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Supplementary

Detection of *pks* Island mRNAs Using Toehold Sensors in *Escherichia coli*

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Detailed Experimental Protocols

Plasmid construction

Plasmids were constructed using circular polymerase extension cloning (CPEC) [1], Gibson assembly [2] and round-the-horn site-directed mutagenesis [3]. All DNA templates for helper-assisted mRNA sensing circuit and our algorithm-based mRNA sensor design were obtained by oligonucleotide synthesis. The synthetic DNA strands were PCR amplified and inserted into plasmid backbones. All plasmids were cloned in the E. coli DH5a strain and validated through DNA Sequencing. Backbones for the plasmids were taken from the commercial vectors pET15b, pCDFDuet, and pCOLADuet (EMD Millipore). GFPmut3b_ASV was used as the reporter [4], and the ASV degradation tag addition were done by round-the-horn site-directed mutagenesis. Sequences of elements commonly used in the plasmids are provided in Table S5-S9.

Microplate reader analysis

For the in vivo validation of *mCherry* mRNA sensors (Figure S1), 200 μL of cell cultures after 0.2 % arabinose (*w*/*v*) induction were added per well on a 96-well Black Plate 33396 (SPL). GFP fluorescence (excitation: 479 nm, emission: 520 nm), mCherry fluorescence (excitation: 587 nm, emission: 610 nm) and OD600 were measured at a Synergy H1 microplate reader (Biotek) running Gen5 3.08 software. GFP and mCherry fluorescence levels were normalized as follows: Fluorescence of LB blank was subtracted for background normalization, and a measured fluorescence value was divided by its OD600. The number of biological replicates was three for in vivo experiments.

Supplementary Tables

Supplementary Table S1. Plasmids used in this study. Abbreviations are as follows: pT7 = T7 promoter, T7term = T7 terminator, AmpR = ampicillin resistance gene, SpecR = spectinomycin resistance gene, kanR = kanamycin resistance gene. All the target mRNAs, sensor RNAs and helper RNAs were cloned into pET15b, pCOLADUET, pCDFDUET plasmid, respectively.

Name	Sequence
Cognate trigger	pT7–Cognate trigger–T7term-AmpR-pBR322 origin-LacI
Sensor	pT7-Sensor-Linker-GFPmut3b_ASV-T7term-kanR-ColA origin-LacI
Helper	pT7-Helper-T7term-SpecR-CloDF origin-LacI

Supplementary Table S2. Examples of DNA plasmid sequences. Replication origin and LacI was reversely oriented to other elements.

Name (architecture)	Sequence
mCherry mRNA (pT7–RBS-mCherry–T7term–(Bla Promoter)–AmpR–pBR322 origin–backbone-LacI-(Lac promoter))	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAG AAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGGGCAGCAGCCAT CATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGCGT AAAATGGTGAGCAAGGGCGAAGAAGATAACATGGCCATCATCAAGGAGT TCATGCGCTTCAAGGTTCACATGGAGGGCTCCGTGAACGGCCACGAGTTCG AGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGC CAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCT GTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGA CATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCG CGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCT CCCTGCAAGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAAC TTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACTATGGGCTGGGAGGC CTCCTCCGAGCGGATGTACCCCGAGGACGGCGCGCTGAAGGGCGAGATCA AGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAA GACCACCTACAAGGCCAAGAAGCCCGTGCAACTGCCCGGCGCGTACAACG TCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTG GAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACG AGCTGTACAAGTAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGA GGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATATCCCGCAAGAGGCC CGGCAGTACCGGCATAACCAAGCCTATGCCTACAGCATCCAGGGTGACGG TGCCGAGGATGACGATGAGCGCATTGTTAGATTTCATACACGGTGCCTGAC TGCGTTAGCAATTTAACTGTGATAAACTACCGCATTAAAGCTTATCGATGA TAAGCTGTCAAACATGAGAATTCTTGAAGACGAAAGGGCCTCGTGATACG CCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGT GGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAA TACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTC AATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCC TTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACG CTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTA CATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGA AGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGT ATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTA TTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTAC GGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTG ATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAG CTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTT GGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCAC

GATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAAC
TACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATA
AAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGC
TGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACT
GGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGA
GTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCC
TCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTT
AGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCT
TTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGA
GCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTT
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GGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGC
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GGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTA
ATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGG
TTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAAC
GGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAAC
TGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGG
AGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGC
GCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCG
GGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGG
GCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGC
CTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGT
GGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCG
AACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTG
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GTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATAC
ACTCCGCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACACCCGCCA
ACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTAC
AGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCG
TCATCACCGAAACGCGCGAGGCAGCTGCGGTAAAGCTCATCAGCGTGGTC
GTGAAGCGATTCACAGATGTCTGCCTGTTCATCCGCGTCCAGCTCGTTGAGT
TTCTCCAGAAGCGTTAATGTCTGGCTTCTGATAAAGCGGGCCATGTTAAGG
GCGGTTTTTTCCTGTTTGGTCACTGATGCCTCCGTGTAAGGGGGATTTCTGTT
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TTACTGATGATGAACATGCCCGGTTACTGGAACGTTGTGAGGGTAAACAAC
TGGCGGTATGGATGCGGCGGGACCAGAGAAAAATCACTCAGGGTCAATGC
CAGCGCTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGCAGCAT
CCTGCGATGCAGATCCGGAACATAATGGTGCAGGGCGCTGACTTCCGCGTT
TCCAGACTTTACGAAACACGGAAACCGAAGACCATTCATGTTGTTGCTCAG
GTCGCAGACGTTTTGCAGCAGCAGTCGCTTCACGTTCGCTCGCGTATCGGT
GATTCATTCTGCTAACCAGTAAGGCAACCCCGCCAGCCTAGCCGGGTCCTC
AACGACAGGAGCACGATCATGCGCACCCGTGGCCAGGACCCAACGCTGCC
CGAGATGCGCCGCGTGCGGCTGCTGGAGATGGCGGACGCGATGGATATGT
TCTGCCAAGGGTTGGTTTGCGCATTCACAGTTCTCCGCAAGAATTGATTGGC
TCCAATTCTTGGAGTGGTGAATCCGTTAGCGAGGTGCCGCCGGCTTCCATTC
AGGTCGAGGTGGCCCGGCTCCATGCACCGCGACGCAACGCGGGGAGGCAG
ACAAGGTATAGGGCGGCGCCTACAATCCATGCCAACCCGTTCCATGTGCTC
GCCGAGGCGGCATAAATCGCCGTGACGATCAGCGGTCCAGTGATCGAAGT
TAGGCTGGTAAGAGCCGCGAGCGATCCTTGAAGCTGTCCCTGATGGTCGTC
ATCTACCTGCCTGGACAGCATGGCCTGCAACGCGGGCATCCCGATGCCGCC

	GGAAGCGAGAAGAATCATAATGGGGAAGGCCATCCAGCCTCGCGTCGCGA ACGCCAGCAAGACGTAGCCCAGCGCGTCGGCCGCCATGCCGGCGATAATG GCCTGCTTCTCGCCGAAACGTTTGGTGGCGGGACCAGTGACGAAGGCTTGA GCGAGGGCGTGCAAGATTCCGAATACCGCAAGCGACAGGCCGATCATCGT CGCGCTCCAGCGAAAGCGGTCCTCGCCGAAAATGACCCAGAGCGCTGCCG GCACCTGTCCTACGAGTTGCATGATAAAGAAGACAGTCATAAGTGCGGCG ACGATAGTCATGCCCCGCGCCCACCGGAAGGAGCTGACTGGGTTGAAGGC TCTCAAGGGCATCGGTCGAGATCCCGGTGCCTAATGAGTGAGCTAACTTAC ATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGC CAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTAT TGGGCGCCAGGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGCTGAT TGCCCTTCACCGCCTGGCCCTGAGAGAGTTGCAGCAAGCGGTCCACGCTGG TTTGCCCCAGCAGGCGAAAATCCTGTTTGATGGTGGTTAACGGCGGGATAT AACATGAGCTGTCTTCGGTATCGTCGTATCCCACTACCGAGATATCCGCAC CAACGCGCAGCCCGGACTCGGTAATGGCGCGCATTGCGCCCAGCGCCATC TGATCGTTGGCAACCAGCATCGCAGTGGGAACGATGCCCTCATTCAGCATT TGCATGGTTTGTTGAAAACCGGACATGGCACTCCAGTCGCCTTCCCGTTCC GCTATCGGCTGAATTTGATTGCGAGTGAGATATTTATGCCAGCCAGCCAGA CGCAGACGCGCCGAGACAGAACTTAATGGGCCCGCTAACAGCGCGATTTG CTGGTGACCCAATGCGACCAGATGCTCCACGCCCAGTCGCGTACCGTCTTC ATGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGAA ATAACGCCGGAACATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGG TCATCCAGCGGATAGTTAATGATCAGCCCACTGACGCGTTGCGCGAGAAG ATTGTGCACCGCCGCTTTACAGGCTTCGACGCCGCTTCGTTCTACCATCGAC ACCACCACGCTGGCACCCAGTTGATCGGCGCGAGATTTAATCGCCGCGAC AATTTGCGACGGCGCGTGCAGGGCCAGACTGGAGGTGGCAACGCCAATCA GCAACGACTGTTTGCCCGCCAGTTGTTGTGCCACGCGGTTGGGAATGTAAT TCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGAAACGTG GCTGGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCAT ACTCTGCGACATCGTATAACGTTACTGGTTTCACATTCACCACCCTGAATTG ACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAAGGTTTTGCGCCATTCG ATGGTGTCCGGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGAA GCAGCCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCCGCAAGGAATG GTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCC ACCATACCCACGCCGAAACAAGCGCTCATGAGCCCGAAGTGGCGAGCCCG ATCTTCCCCATCGGTGATGTCCGCGAAAT
Sensor RNA (pT7–Sensor C(RBS)–Linker–GFPmut3b_ASV–T7term–kanR–(Bla Promoter)–ColA origin-backbone-LacI-(Lac promoter))	TAATACGACTCACTATAGGGTGTGGGAGGTGATGTCCAACTTGATGTTGAC GTTGTGTTATAGTTATGAGACAAGAACAGAGGAGACATAACATGAACACA ACGAACTGTGTTAACCTGGCGGCAGCGCAAAAGATGCGTAAAGGAGAAG AACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAA TGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACG GAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCGTG GCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATAC CCAGATCACATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGT TACGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGAC ACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTT AAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGG AATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAG AATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAG CGTTCAACTAGCAGACCATTATCAACAAAATACTCCGATTGGCGATGGCCC TGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAA

GATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACCGCT
GCTGGGATTACACATGGCATGGATGAACTATACAAAAGGCCTGCAGCAAA
CGACGAAAACTACGCTGCATCAGTTTAATAAGATAAACCAGAGCGGCACG
GCAAGCAGAGTATACGAGATTCGGTAGCCACCGCTGAGCAATAACTAGCA
TAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAACCTC
AGGCATTTGAGAAGCACACGGTCACACTGCTTCCGGTAGTCAATAAACCG
GTAAACCAGCAATAGACATAAGCGGCTATTTAACGACCCTGCCCTGAACC
GACGACAAGCTGACGACCGGGTCTCCGCAAGTGGCACTTTTCGGGGAAAT
GTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATC
CGCTCATGAATTAATTCTTAGAAAAACTCATCGAGCATCAAATGAAACTGC
AATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCT
GTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCC
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TCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACG
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CAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAA
CCGTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGGTCGCTG
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CTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATAC
CTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATC
AGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCA
GCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTT
GCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAATCGATA
GATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATAT
AAATCAGCATCCATGTTGGAATTTAATCGCGGCCTAGAGCAAGACGTTTCC
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GGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAA
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CGACGATCGTAAAAGGATCTCAAGAAGATCCTTTACGGATTCCCGACACC
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CCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGC
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GTCGTATCCCACTACCGAGATGTCCGCACCAACGCGCAGCCCGGACTCGGT
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	AGTGGGAACGATGCCCTCATTCAGCATTTGCATGGTTTGTTGAAAACCGGA CATGGCACTCCAGTCGCCTTCCCGTTCCGCTATCGGCTGAATTTGATTGCGA GTGAGATATTTATGCCAGCCAGCCAGACGCAGACGCGCCGAGACAGAACT TAATGGGCCCGCTAACAGCGCGATTTGCTGGTGACCCAATGCGACCAGAT GCTCCACGCCCAGTCGCGTACCGTCTTCATGGGAGAAAATAATACTGTTGA TGGGTGTCTGGTCAGAGACATCAAGAAATAACGCCGGAACATTAGTGCAG GCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGATAGTTAATGATC AGCCCACTGACGCGTTGCGCGAGAAGATTGTGCACCGCCGCTTTACAGGCT TCGACGCCGCTTCGTTCTACCATCGACACCACCACGCTGGCACCCAGTTGA TCGGCGCGAGATTTAATCGCCGCGACAATTTGCGACGGCGCGTGCAGGGC CAGACTGGAGGTGGCAACGCCAATCAGCAACGACTGTTTGCCCGCCAGTT GTTGTGCCACGCGGTTGGGAATGTAATTCAGCTCCGCCATCGCCGCTTCCA CTTTTTCCCGCGTTTTCGCAGAAACGTGGCTGGCCTGGTTCACCACGCGGGA AACGGTCTGATAAGAGACACCGGCATACTCTGCGACATCGTATAACGTTAC TGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGGCGCTATCATGCC ATACCGCGAAAGGTTTTGCGCCATTCGATGGTGTCCGGGATCTCGACGCTC TCCCTTATGAAGTCTAACGCTGCTCTGGGCTAACTGTC
Helper RNA (pT7–Helper2–T7term–pT7–Helper1–T7term–SpecR–(Bla Promoter)–CloDF13 origin–backbone- LacI-(Lac promoter))	TAATACGACTCACTATAGGGGGACCGGTCTTTGCAGAGACCGGTTGCCGCC GGTGGAGTGGCGGCCCTCGGCGCGGGGGTACTGTTCCACGATGGTGTAGTC CTACTTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG CTGAAACCTCAGGCATTTGAGAAGCACACGGTCACACTGCTTCCGGTAGTC AATAAATAATACGACTCACTATAGGGCGTGAGATAAGCACATCTCACGTG TACGCGCCGGGCAGTTGCACGGGCTTCTTAAGCTTGTAGGTGGTCTTGACCT CAGCGACTTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTT TTTGCCGGTAAACCAGCAATAGACATAAGCGGCTATTTAACGACCCTGCCC TGAACCGACGACCGGGTCATCGTGGCCGGATCTTGCGGCCCCTCGGCTTGA ACGAATTGTTAGACATTATTTGCCGACTACCTTGGTGATCTCGCCTTTCACG TAGTGGACAAATTCTTCCAACTGATCTGCGCGCGAGGCCAAGCGATCTTCT TCTTGTCCAAGATAAGCCTGTCTAGCTTCAAGTATGACGGGCTGATACTGG GCCGGCAGGCGCTCCATTGCCCAGTCGGCAGCGACATCCTTCGGCGCGATT TTGCCGGTTACTGCGCTGTACCAAATGCGGGACAACGTAAGCACTACATTT CGCTCATCGCCAGCCCAGTCGGGCGGCGAGTTCCATAGCGTTAAGGTTTCA TTTAGCGCCTCAAATAGATCCTGTTCAGGAACCGGATCAAAGAGTTCCTCC GCCGCTGGACCTACCAAGGCAACGCTATGTTCTCTTGCTTTTGTCAGCAAG ATAGCCAGATCAATGTCGATCGTGGCTGGCTCGAAGATACCTGCAAGAAT GTCATTGCGCTGCCATTCTCCAAATTGCAGTTCGCGCTTAGCTGGATAACGC CACGGAATGATGTCGTCGTGCACAACAATGGTGACTTCTACAGCGCGGAG AATCTCGCTCTCTCCAGGGGAAGCCGAAGTTTCCAAAAGGTCGTTGATCAA AGCTCGCCGCGTTGTTTCATCAAGCCTTACGGTCACCGTAACCAGCAAATC AATATCACTGTGTGGCTTCAGGCCGCCATCCACTGCGGAGCCGTACAAATG TACGGCCAGCAACGTCGGTTCGAGATGGCGCTCGATGACGCCAACTACCTC TGATAGTTGAGTCGATACTTCGGCGATCACCGCTTCCCTCATACTCTTCCTT TTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACA TATTTGAATGTATTTAGAAAAATAAACAAATAGCTAGCTCACTCGGTCGCT ACGCTCCGGGCGTGAGACTGCGGCGGGCGCTGCGGACACATACAAAGTTA CCCACAGATTCCGTGGATAAGCAGGGGACTAACATGTGAGGCAAAACAGC AGGGCCGCGCCGGTGGCGTTTTTCCATAGGCTCCGCCCTCCTGCCAGAGTT CACATAAACAGACGCTTTTCCGGTGCATCTGTGGGAGCCGTGAGGCTCAAC CATGAATCTGACAGTACGGGCGAAACCCGACAGGACTTAAAGATCCCCAC CGTTTCCGGCGGGTCGCTCCCTCTTGCGCTCTCCTGTTCCGACCCTGCCGTTT ACCGGATACCTGTTCCGCCTTTCTCCCTTACGGGAAGTGTGGCGCTTTCTCA

	TAGCTCACACACTGGTATCTCGGCTCGGTGTAGGTCGTTCGCTCCAAGCTG GGCTGTAAGCAAGAACTCCCCGTTCAGCCCGACTGCTGCGCCTTATCCGGT AACTGTTCACTTGAGTCCAACCCGGAAAAGCACGGTAAAACGCCACTGGC AGCAGCCATTGGTAACTGGGAGTTCGCAGAGGATTTGTTTAGCTAAACACG CGGTTGCTCTTGAAGTGTGCGCCAAAGTCCGGCTACACTGGAAGGACAGAT TTGGTTGCTGTGCTCTGCGAAAGCCAGTTACCACGGTTAAGCAGTTCCCCA ACTGACTTAACCTTCGATCAAACCACCTCCCCAGGTGGTTTTTTCGTTTACA GGGCAAAAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGAT CTTTTCTACTGAACCGCTCTAGATTTCAGTGCAATTTATCTCTTCAAATGTA GCACCTGAAGTCAGCCCCATACGATATAAGTTGTAATTCTCATGTTAGTCA TGCCCCGCGCCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGC ATCGGTCGAGATCCCGGTGCCTAATGAGTGAGCTAACTTACATTAATTGCG TTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATT AATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCCAG GGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCACC GCCTGGCCCTGAGAGAGTTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGC AGGCGAAAATCCTGTTTGATGGTGGTTAACGGCGGGATATAACATGAGCTG TCTTCGGTATCGTCGTATCCCACTACCGAGATGTCCGCACCAACGCGCAGC CCGGACTCGGTAATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCA ACCAGCATCGCAGTGGGAACGATGCCCTCATTCAGCATTTGCATGGTTTGT TGAAAACCGGACATGGCACTCCAGTCGCCTTCCCGTTCCGCTATCGGCTGA ATTTGATTGCGAGTGAGATATTTATGCCAGCCAGCCAGACGCAGACGCGCC GAGACAGAACTTAATGGGCCCGCTAACAGCGCGATTTGCTGGTGACCCAA TGCGACCAGATGCTCCACGCCCAGTCGCGTACCGTCTTCATGGGAGAAAAT AATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATAACGCCGGAA CATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGAT AGTTAATGATCAGCCCACTGACGCGTTGCGCGAGAAGATTGTGCACCGCC GCTTTACAGGCTTCGACGCCGCTTCGTTCTACCATCGACACCACCACGCTG GCACCCAGTTGATCGGCGCGAGATTTAATCGCCGCGACAATTTGCGACGGC GCGTGCAGGGCCAGACTGGAGGTGGCAACGCCAATCAGCAACGACTGTTT GCCCGCCAGTTGTTGTGCCACGCGGTTGGGAATGTAATTCAGCTCCGCCAT CGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGAAACGTGGCTGGCCTGGTTC ACCACGCGGGAAACGGTCTGATAAGAGACACCGGCATACTCTGCGACATC GTATAACGTTACTGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGG CGCTATCATGCCATACCGCGAAAGGTTTTGCGCCATTCGATGGTGTCCGGG ATCTCGACGCTCTCCCTTATGAGTGATAGCCGTTTGTCTGGTGTCTACGCCG CGCGGGCTAACTGTC

Supplementary Table S3. *mCherry* mRNA sensor sequences used in this study. Common sequence element from high-performance first-generation toehold switch was colored in yellow [3]. Conserved linker sequence was indicated as gray. Trigger binding region was covered in green and its complementary region was colored in blue. Plasmid sequences of *mCherry* sensor can be constructed by replacing the sensor RNA region in the example plasmids in Table S2 with the *mCherry* sensor sequences described underneath.

Name	Target site	Sequence
Sensor A	448:477	AGGGGAAGTTGGTGCCGCGCAGCTTCACGTTATAGTTATGAGACAAGAACAGAGGAGACATAACATGAACGTGAAGAACCACGTTAACCTGGCGGCAGCGCAAAAG
Sensor C	662:697	TGTGGGAGGTGATGTCCAACTTGATGTTGACGTTGTGTTATAGTTATGAGACAAGAACAGAGGAGACATAACATGAACACAACGAACTGTGTTAACCTGGCGGCAGCGCAAAAG
Sensor Variant 1	568:602	TAGTGGCCGCCGTCCTTCAGCTTCAGCCTCTGCTTGTTATAGTTATGAGACAAGAACAGAGGAGACATAACATGAACAAGCAGAACCTTGTTAACCTGGCGGCAGCGCAAAAG
Sensor Variant 2	463:498	CTTCTGCATTACGGGGCCGTCGGAGGGGAAGTTGGTGTTATAGTTATGAGACAAGAACAGAGGAGACATAACATGAACACCAACAACGGTGTTAACCTGGCGGCAGCGCAAAAG

Supplementary Table S4. Helper sequences for *mCherry* mRNA sensor used in this study. Plasmid sequences of *mCherry* helper can be constructed by replacing the Helper 1 and Helper 2 region in the example plasmids in Table S2 with the appropriate helper sequences described underneath. 5′ structure was colored in green. mRNA binding regions were colored in yellow, and 3 nt bulge was colored in gray.

Name	Target site	Position	Sequence
Helper C			
H1-60	599:628 + 629:658	Upstream	CGTGAGATAAGCACATCTCACGCGCCGGGCAGTTGCACGGGCTTCTTGGCCTACATGTAGGTGGTCTTGACCTCAGCGTCGTAGT
H1-30	701:730 + 731:760	Upstream	CGTGAGATAAGCACATCTCACGCGCCGGGCAGTTGCACGGGCTTCTTGGCCT
H1-15	629:658	Upstream	CGTGAGATAAGCACATCTCACGCGCCGGGCAGTTGCA
H2-60	701:730	Downstream	ACCGGTCTTTGCAGAGACCGGTTGCCGCCGGTGGAGTGGCGGCCCTCGGCGCCGGGTTCGTACTGTTCCACGATGGTGTAGTCCT
H2-30	644:658	Downstream	ACCGGTCTTTGCAGAGACCGGTGTTCGTACTGTTCCACGATGGTGTAGTCCT
H2-15	701:715	Downstream	ACCGGTCTTTGCAGAGACCGGTCGATGGTGTAGTCCT
Helper variant 1			
H1-60	505:534 + 535:564	Upstream	GTCCCACCAAAACAGGTGGGACACACTCGCCCTTCAGCGCGCCGTCCTCGGGGTAGGGCATCCGCTCGGAGGAGGCCTCCCAGCCCAT
H2-60	606:635 + 636:665	Downstream	GACCGTCCGGGCTAGGACGGTCATTTTGTACGCGCCGGGCAGTTGCACGGGCTTCGGGTTGGCCTTGTAGGTGGTCTTGACCTCAGCG
H2-30	606:635	Downstream	GACCGTCCGGGCTAGGACGGTCATTTTGGCCTTGTAGGTGGTCTTGACCTCAGCG
H2-15	606:620	Downstream	GACCGTCCGGGCTAGGACGGTCATTGTCTTGACCTCAGCG

Supplementary Table S5. *clb* mRNA sequences used in this study. Plasmid sequences of *clb* mRNA can be constructed by replacing Table S2. with the *clb* mRNA sequences described underneath.

Name	Sequence
clbA	ATGAGGATTGATATATTAATTGGACATACTAGTTTTTTTCATCAAACCAGTAGAGATAACTTCCTT CACTATCTCAATGAGGAAGAAATAAAACGCTATGATCAGTTTCATTTTGTGAGTGATAAAGAACT CTATATTTTAAGCCGTATCCTGCTCAAAACAGCACTAAAAAGATATCAACCTGATGTCTCATTAC AATCATGGCAATTTAGTACGTGCAAATATGGCAAACCATTTATAGTTTTTCCTCAGTTGGCAAAA AAGATTTTTTTTAACCTTTCCCATACTATAGATACAGTAGCCGTTGCTATTAGTTCTCACTGCGAG CTTGGTGTCGATATTGAACAAATAAGAGATTTAGACAACTCTTATCTGAATATCAGTCAGCATTTT TTTACTCCACAGGAAGCTACTAACATAGTTTCACTTCCTCGTTATGAAGGTCAATTACTTTTTTGG AAAATGTGGACGCTCAAvAGAAGCTTACATCAAATATCGAGGTAAAGGCCTATCTTTAGGACTG GATTGTATTGAATTTCATTTAACAAATAAAAAACTAACTTCAAAATATAGAGGTTCACCTGTTTAT TTCTCTCAATGGAAAATATGTAACTCATTTCTCGCATTAGCCTCTCCACTCATCACCCCTAAAATA ACTATTGAGCTATTTCCTATGCAGTCCCAACTTTATCACCACGACTATCAGCTAATTCATTCGTCA AATGGGCAGAATTGA
clbE	ATGAAAAAGCAAGATATGAAAGCCGCCATTCGGGAATTTCTTTCACGCTCATTACGTGGGCATAC GTTGAACGATGATGACGATATTTTTTCTCTCGGGCTTGTCCATTCGTTATTTACTGTGCAAATCATA CTGTTTATAGAAAAAAATTTTCAGGTTGAGCTGGAAGTGAGTGAGTTGAAAACAGAACAGATTG CTACCGTCAATAAAATAGTGGAGCTCATTCAGCGACAAACAGGCCTGGAGTAA
clbP	ATGACAATAATGGAACACGTTAGCATTAAAACATTATATCATCTCCTGTGCTGTATGCTGCTCTTT ATTTCCGCTATGTGCGCTTTGGCGCAAGAACATGAGCCTATCGGGGCGCAAGATGAGCGCCTGTC GACATTAATTCACCAACGGATGCAGGAGGCCAAGGTCCCAGCCCTTTCCGTAAGTGTGACCATT AAGGGGGTACGTCAGCGATTTGTCTACGGTGTTGCCGATGTGGCTAGTCAGAAAGCGAATACTCT AGACACAGTTTACGAGCTGGGATCGATGAGTAAGGCGTTTACCGGACTTGTGGTGCAAATACTG ATTCAGGAAGGCAGACTCCGGCAAGGGGATGATATCATTACCTATCTGCCGGAAATGCGCTTGA ATTATCAGGGAAAACCTGCTTCCCTGACCGTGGCTGATTTCCTTTATCATACATCAGGATTGCCTT TTTCAACACTGGCTCGGCTGGAAAACCCTATGCCTGGGAGCGCTGTGGCACAGCAACTGCGCAA CGAGAATCTGCTGTTTGCGCCGGGTGCGAAGTTTAGCTATGCCTCCGCCAATTATGATGTGTTGGG CGCGGTGATTGAAAATGTGACGGGAAAAACCTTTACAGAGGTCATTGCGGAACGACTCACGCAG CCGCTGGGCATGTCGGCGACTGTGGCAGTTAAGGGGGATGAGATTATTGTCAACAAGGCAAGCG GCTATAAACTGGGATTCGGCAAACCCGTTCTGTTTCATGCGCCTCTGGCCCGGAACCATGTTCCTG CCGCCTATATCCATAGCACTCTGCCTGATATGGAAATATGGATAGACGCCTGGTTGCACAGAAAG GCTTTGCCGGCAACGCTGCGTGAGGCGATGAGTAACAGTTGGCGTGGTAATAGTGATGTTCCGCT TGCCGCAGACAATCGTATCCTCTATGCCAGCGGTTGGTTTATCGACCAGAATCAAGGCCCTTACA TCAGTCACGGTGGGCAGAATCCAAACTTTTCTTCTTGCATTGCGTTGCGACCGGATCAGCAGATT GGCATTGTTGCGCTGGCAAATATGAATTCGAATCTGATACTACAGCTTTGCGCGGATATCGATAA TTATCTGCGCATTGGCAAATATGCTGACGGCGCTGGTGATGCAATTACAGCCACCGATACCCTTT TCGTCTACCTCACGTTGTTGCTGTGTTTTTGGGGGGCGGTGGTTGTAGTGCGCGGTGCTTTCCGTGT TTATCGCGCAACGGCGCATGGCCCTGGAAAACAGCAGAGGTTACGTTTACGCGTACGTGACTAT ATCATCGCCTTGGCGGTTCCTGGGCTCGTGGCCGCCATGCTCTATGTCGCACCGGGTATACTATCT CCAGGACTTGACTGGCGTTTTATCTTGGTATGGGGTCCATCGAGCGTGTTGGCGATACCGTTCGGA ATTATCCTGTTAGCTTTCGTTCTGACATTAAATCATCAAATTAAACGAATTCTATTACACAACAAG GAGTGGGACGATGAGTAA
clbQ	ATGAGTAATATCAGTTTGTATTGTTTGCCATATTCAGGTGGTTCTGCCGCCATGTATTATAAATGG CGTAGCGTGCTGTCGGACAATATTACTTTGCGGCCTTTAGAACCTGCGGGGAGGGGAACTAGAAT ACGCCAGCCGCTGTGTCTTACGATGGTGGATGCCGTCGCTGACCTTTATCAACAATTTGTGAAAC ACTACACAGGTGGAGACTACGCCATTTTTGGGCATAGTCTCGGAGGGATCATGGCCTTCGAACTG GTGCATTATATTCTCGATCATGGACATGACATGCCATGCGCGCTGTTTTTTTCCGGCTGTCGCCCA CCCGATCGGGCCTCTCATGAAGTAATACTGCATACCTTGCCCGATCAGGCGTTTATGGAAGAGAT CGTCAAGCTGGGCGGAACTCCGGTTGATGTCTTTCGTAATAAAGAGTTAATGACAATTTTCACCC

	CCATCATTAAAAACGATTATCGGCTCTATGAGCAGTATGTATTTCAGGCCAAGGCGCGCACATTA ACCTGTCCGATCGTGCTATTTCATGGCGATGCTGACAATCTGGTAATGCAGGATGAATTACTTGC ATGGGAAAAATTCACCACACGAAAGACGCGGACTATTATATTTCCTGCTGCCGATCATTTTTTTGT CGATAAGCATTTTGAACAGGTGGTAGGTTATGTGAACCAGACGATTGAATCACTCGAAATAGTA GGGTAG

Supplementary Table S6. *clb* synthetic short trigger sequences used in this study.

Name	Target site	Classification	Sequence
clbA	390:419	open	TTTTTTTACTCCACAGGAAGCTACTAACAT
	477:506	open	AGAAGCTTACATCAAATATCGAGGTAAAGG
	447:476	structured	ATTACTTTTTTGGAAAATGTGGACGCTCAA
	513:542	structured	TTTAGGACTGGATTGTATTGAATTTCATTT
	630:659	intermediate	CTCTCCACTCATCACCCCTAAAATAACTAT
clbE	1:30	open	ATGAAAAAGCAAGATATGAAAGCCGCCATT
	219:248	open	GCTCATTCAGCGACAAACAGGCCTGGAGTA
	95:124	structured	TCGGGCTTGTCCATTCGTTATTTACTGTGC
	40:69	structured	CTTTCACGCTCATTACGTGGGCATACGTTG
	83:112	intermediate	ATATTTTTTCTCTCGGGCTTGTCCATTCGT
clbP	969:998	open	TTACATCAGTCACGGTGGGCAGAATCCAAA
	992:1021	open	ATCCAAACTTTTCTTCTTGCATTGCGTTGC
	1205:1234	structured	CGGTGGTTGTAGTGCGCGGTGCTTTCCGTG
	846:875	structured	TTTGCCGGCAACGCTGCGTGAGGCGATGAG
	160:189	intermediate	GCCAAGGTCCCAGCCCTTTCCGTAAGTGTG
clbQ	256:285	open	GAACTGGTGCATTATATTCTCGATCATGGA
	506:535	open	CCAAGGCGCGCACATTAACCTGTCCGATCG
	133:162	structured	CGCCAGCCGCTGTGTCTTACGATGGTGGAT
	475:504	structured	TATCGGCTCTATGAGCAGTATGTATTTCAG
	86:115	intermediate	ATATTACTTTGCGGCCTTTAGAACCTGCGG

Supplementary Table S7. *clb* mRNA sensor sequences used in this study. Common sequence element from high-performance first-generation toehold switch was colored in yellow [3]. Conserved linker sequence was colored as gray. Trigger binding region was covered in green and its complementary region was colored in cyan. Plasmid sequences of *clb* sensor can be constructed by replacing the sensor RNA region in the example plasmids in Table S2 with the *clb* sensor sequences described underneath.

Name	Target site	Classification	Sequence
clbA	390:419	open	ATGTTAGTAGCTTCCTGTGGAGTAAAAAAAGTTATAGTTATGAGACAA GAACAGAGGAGACATAACATGAACTTTTTTAACAAAGTTAACCTGGC GGCAGCGCAAAAG
	477:506	open	CCTTTACCTCGATATTTGATGTAAGCTTCTGTTATAGTTATGAGACAAG AACAGAGGAGACATAACATGAACAGAAGCAACTCTGTTAACCTGGCG GCAGCGCAAAAG
	447:476	structured	TTGAGCGTCCACATTTTCCAAAAAAGTAATGTTATAGTTATGAGACAA GAACAGAGGAGACATAACATGAACATTACTAACAATGTTAACCTGGC GGCAGCGCAAAAG
	513:542	structured	AAATGAAATTCAATACAATCCAGTCCTAAAGTTATAGTTATGAGACA AGAACAGAGGAGACATAACATGAACTTTAGGAACAAAGTTAACCTG GCGGCAGCGCAAAAG

	630:659	intermediate	ATAGTTATTTTAGGGGTGATGAGTGGAGAGGTTATAGTTATGAGACAA GAACAGAGGAGACATAACATGAACCTCTCCAACGAGGTTAACCTGGC GGCAGCGCAAAAG
clbE	1:30	open	AATGGCGGCTTTCATATCTTGCTTTTTCATGTTATAGTTATGAGACAAG AACAGAGGAGACATAACATGAACATGAAAAACCATGTTAACCTGGC GGCAGCGCAAAAG
	219:248	open	TACTCCAGGCCTGTTTGTCGCTGAATGAGCGTTATAGTTATGAGACAA GAACAGAGGAGACATAACATGAACGCTCATAACAGCGTTAACCTGGC GGCAGCGCAAAAG
	95:124	structured	GCACAGTAAATAACGAATGGACAAGCCCGAGTTATAGTTATGAGACA AGAACAGAGGAGACATAACATGAACTCGGGCAACCGAGTTAACCTG GCGGCAGCGCAAAAG
	40:69	structured	CAACGTATGCCCACGTAATGAGCGTGAAAGGTTATAGTTATGAGACA AGAACAGAGGAGACATAACATGAACCTTTCAAACAAGGTTAACCTGG CGGCAGCGCAAAAG
	83:112	intermediate	ACGAATGGACAAGCCCGAGAGAAAAAATATGTTATAGTTATGAGACA AGAACAGAGGAGACATAACATGAACATATTTAACTATGTTAACCTGG CGGCAGCGCAAAAG
clbP	969:998	open	TTTGGATTCTGCCCACCGTGACTGATGTAAGTTATAGTTATGAGACAA GAACAGAGGAGACATAACATGAACTTACATAACCTGGCGGCAGCGC AAAAG
	992:1021	open	GCAACGCAATGCAAGAAGAAAAGTTTGGATGTTATAGTTATGAGACA AGAACAGAGGAGACATAACATGAACATCCAAAACGATGTTAACCTG GCGGCAGCGCAAAAG
	1205:1234	structured	CACGGAAAGCACCGCGCACTACAACCACCGGTTATAGTTATGAGACA AGAACAGAGGAGACATAACATGAACCGGTGGAACCCGGTTAACCTG GCGGCAGCGCAAAAG
	846:875	structured	CTCATCGCCTCACGCAGCGTTGCCGGCAAAGTTATAGTTATGAGACAA GAACAGAGGAGACATAACATGAACTTTGCCAACAAAGTTAACCTGGC GGCAGCGCAAAAG
	160:189	intermediate	CACACTTACGGAAAGGGCTGGGACCTTGGCGTTATAGTTATGAGACA AGAACAGAGGAGACATAACATGAACGCCAAGAACGGCGTTAACCTG GCGGCAGCGCAAAAG
clbQ	256:285	open	TCCATGATCGAGAATATAATGCACCAGTTCGTTATAGTTATGAGACAA GAACAGAGGAGACATAACATGAACGAACTGAACTTCGTTAACCTGGC GGCAGCGCAAAAG
	506:535	open	CGATCGGACAGGTTAATGTGCGCGCCTTGGGTTATAGTTATGAGACAA GAACAGAGGAGACATAACATGAACCCAAGGAACTGGGTTAACCTGG CGGCAGCGCAAAAG
	133:162	structured	ATCCACCATCGTAAGACACAGCGGCTGGCGGTTATAGTTATGAGACA AGAACAGAGGAGACATAACATGAACCGCCAGAACGCGGTTAACCTG GCGGCAGCGCAAAAG
	475:504	structured	ATCCACCATCGTAAGACACAGCGGCTGGCGGTTATAGTTATGAGACA AGAACAGAGGAGACATAACATGAACCGCCAGAACGCGGTTAACCTG GCGGCAGCGCAAAAG
	86:115	intermediate	CCGCAGGTTCTAAAGGCCGCAAAGTAATATGTTATAGTTATGAGACA AGAACAGAGGAGACATAACATGAACATATTAAACTATGTTAACCTGG CGGCAGCGCAAAAG

Supplementary Table S8. Helper sequences for *clb* mRNA sensor used in this study. Plasmid sequences of *clb* helper can be constructed by replacing the Helper 1 and Helper 2 region in the example plasmids in Table S2 with the appropriate helper sequences described underneath. 5′ structure was colored in green. mRNA binding regions were covered in yellow.

Name	Target site	Position	Sequence
Open			
*clbA*1	357:386	upstream	GCTCCAGCCCGCTCGCTGGAGCACCTGACTGATATTCAGATAA GAGTTGTCTAAAACC
	423:452	downstream	GGCTGCTCGGGGAGGAGCAGCCGAAAGTAATTGACCTTCATA ACGAGGAAGTGAAACC
*clbA*2	444:473	upstream	GCACCGCCAAGTACGGCGGTGCCCCAGCGTCCACATTTTCCAA AAAAGTAATTGAATT
	510:539	downstream	GCCCCTACAAGTCCGTAGGGGCCTCTGAAATTCAATACAATCC AGTCCTAAAGATCCC
*clbP*1	936:965	upstream	GGTCGAGGAAAACGCCTCGACCGTACCTTGATTCTGGTCGATA AACCAACCGCTGCGC
	1002:1031	downstream	GCCCCCGCACATCAGCGGGGGCAAATGATCCGGTCGCAACGC AATGCAAGAAGAAAAG
*clbP*2	959:988	upstream	GGAGCTGCAACGGAGCAGCTCCACCGCCCACCGTGACTGATG TAAGGGCCTTGATCTA
	1025:1054	downstream	GCCGCGTCGACTAAGACGCGGCACGCCAGCGCAACAATGCCA ATCTGCTGATCCGACA
*clbQ*1	223:252	upstream	GCGGTCGCTCCGCCGCGACCGCAACGGCCATGATCCCTCCGAG ACTATGCCCAAAGTC
	289:318	downstream	CGCCTCGCAAAAAAGCGAGGCGTAAGCCGGAAAAAAACAGC GCGCATGGCATGTCGAT
*clbQ*2	473:502	upstream	GGACCCTCTTTGCTGAGGGTCCACCGAAATACATACTGCTCAT AGAGCCGATAATACC
	539:568	downstream	GCCCAAGCCCACCCGCTTGGGCATATTACCAGATTGTCAGCAT CGCCATGAAATACCC
Intermediate			
clbA	597:626	upstream	GGACGTACCATTCAGTACGTCCTTAAATGCGAGAAATGAGTTA CATATTTTCCATCGG
	663:692	downstream	GACGGTCCGGCGGAGGACCGTCACATGATAAAGTTGGGACTG CATAGGAAATAGCGAA
clbE	127:156	upstream	GGGCGGCCTAACCCGGCCGCCCTTTCATCATCGTTCAACGTAT GCCCACGTAATGGCT
	193:222	downstream	GCGCCCTGGCGCGACAGGGCGCACCTTTCTATAAACAGTATGA TTTGCACAGTAACCC
clbP	50:79	upstream	GGGTGAGGGCGAAACCTCACCCAACCTGCATCCGTTGGTGAAT TAATGTCGACAGTCC
	116:145	downstream	CAGTAGGCGACCCAGCCTACTGAGCGTAGACAAATCGCTGAC GTACCCCCTTAATTAA
clbQ	53:82	upstream	GGCGGCGCGATGGAGCGCCGCCAAACCGACAGCACGCTACGC CATTTATAATACACAC
	119:148	downstream	GCCGGAGCATATCCGCTCCGGCTTCGACACAGCGGCTGGCGTA TTCTAGTTCCCCCCA

Supplementary Table S9. Other accessory sequences used in this study. Accessory sequences used for constructing plasmids are indicated here.

Name	**Sequence**
RBS	AGAAGGAGA or AGAGGAGA
Linker	AACCTGGCGGCAGCGCAAAAG
T7term	TAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
GFPmut3b_ASV	ATGCGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCGTGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATACCCAGATCACATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTACGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCGATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACCGCTGCTGGGATTACACATGGCATGGATGAACTATACAAAAGGCCTGCAGCAAACGACGAAAACTACGCTGCATCAGTTTAATAA
mCherry	ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCataTgCGtaAAATGGTGAGCAAGGGCGAAGAAGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTTCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAAGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACTATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCGCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAACTGCCCGGCGCGTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAA

Supplementary Table S10. Primers used for qPCR.

Primer Name	Sequence (5′ to 3′)
*clbA*_fwd	ATTAGTTCTCACTGCGAGC
*clbA*_rev	TCTTTGAGCGTCCACATTT
*clbP*_fwd	GAGTGCTATGGATATAGGCG
*clbP*_rev	CCAATTATGATGTGTTGGGC
*clbE*_fwd	GTTTTCAACTCACTCACTTCC
*clbE*_rev	ATTTCTTTCACGCTCATTACG
*clbQ*_fwd	GGTGAATTTTTCCCATGCAA
*clbQ*_rev	AGGCGTTTATGGAAGAGATC
*mCherry*_fwd	GAAGATAACATGGCCATCATCAAGG
*mCherry*_rev	TACATGAACTGAGGGGACAGGAT
16S_rRNA_fwd	TGGTAGTCCACGCCGTAAAC
16S_rRNA_rev	TTTAACCTTGCGGCCGTACT
*cysG*_fwd	ATGCGGTGAACTGTGGAATAAACG
*cysG*_rev	CTCTTGCCATCGGATGTGCCCA

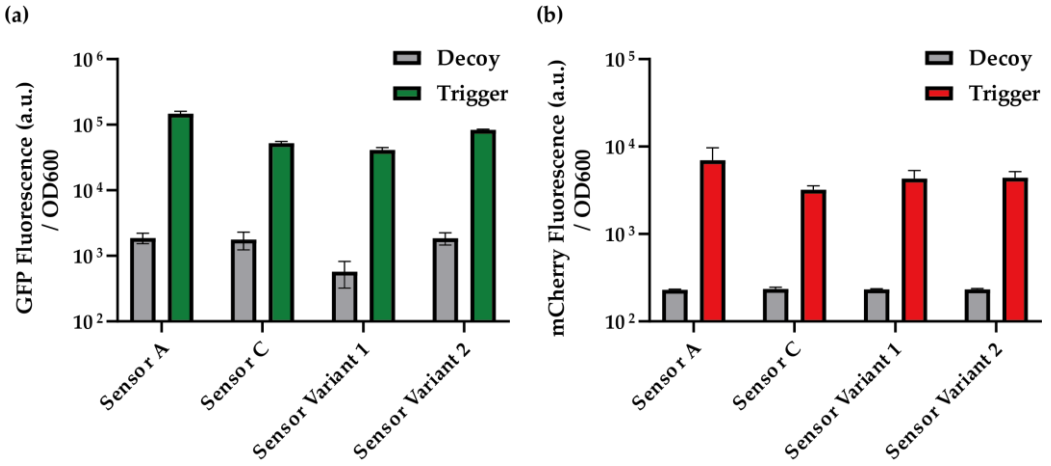
Supplementary Table S11. Gibbs free energy of mRNAs, mRNA sensors, and RNA complexes studied in this paper (unit: kcal/mol). For this calculation, full length mRNAs were used. ※ ΔΔΔG = ΔΔG_helper – ΔΔG_original.

1) [mRNA] + [toehold sensor] → [mRNA + toehold sensor].								
(kcal/mol)	*mCherry* sensor C			A_open1	A_open2	P_open1	P_open2	Q_open1	Q_open2
ΔG[mRNA]	-290.431			-131.42	-131.42	-489.438	-489.438	-202.712	-202.712
ΔG[toehold sensor]	-28.137			-19.704	-26.481	-24.346	-24.507	-22.88	-37.196
ΔG[mRNA + toehold sensor]	-371.688			-187.65	-192.459	-551.231	-546.148	-267.209	-275.103
ΔΔG original	**-53.12**			**-36.526**	**-34.558**	**-37.447**	**-32.203**	**-41.617**	**-35.195**
2) [mRNA + h1 + h2] + [toehold sensor] → [mRNA + h1 + h2 + toehold sensor]									
(kcal/mol)	*mCherry* sensor C			A_open1	A_open2	P_open1	P_open2	Q_open1	Q_open2
	Helper_60	Helper_30	Helper_15						
ΔG[mRNA+ h1 + h2]	-542.652	-428.143	-367.885	-256.599	-249.457	-630.618	-636.051	-342.383	-331.403
ΔG[toehold sensor]		-28.137		-19.704	-26.481	-24.346	-24.507	-22.88	-37.196
ΔG[mRNA+h1 +h2+toehold sensor]	-627.23	-512.721	-452.463	-319.381	-313.799	-699.381	-700.853	-411.982	-409.83
ΔΔG helper		**-56.441**		**-43.078**	**-37.861**	**-44.417**	**-40.295**	**-46.719**	**-41.231**
ΔΔΔG		**-3.321**		**-6.552**	**-3.303**	**-6.97**	**-8.092**	**-5.102**	**-6.036**

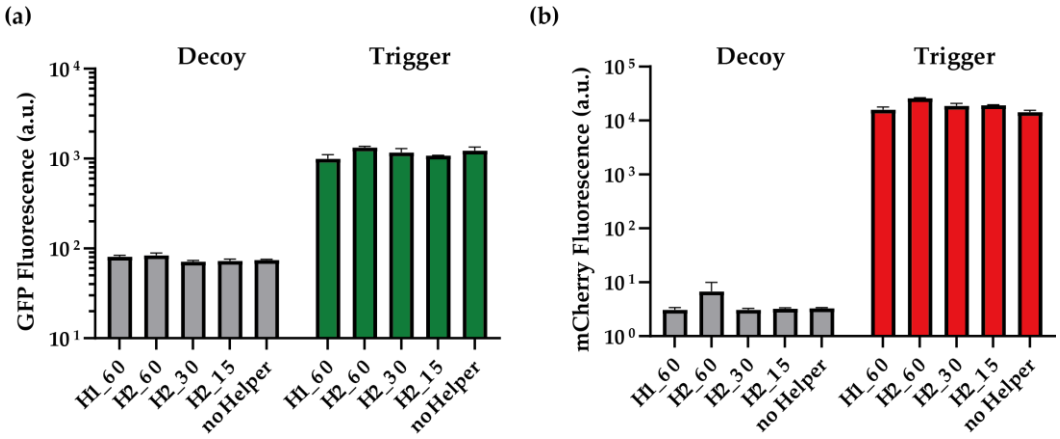
Supplementary Table S12. Performance information for a selection of toehold switches, toehold repressors, 3WJ repressors, and *clb* sensors.

Name	Type	Fold change
Toehold switch		
First-generation Toehold switch #1	Short trigger	292.0 ± 19.5
First-generation Toehold switch #2	Short trigger	279.6 ± 17.6
First-generation Toehold switch #3	Short trigger	265.3 ± 28.2
Second-generation Toehold switch #1	Short trigger	665 ± 135
Second-generation Toehold switch #2	Short trigger	586 ± 92
Second-generation Toehold switch #3	Short trigger	557 ± 68
mRNA sensor derived from toehold switch		
mCherry mRNA sensor #1	Long trigger	57 ± 8
mCherry mRNA sensor #2	Long trigger	38 ± 2
mCherry mRNA sensor #3	Long trigger	17 ± 1
cat mRNA sensor	Long trigger	7 ± 3
aadA mRNA sensor	Long trigger	6 ± 1
Toehold repressor		
Toehold repressor #1	Short trigger	172.8 ± 29.4
Toehold repressor #2	Short trigger	93.3 ± 26.6
Toehold repressor #3	Short trigger	88.5 ± 17.4
mRNA sensor derived from toehold repressor		
kanR mRNA sensor	Long trigger	14 ± 2
bla mRNA sensor	Long trigger	6 ± 3
3WJ repressor		
3WJ repressor #1	Short trigger	134.2 ± 8.1
3WJ repressor #2	Short trigger	85.2 ± 4.9
3WJ repressor #3	Short trigger	66.1 ± 3.2
mRNA sensor derived from 3WJ repressor		
kanR mRNA sensor	Long trigger	20 ± 4
aadA mRNA sensor	Long trigger	7 ± 1
Switch used in this paper		
clbP sensor open #1	Short trigger	942.5 ± 99.9
clbP sensor open #2	Short trigger	75.9 ± 11.2
clbQ sensor open #1	Short trigger	259.2 ± 22.3
Switch with full length mRNA without helper		
clbP sensor open #1 without helper	Long trigger	35.8 ± 9.8
clbP sensor open # without helper	Long trigger	4.7 ± 0.8
clbQ sensor open #1 without helper	Long trigger	11.3 ± 1.1
Switch with full length mRNA with helper		
clbP sensor open #1 with helper	Long trigger	62.3 ± 16.8
clbP sensor open #2 with helper	Long trigger	57.0 ± 9.2
clbQ sensor open #1 with helper	Long trigger	15.8 ± 1.5

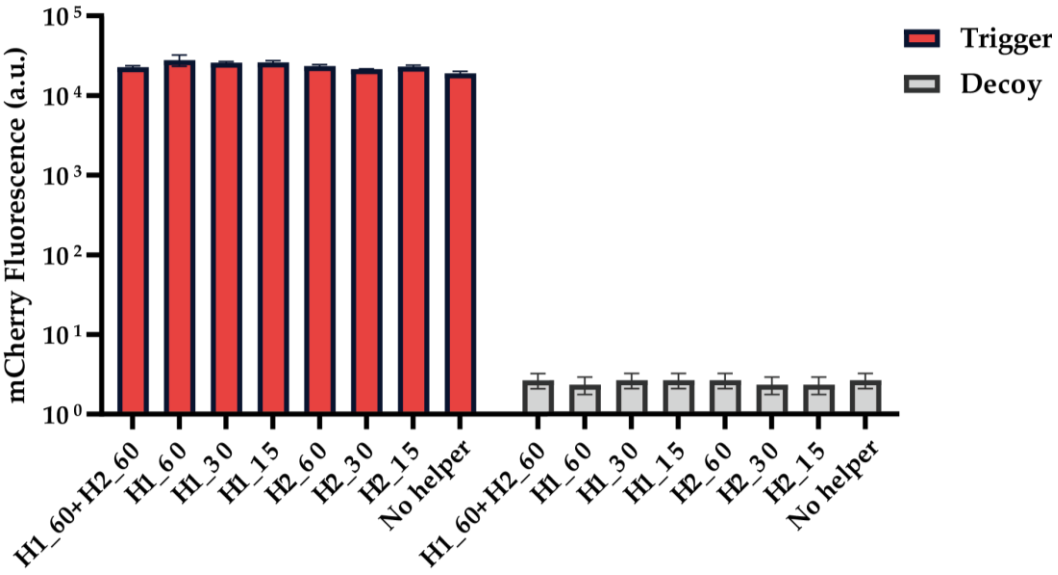
Supplementary Figures



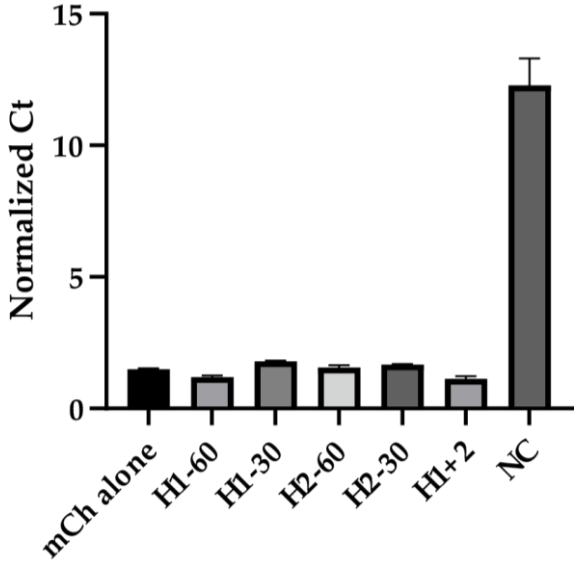
Supplementary Figure S1. *mCherry* mRNA sensor variants tested in this study. Sensor A and Sensor C were adopted from the previous research [5]. Among four sensors, sensor C and sensor variant 1 were selected for further helper tests. 0.2% (*w*/*v*) Arabinose was treated for induction of T7 RNA polymerase in *E. coli* BL21 AI strain. GFP and mCherry fluorescence were measured on the microplate reader (error bars indicate ±SD).



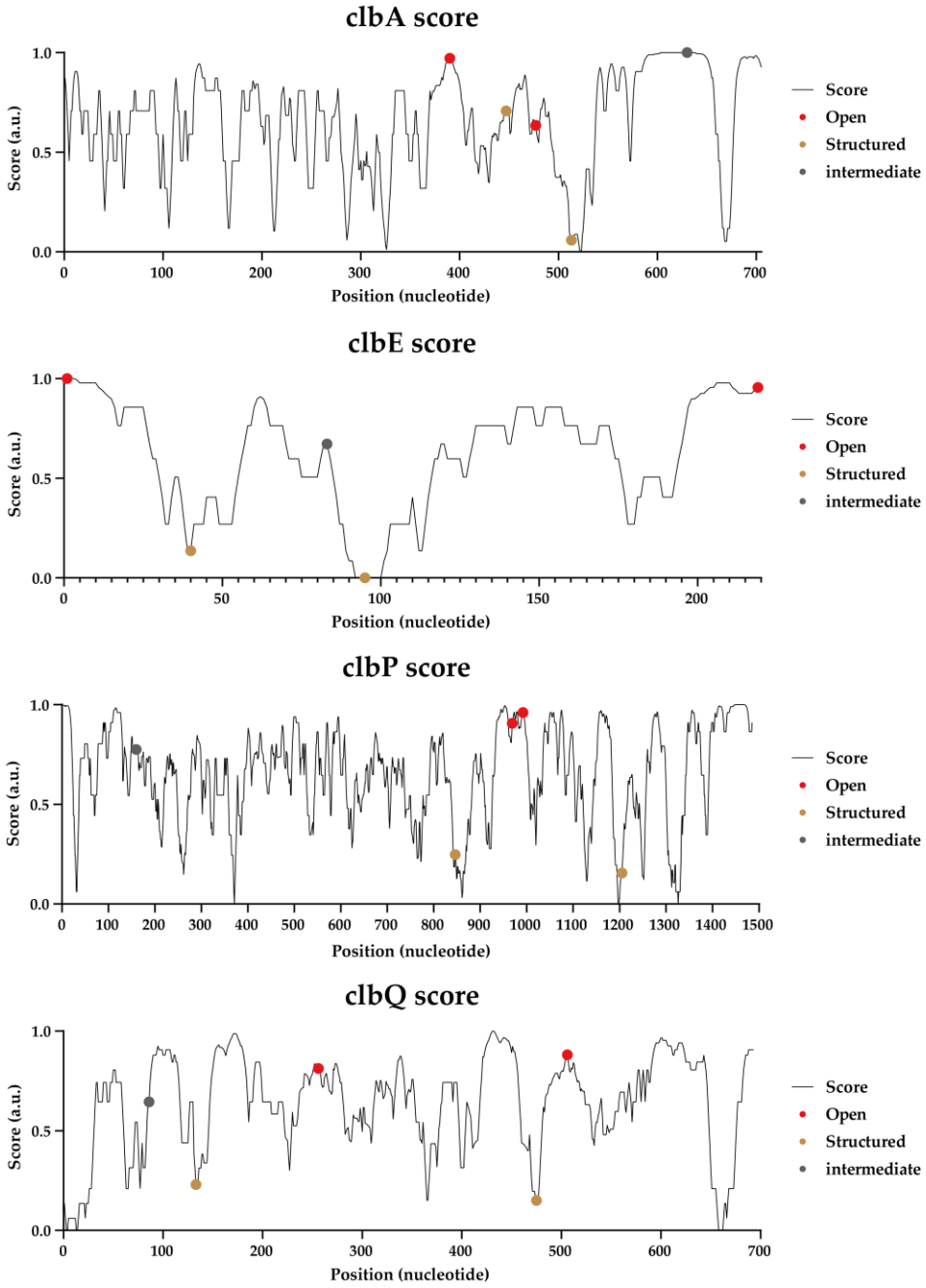
Supplementary Figure S2. Performance of *mCherry* sensor variant 1 with helper RNAs. H1 and H2 mean a helper RNA strand that stretching the upstream or downstream of a target site, respectively, and the following number means the length of the helper, 60, 30, and 15 nt. 0.2% (*w*/*v*) Arabinose was treated for induction of T7 RNA polymerases in *E. coli* BL21 AI strain. GFP and mCherry fluorescence were measured on flow cytometry (error bars indicate ±SD).



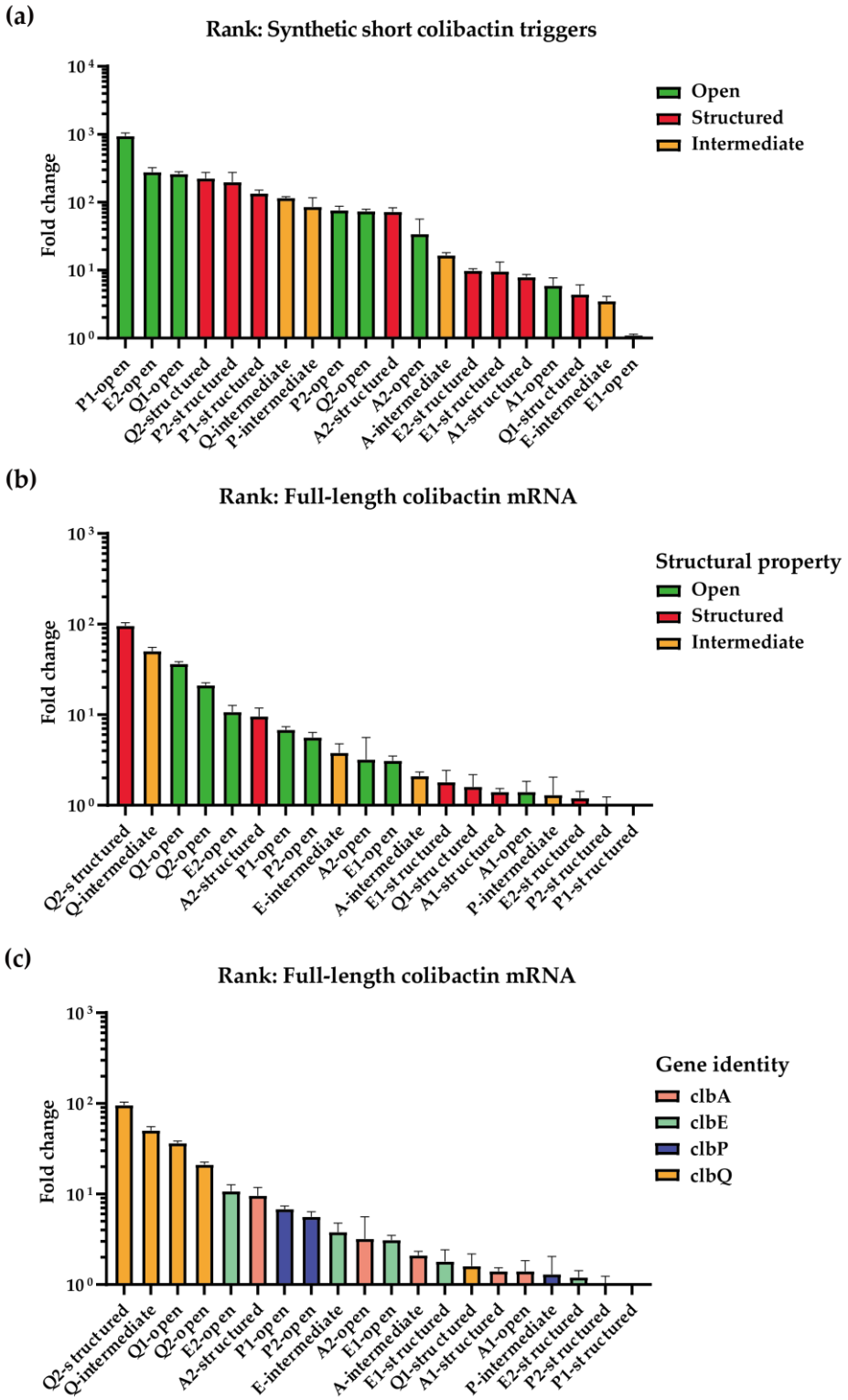
Supplementary Figure S3. mCherry expression levels in the presence of helper RNA variants. No significant inhibition of mCherry mRNA translation was observed regardless of the length or position of helper RNAs. H1 and H2 mean a helper that stretching the upstream or downstream of a toehold switch target site, respectively, and the following number means the length of the helper, 60, 30, and 15 nt. 0.2% (*w*/*v*) Arabinose was treated for induction, and GFP and mCherry fluorescence were measured on flow cytometry (error bars indicate ±SD).



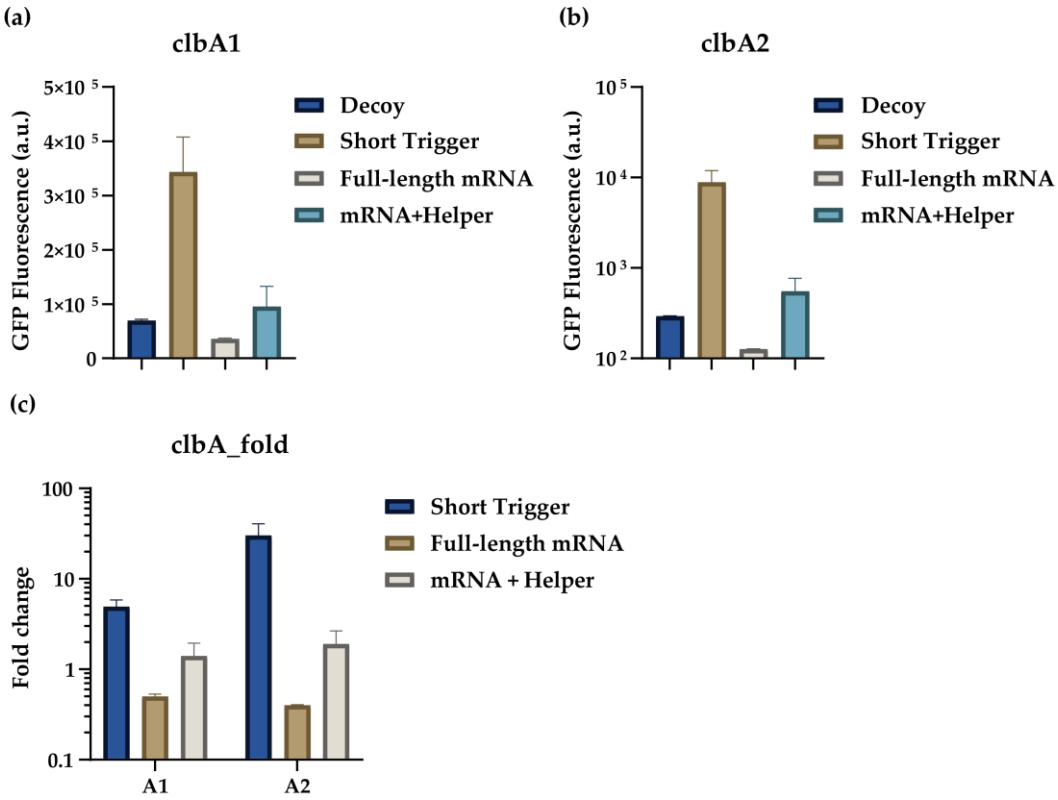
Supplementary Figure S4. RT-qPCR data for *mCherry* mRNA levels. 16s rRNA was selected as reference RNA for normalization of Ct values. *mCherry* mRNA amount was not significantly changed when it was compared to no helper (*mCherry* only) sample. Primer sequences used are listed in Table S10 (error bars indicate ±SEM).



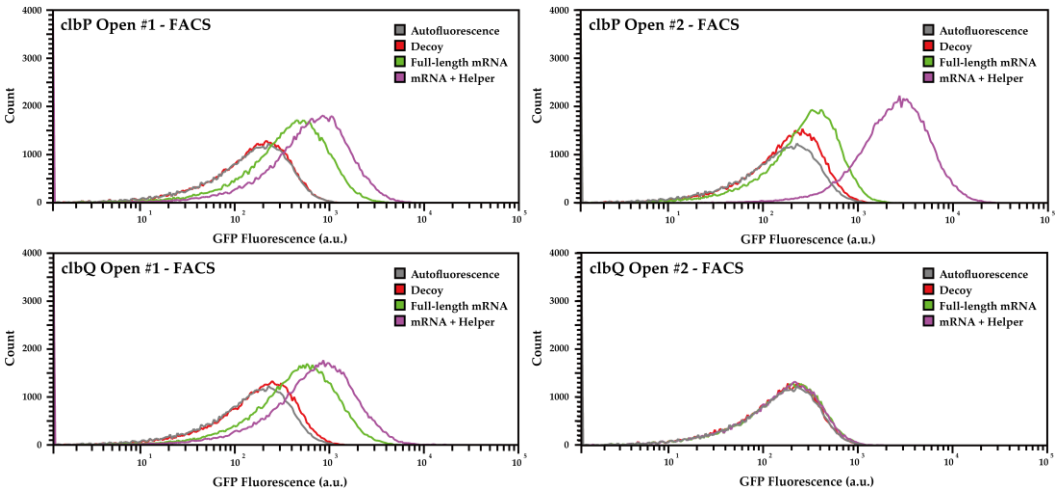
Supplementary Figure S5. Accessibility calculation of *clbA*, *clbE*, *clbP*, and *clbQ*. High score means highly single-stranded and low score means highly structured. Each point indicates the start position of the target region on a designed sensor. Color of the dot indicates the type of sensor; red: open structure, brown: highly structured, and gray: intermediate region.



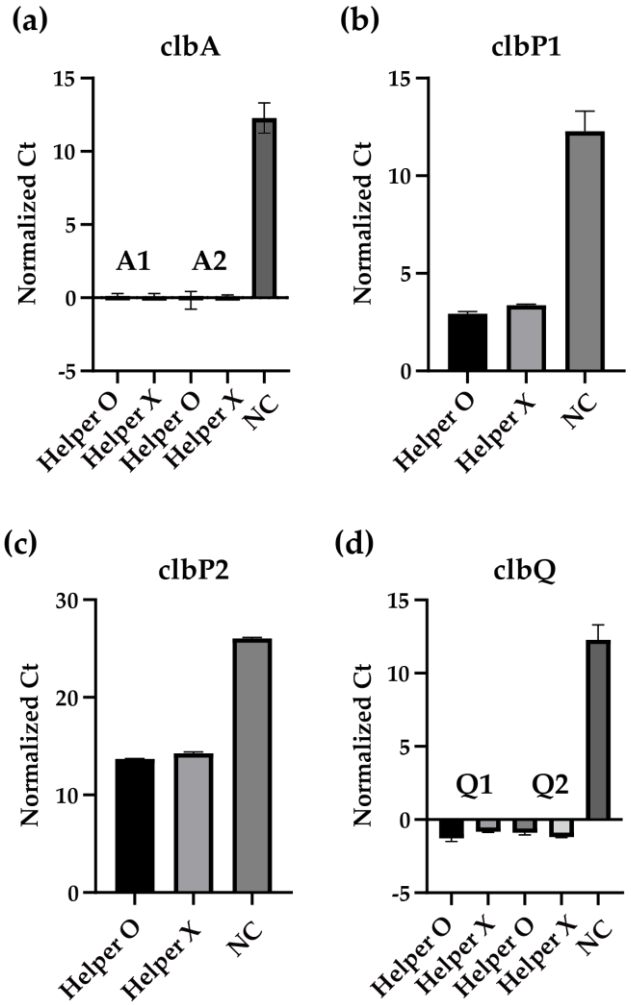
Supplementary Figure S6. In vivo performance of *clb* mRNA sensors. When cognate short triggers were treated, there were no significant difference between open region targeting sensors, close region targeting sensors and intermediated region targeting sensors was observed. However, when cognate full-length mRNAs were treated with sensors, open region targeting sensors generally showed higher performance (error bars indicate ±SEM).



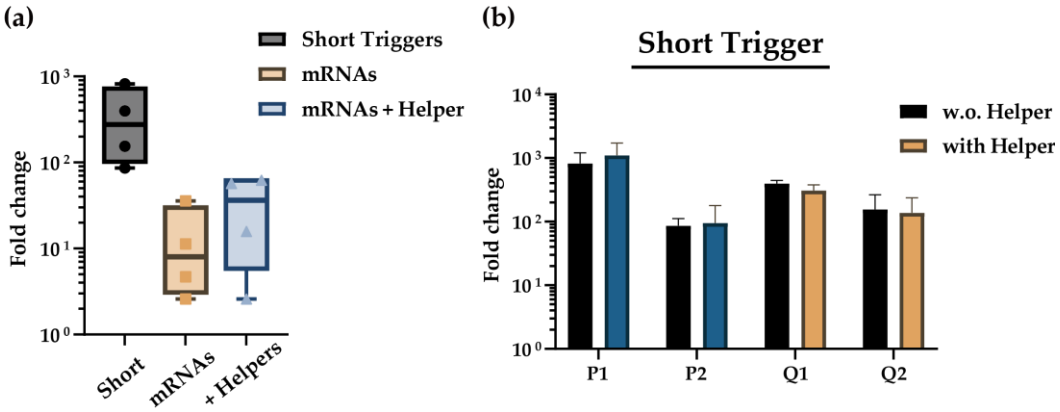
Supplementary Figure S7. In vivo performance of *clbA* open sensors with helper RNAs. Although *clbA*1 (open) and *clbA*2 (open) sensors worked well with synthetic short triggers but did not detect a full-length mRNA. Fold change was partly restored by introducing helper RNAs (error bars indicate ±SEM).



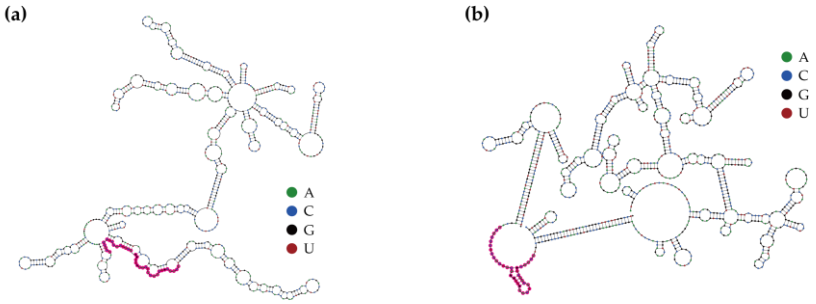
Supplementary Figure S8. Flow cytometry data for *clbP* and *clbQ* open sensors with helper RNAs. Cell population was unimodal, and GFP fluorescence increased with the introduction of helper RNAs. In all in vivo experiments, the number of biological replicates is three, and the figure showed a representative case.



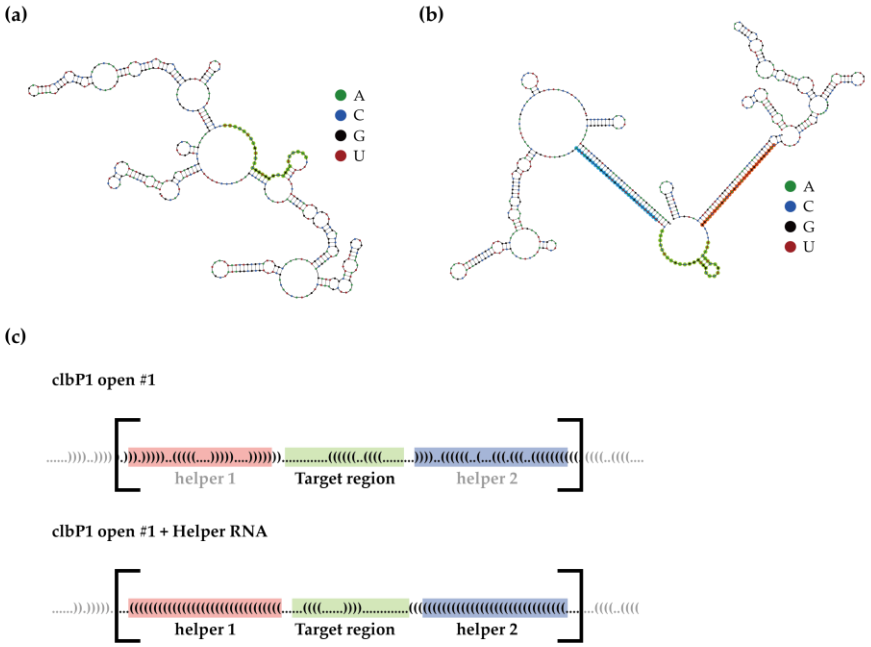
Supplementary Figure S9. RT-qPCR data for colibactin mRNA levels. 16s rRNA (for *clbA*, *clbP*1 and *clbQ*) or *CysG* (*clbP*2) were selected as reference RNA for normalization of Ct values. No significant change of target mRNA amount was observed between 'without helper' samples and 'with helper' samples. Primer sequences used are listed in Table S10 (error bars indicate ±SEM).



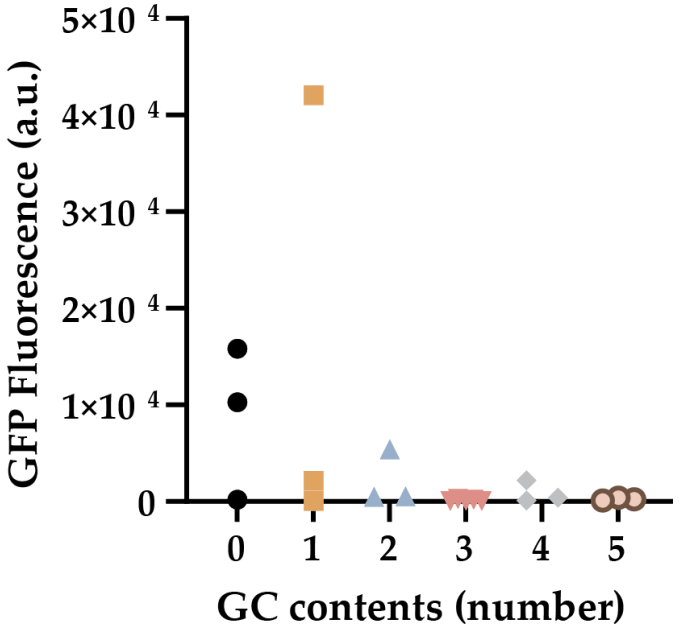
Supplementary Figure S10. In vivo performance of *clbP* and *clbQ* open sensors with short triggers and helper RNAs. (a) The sensing ability of toehold switch sensors was reduced in the full-length mRNA compared to synthetic short trigger. However, it was partially restored with the introduction of helper RNAs. (b) The sensing ability against synthetic short trigger was not affected by helper RNAs (error bars indicate ±SEM).



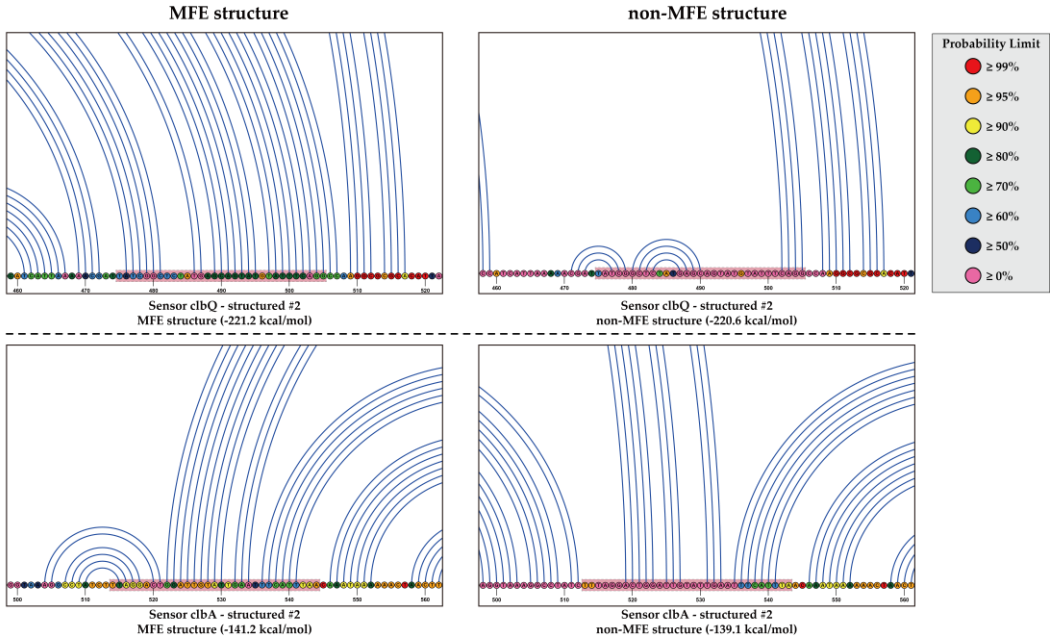
Supplementary Figure S11. Predicted MFE structures for *mCherry* mRNA without (a) and with (b) helper RNAs. Target sequences for toehold sensors are highlighted with pink. NUPACK was used to calculate MFE structures for each complex.



Supplementary Figure S12. Predicted MFE structure for *clb* mRNAs without and with helper RNAs. (a) *clbP* mRNA without helper RNAs for *clbP* open 1 sensor. (b) *clbP* mRNA with helper RNAs for *clbP* open1 sensor. (c) Dot-bracket model-expressed secondary structure of *clbP* open 1 sensor targeting *clbP* mRNA without or with helper RNAs. NUPACK was used to calculate MFE structures for each complex.



Supplementary Figure S13. The OFF-state fluorescence of *clb* sensors and the GC content of lower stem. The two cases showing highest leakages have 0 or 1 GC base-pair. The first one was *clbA* open Scheme 1. and the second one was *clbE* open sensor 1.



Supplementary Figure S14. Predicted alternative secondary structures for *clbQ* and *clbA* mRNAs. Secondary structure nearby target site (emphasized with red box) is dramatically changed with a small energy penalty. Structures were calculated using the RNAstructure 6. 2 [6,7] to figure out non-minimum free energy (MFE) structures.

References

1. Quan, J.; Tian, J. Circular polymerase extension cloning for high-throughput cloning of complex and combinatorial DNA libraries. *Nat Protoc* **2011**, *6*, 242–251, doi:10.1038/nprot.2010.181.
2. Gibson, D.G.; Young, L.; Chuang, R.-Y.; Venter, J.C.; Hutchison, C.A.; Smith, H.O. Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nature methods* **2009**, *6*, 343–345.
3. Liu, H.; Naismith, J.H. An efficient one-step site-directed deletion, insertion, single and multiple-site plasmid mutagenesis protocol. *BMC Biotechnol* **2008**, *8*, 91, doi:10.1186/1472-6750-8-91.
4. Andersen, J.B.; Sternberg, C.; Poulsen, L.K.; Bjørn, S.P.; Givskov, M.; Molin, S. New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. *Applied and environmental microbiology* **1998**, *64*, 2240–2246.
5. Green, A.A.; Silver, P.A.; Collins, J.J.; Yin, P. Toehold switches: de-novo-designed regulators of gene expression. *Cell* **2014**, *159*, 925–939, doi:10.1016/j.cell.2014.10.002.
6. Reuter, J.S.; Mathews, D.H. RNAstructure: software for RNA secondary structure prediction and analysis. *BMC Bioinformatics* **2010**, *11*, 129, doi:10.1186/1471-2105-11-129.
7. Mathews, D.H.; Disney, M.D.; Childs, J.L.; Schroeder, S.J.; Zuker, M.; Turner, D.H. Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure. *Proc Natl Acad Sci U S A* **2004**, *101*, 7287–7292, doi:10.1073/pnas.0401799101.