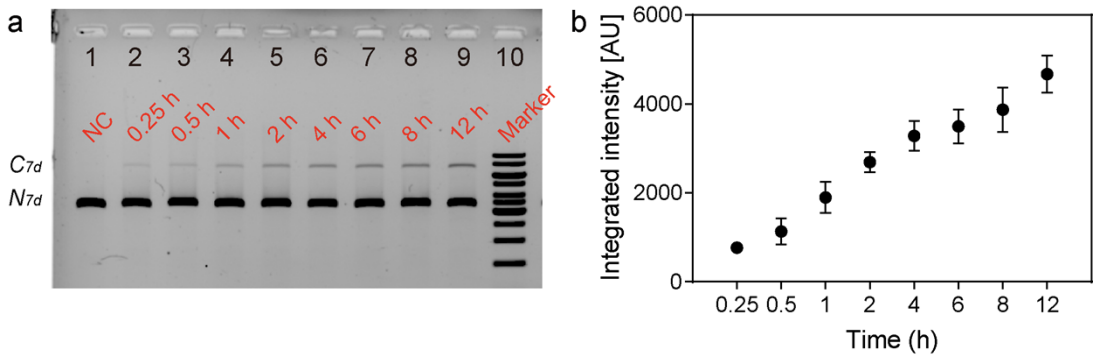

Fig. S6. Influence of incubation time for the nanowire-based strategy for let-7d response. (a) Image of agarose gel readout of 100 pM let-7d incubated with 300 pM of $N_{7d\text{-}1}$ and $N_{7d\text{-}2}$ at different times. Lane 1, negative control (NC); lane 2 to 9: 0.25, 0.5, 1, 2, 4, 6, 8, 12 h; lane 10, 1 kb DNA ladder. (b) Plots of the corresponding C_{7d} band intensities changed with the incubation time.

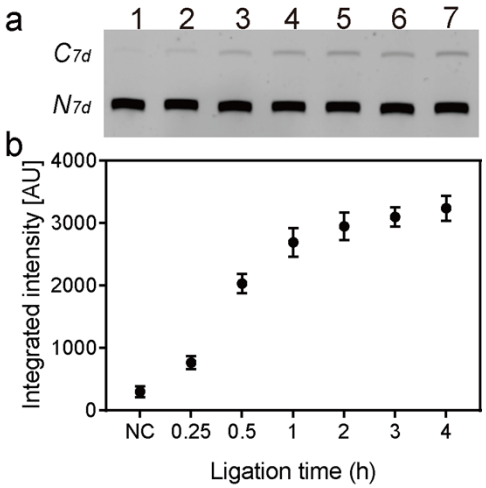

Fig. S7. Influence of ligation time of the nanowire-based strategy. (a) Image of agarose gel readout by the let-7d response with nanowires generated by different ligation time at 16 °C. The 300 pM of $N_{7d\text{-}1}$ and $N_{7d\text{-}2}$ generated by different ligation times interacted with 100 pM let-7d for 4 h at room temperature. NC, negative control with no T4 ligase treatment. Lane 2-7 represents 0.25, 0.5, 1, 2, 3, 4 h. (b) Plots of the corresponding C_{7d} band intensities changed with the ligation time.

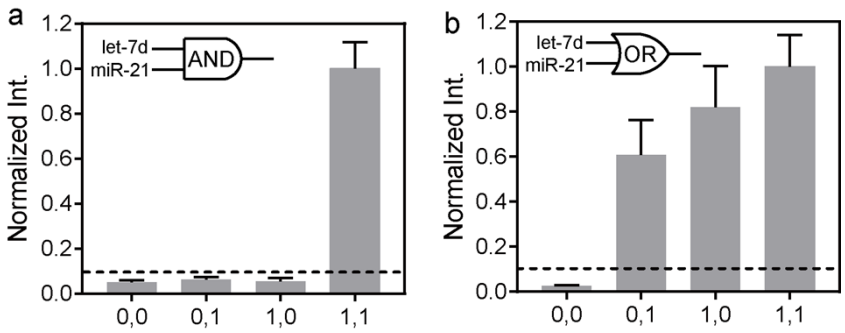

Fig. S8. Normalized gel band intensity of nanowire conjugate complexes of logic gates. (a) The AND gate. (b) The OR gate. The dotted lines represent the normalized gel band intensity threshold value.

S3. Supplementary Tables

Name	Sequence (5' to 3')
let-7d	AGAGGUAGUAGGUUGCAUAGUU
let-7a	UGAGGUAGUAGGUUGUAUAGUU
let-7b	UGAGGUAGUAGGUUGUAUAGUU
let-7c	UGAGGUAGUAGGUUGUAUAGUU
let-7i	UGAGGUAGUAGUUUGUGCUGUU
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-224	UCAAGUCACUAGUGGUUCCGUUUAG
C-U1	p-GGAGAAGACGACTACTTAACAA
U2	TTCTTCTTCTTCTTT
C-let7d-L1	CTACTACCTCTTTGTTAAGTAGTCGTCTT
C-let7d-L2	p-ATGGAAAGAAGAAGAAGAAAACTATGCAAC
C-miR21-L1	CTGATAAGCTATTGTTAAGTAGTCGTCTT
C-miR21-L2	p-ATGGAAAGAAGAAGAAGAATCAACATCAGT
Helper DNA	AACTATGCAACCTGATAAGCTA
A-U1	p-AAGACGACTACTTAACAA
A-miR224-L1	ACTAGTGACTTGATTGTTAAGTAGTCGTCTTTGCA
A-U2	TTCTTCTTCTTCTTC
A-miR224-L2	p-GTACGAAGAAGAAGAAGAACTAAACGGAACCA
B-U1	p-CTAGAAGACGACTACTTAACAA
B-miR21-L1	CTGATAAGCTATTGTTAAGTAGTCGTCTT
B-miR21-L2	p-CTGGAAAGAAGAAGAAGAATCAACATCAGT

Table S1. miRNA and DNA sequences used in this work.

The sequence of dsDNA fragment A (5' to 3'; 1343 bp)
GTACAAGGAATTCTAATCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTG
CCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAA
GGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAG
TAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTG
GGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGA
AAGAACCAGCTGGGGCTCTAGGGGGTATCCCCACGCGCCCTGTAGCGGCGCATTAAGC
GCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGC
CCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAG
CTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCC
AAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTT
TTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGA
ACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCG
GCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTAATTCTGTG
GAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATG
CAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAG
CAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCT
AACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCT
GACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGAGCTATTCCAG
AAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTCCCGGGAGCTT
GTATATCCATTTTCGGATCTGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGA
ACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTAT
GACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGC
AGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCA

Table S2. The sequence of the 1343-bp dsDNA fragment A obtained from pcDNA 3.1+ EGFP plasmid. The 4-nt sticky ends obtained by *PstI* and *BsrGI* digestion were underlined.

The sequence of dsDNA fragment B (5' to 3'; 1547 bp)
CCACCGAGACCCCATTGGGGCCAATACGCCCGCGTTTCTTCCTTTTCCCCACCCCACCC
CCCAAGTTCGGGTGAAGGCCCAGGGCTCGCAGCCAACGTCGGGGCGGCAGGCCCTGC
CATAGCCTCAGGTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAA
AAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGT
TTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCT
TTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGT
TTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAG
CGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAA
CTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCA
GTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGC
GCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGAC
CTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAA
GGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCAC
GAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCAC
CTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAA
ACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATG
TTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCATGCATTAGTTA
TTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTA
CATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGAC
GTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAAT
GGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCC
AAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAG
TACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTAT
TACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCA
CGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAA
TCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGT
AGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGGCTAG

Table S3. The sequence of the 1547-bp dsDNA fragment B obtained from pMCS-EGFP plasmid. The 4-nt sticky ends obtained by *BsaI* and *NheI* digestion are underlined.

The sequence of dsDNA fragment C (5' to 3'; 2137 bp)
CCATTCCTGTAGTCTTCTTAATTAAGACGTCAGAATTCTCGAGGCGGCCGCATGTGCGT
CTCCCTATAGTGAGTCGTATTAATTTCGCGGGCGGAACCCCTATTTGTTTATTTTTCTAA
ATACATTCAAATATGTATCCGCTCATGAGTAGCACCAGGCGTTTAAGGGCACCAATAA
CTGCCTTAAAAAAATTACGCCCCGCCCTGCCACTCATCGCAGTACTGTTGTAATTCATT
AAGCATTCTGCCGACATGGAAGCCATCACAAACGGCATGATGAACCTGAATCGCCAGC
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GGCTGAGACGAAAAACATATTCTCAATAAACCCTTTAGGGAAATAGGCCAGGTTTTCA
CCGTAACACGCCACATCTTGCGAATATATGTGTAGAAACTGCCGGAAATCGTCGTGGT
ATTCACTCCAGAGCGATGAAAACGTTTCAGTTTGCTCATGGAAAACGGTGTAACAAGG
GTGAACACTATCCCATATCACCAGCTCACCGTCTTTCATTGCCATACGGAATTCCGGAT
GAGCATTCATCAGGCGGGCAAGAATGTGAATAAAGGCCGGATAAAACTTGTGCTTATT
TTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCAGCTGAACGGTCTGGTTATAGGTAC
ATTGAGCAACTGACTGAAATGCCTCAAAATGTTCTTTACGATGCCATTGGGATATATCA
ACGGTGGTATATCCAGTGATTTTTTTCTCCATTTTAGCTTCCTTAGCTCCTGAAAATCTC
GATAACTCAAAAAATACGCCCGGTAGTGATCTTATTTCATTATGGTGAAAGTTGGAAC
CTCTTACGTGCCGATCAACGTCTCATTTTCGCCAAAAGTTGTCATGACCAAAATCCCTT
AACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTC
TTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTAC
CAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGC
TTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACC
ACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTG
GCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTAC
CGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGG
AGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCA
CGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAG
GAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGG
GTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCC
TATGGAAAAACGCCAGCAATGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTT
GCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTT
GAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGC
GAGGAAGCCGAAAAATCAATAATCAGACAACAAGATGTGCGAACTCGATATTTTACA
CGACTCTCTTTACCAATTCTGCCCCGAATTACACTTAAAACGACTCAACAGCTTAACGT
TGGCTTGCCACGCATTACTTGACTGTAAAACTCTCACTCTTACCGAACTTGGCCGTAAC
CTGCCAACCAAAGCGAGAACAAAACATAACATCAAACGAATCGACCGATTGTTAGGT
AATCGTCACCTGCAGGAAGGTTTAAACGCATTTAGGTGACACTATAGAAGTGTGTATC
GCTCGAGGGATCCGAATTCGAAGACTTGGTACGGAG

Table S4. The sequence of the 2137-bp dsDNA fragment C obtained from pGGA plasmid. The 4-nt sticky ends obtained by *BsaI* digestion are underlined.

Technique	Strategy	Sensitivity	Advantages	Ref.
Fluorescence	GO, AgNCs	0.31 nM	Label-free, high selectivity	1
CERT	CHA, HCR	0.72 pM	Enzyme-free, high sensitivity	2
FRET	HCR	76 pM	Isothermal, enzyme-free	3
SPR	Hairpin stacking circuits	1.5 pM	Decreased background noise, high sensitivity	4
Colorimetry	SDR, HCR	1 pM	Enzyme-free, good specificity	5
Colorimetry	AuNPs, HCR	23 pM	Label-free	6
Colorimetry	AuNPs, CHA	5 pM	Label-free	7
Gel electrophoresis	DNA-RNA hybridization	3 pM	Label-free, amplification-free, low cost	This work

*GO, Graphene oxide; AgNCs, silver nanoclusters; CERT, chemiluminescence resonance energy transfer; CHA, catalyzed hairpin assembly; HCR, hybridization chain reaction; FRET, fluorescence resonance energy transfer; SPR, surface plasmon resonance; SDR, strand displacement reaction; AuNPs, gold nanoparticles.

Table S5. Comparison of sensitivity and advantages of proposed strategy with some representative miRNA logic systems.

References

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