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Programming Quorum Sensing-based AND Gate in *Shewanella oneidensis* for Logic Gated-Microbial Fuel Cells

Yidan Hu,[‡a] Yun Yang,[‡b] Evgeny Katz,[c] and Hao Song *[a]

[a]*Key Laboratory of Systems Bioengineering (Ministry of Education), SynBio Research Platform, Collaborative Innovation Centre of Chemical Science and Engineering (Tianjin), School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China. Tel.: +86 22 6097-7327; E-mail: hsong@tju.edu.cn.*

[b]*School of Chemical and Biomedical Engineering, Nanyang Technological University, 70 Nanyang Drive, 637457, Singapore.*

[c]*Department of Chemistry and Biomolecular Science, Clarkson University, Potsdam, NY 13699, USA.*

[‡] *Equal contribution*

Procedure of gene circuit construction

To accelerate construction of complex gene circuit with multiple components, every element was standardly designed as a biobrick. Each biobrick contains an upstream prefix (including EcoRI and XbaI) and a downstream suffix (with SpeI and PstI). To confer precise programing of our gene circuit and prevent leaking signals from upstream promoters, a double terminator (TT) (BBa_B0015, iGEM) was incorporated into the biobricks of key elements belong to the gene circuit. (See Figure S1) Strong ribosome bonding sites (RBS) (BBa_B0034, iGEM) were placed in front of *luxR* and *mtrA* to ensure substantial translation level. Multiple biobricks were assembled through sequential routine ligation processes in the biobrick compatible backbone pSB1AK3 (iGEM).[1]

The IPTG responding module P*lacIq*-*lacI*-P*tac* was cloned from pMAL-c vector (New England Biolabs, USA). The quorum-sensing module was *in vitro* synthesized. For the rapid and substantial respond to 3-oxo-C6HSL by the quorum-sensing module, the sequence of *luxR* originated from *Vibrio fischeri* was codon optimized before synthesis, in order to prevent insufficient translation rate due to rare codon. The procedure of *in vitro* gene synthesis is illustrated in Figure S2. The nucleotide sequences of *luxR* and *mtrA* were extracted from database KEGG.[2] RBS-*luxR*-TT-p*lux* (p*lux*: BBa_R0062) and RBS-*mtrA*-TT were *in vitro* synthesized separately. Codon optimization for expressing in *Shewanella* was performed by JCAT,[3] and restriction enzyme sites of EcoRI, XbaI, SpeI, and PstI were avoided at the mean time.

Sequentially, to avoid high error rate of long fragment synthesis, GeneDesign[4] was used to divide each to be synthesized sequence into several building blocks (less than 750 bp) with 50bp overlaps and design the oligonucleotide sets with ~10bp overlaps. Each building block was assembled by polymerase chain reaction (PCR) with corresponding set of primers and cloned into peasyblunt vector (Transgen biotech, China) for further sequencing confirmation (Genewiz, China). Correct building blocks for one biobrick were ligated through overlap extension PCR. Correct building blocks were introduced into pSB1AK3 sequentially according to the gene circuit design. Complete gene circuit was inserted into pHG101, a mobile and wide host vector, by using restriction enzyme to cut and ligate EcoRI and SpeI.

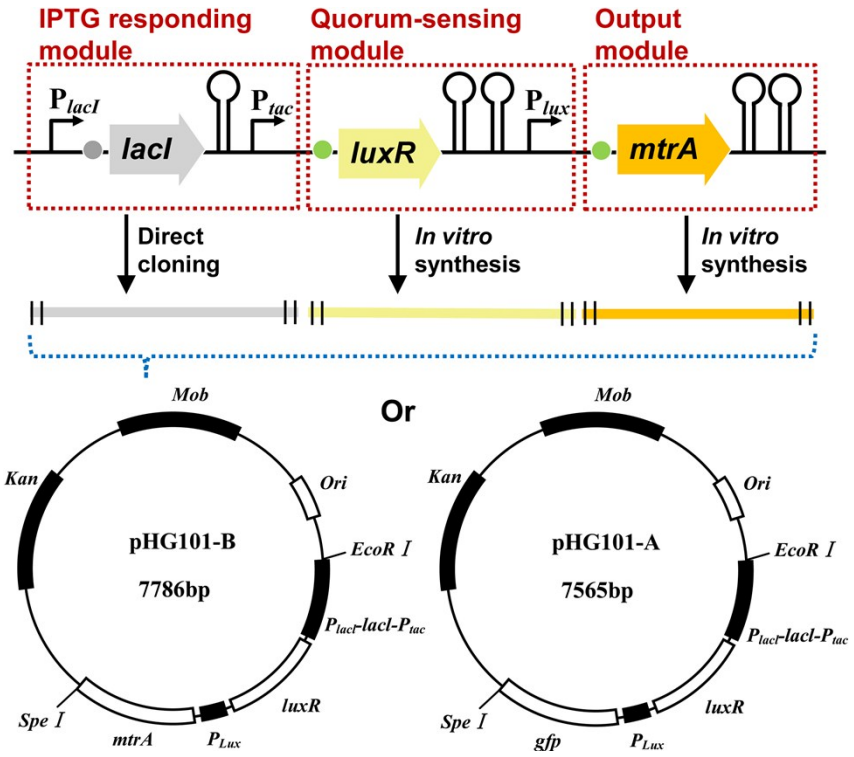

Figure S1. Schematic of the gene circuit components and the procedure for the gene circuit construction. The gene circuit contains three modules: IPTG responding module, quorum-sensing module and output signal module. The IPTG responding module was directly cloned from pMAL-c vector with biobrick restriction enzyme site prefix (EcoRI and XbaI) and suffix (SpeI and PstI). The quorum-sensing module and output signal module were *in vitro* synthesized as biobricks. Green circles represent the ribosome binding site (RBS), while stem loops stand for the terminators. Double terminators (BBa_B0015, iGEM) were placed after *luxR* and *mtrA* in order to minimize background signals from leakage. Sufficient translation of key components in the gene circuit was ensured by the strong RBS (BBa_B0034, iGEM) incorporated in front of *luxR* and *mtrA*. The fully assembled gene circuit was inserted into pHG101, which are named as pHG101-A and pHG101-B. The procedure for the gene circuit construction with *gfp* as the reporter gene was similar, except that the output signal module was constructed by ligating existing RBS (BBa_B0034) with the *gfp* gene (BBa_J04630).

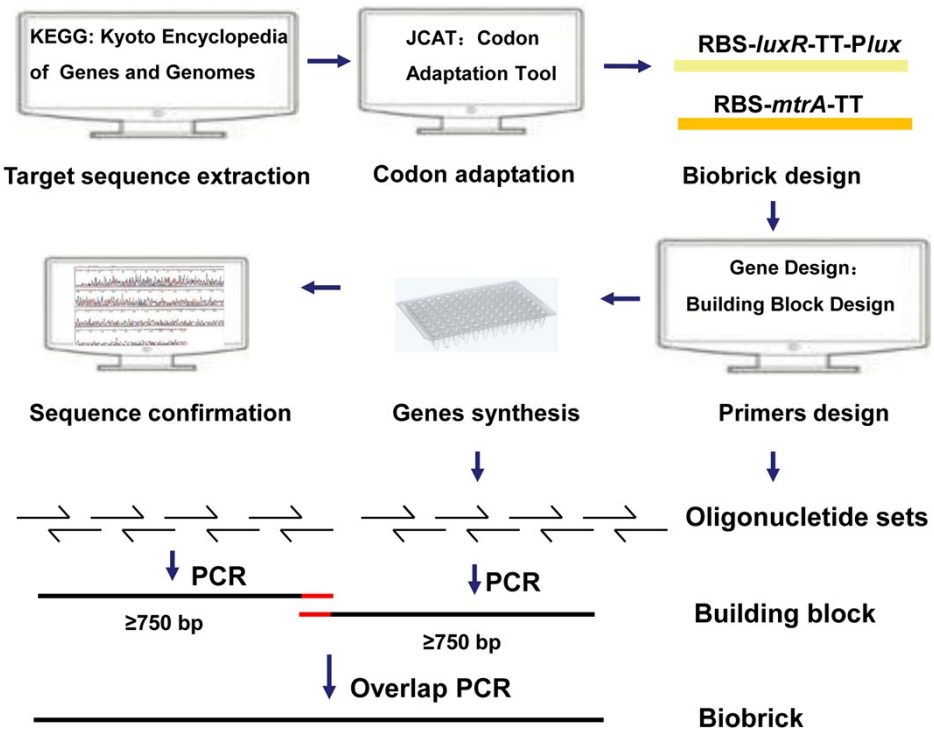

Figure S2. Flowchart of *in vitro* gene synthesis. The sequences of RBS-*luxR*-TT-P*lux* and RBS-*mtrA*-TT biobricks were designed according to genome information provided by KEGG and codon adaptation results performed by JCAT. Gene Design was applied to divide biobrick sequence into several building blocks (less than 750 bp), and give out the oligonucleotide sets for PCR synthesis. Correct building blocks with 50 bp overlap were ligated through overlap PCR.

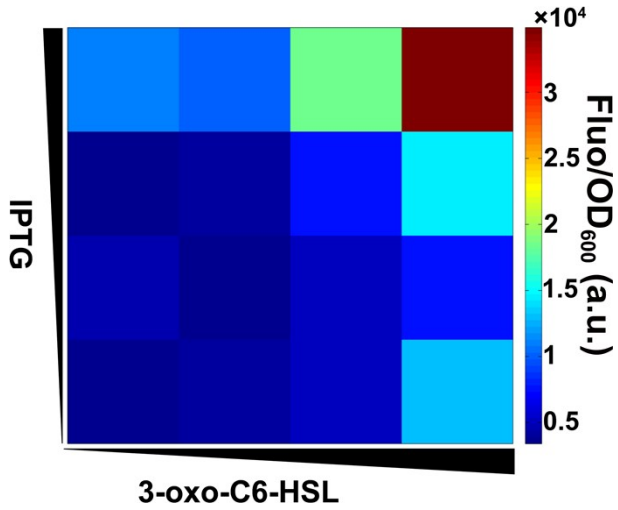

Figure S3. The performance of *S. oneidensis* MR-1 harboring pHG101-A with GFP as output. The inducer concentrations for AND gate characterizations are 0, 10^{-1}, 1, 10 nM 3-oxo-C6-HSL (squares from left to right) and 0, 10^{-4}, 10^{-3} and 10^{-2} mM IPTG (squares from bottom to top).

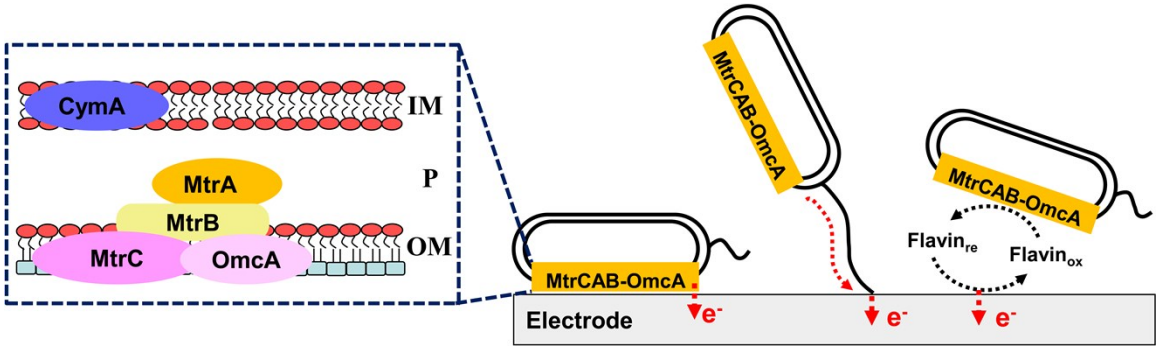

Figure S4. Schematic diagram of the extracellular electron transfer (EET) pathways of *Shewanella oneidensis* MR-1. Intracellular electrons pass the MtrCAB-OmcA membrane conduit, and go to external electron acceptor by direct contact-based EET *via* the outer membrane (OM) *c*-Cyts or nanowires (pilus-like filaments that could conduct long-range electron transfer), and electron shuttle (flavins)-mediated EET.

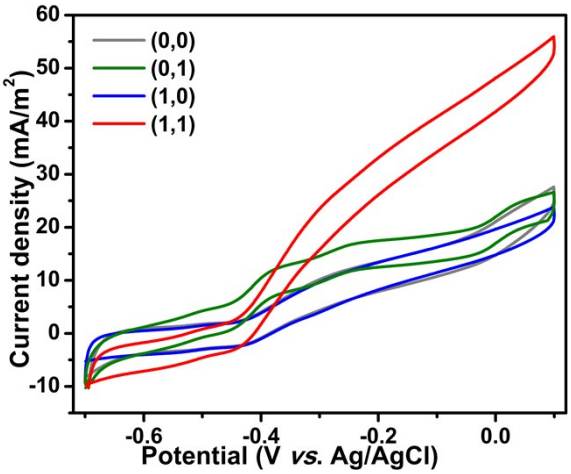

Figure S5. Turnover cyclic voltammetry (CV) (at scan rate of 1 mV/s) of *S. oneidensis mtrA* knockout mutant harboring the pHG101-B plasmid with the different input combinations: (1) None (grey curve); (2) IPTG (blue curve); (3) 3-oxo-C6HSL (green curve); and (4) IPTG and 3-oxo-C6HSL (red curve), respectively.

Table S1. Primer sequences of genes synthesis

luxR- P_{Lux}	1050bp
luxR- P_{Lux} -BB01	545 bp
01.o01	GCAGAATTCGCGGCCGCTTCTAGAGAAAGAGGAGAAATACTAGAGATGAAAAACATCAACG
01.o02	TGATTTTGTTGATGATAGGTAAGTATCATCAGCGTTGATGTTTTTCATCTCTAGTATTTC
01.o03	GATACTTACCGTATCATCAACCAAAATCAAAGCTTGTCGTTCTAACAACGATATCAACC
01.o04	CACAGTGAACCATTTTAGTCATATCAGATAAACATTGGTTGATATCGTTGTTAGAACGACA
01.o05	TCTGATATGACTAAAATGGTTCACTGTGAATACTACTTATTAGCTATCATCTACCCACAC
01.o06	GGTAGTTATCTAAGATAGAGATATCAGATTTAACCATAGAGTGTGGGTAGATGATAGCTAATAAGT
01.o07	TAAATCTGATATCTCTATCTTAGATAACTACCCAAAAAAATGGCGTCAATACTACGATGATG
01.o08	GTAATCAACGATTGGATCGTATTTGATTAAGTTAGCATCATCGTAGTATTGACGCCATTTTTT
01.o09	TAATCAAATACGATCCAATCGTTGATTACTCTAACTCTAACCACTCTCCAATCAACT
01.o10	GAGATTTTTTGTTAACAGCGTTGTTTTCGAAGATGTTCCAGTTGATTGGAGAGTGGTTAGAG
01.o11	GAAAACAACGCTGTTAACAAAAAATCTCCAAACGTTATCAAAGAAGCTAAAACTTCGGGTC
01.o12	GTGGATTGGGAAGCTGAAACCAGTTATGAGACCCGAAGTTTTAGCTTCTTTGATAA
01.o13	TGGTTTCAGCTTCCCAATCCACACTGCTAACAACGGTTTCGGTATGTTATCTTTCGC
01.o14	GTGTAAGAATAAAGAATCGATGTAGTTATCTTTTTCAGAGTGAGCGAAAGATAACATACCGAAACC
01.o15	AAGATAACTACATCGATTCTTTATTCTTACACGCTTGTATGAACATCCCATTAATCGTTCC
01.o16	CAACTAAAGATGGAACGATTAATGGGATGTTCATACAA
luR- P_{Lux} -BB02	544bp
02.o01	GCTTGTATGAACATCCCATTAATCGTTCCATCTTTAGTTGATAACTACCGTAAAATCAACATC
02.o02	CGTTTAGTTAAATCGTTGTTAGATTTGTTGTTAGCGATGTTGATTTTACGGTAGTTATCAACTAA
02.o03	AACAACAAATCTAACAACGATTTAACTAAACGTGAAAAAGAATGTTTAGCTTGGGCTTG
02.o04	CTAAGATTTTAGAGATATCCCAAGAAGATTTACCTTCACAAGCCCAAGCTAAACATTCTTTTTC
02.o05	AAATCTTCTTGGGATATCTCTAAAATCTTAGGTTGTTCTGAACGTACTGTTACTTTCC
02.o06	GTTAGTAGTGTTTAATTTCATTTGAGCGTTAGTTAAGTGGAAAGTAACAGTACGTTCAGAAC
02.o07	CGCTCAAATGAAATTAAACACTACTAACCGTTGTCAATCTATCTCTAAAGCTATCTTAACTG
02.o08	GTTATTAGTTTTTGAAGTATGGACAATCGATAGCACCAGTTAAGATAGCTTTAGAGATAGATTG
02.o09	TCGATTGTCCATACTTCAAAAACTAATAACACTAATAATGTTAATGTCGTTCTTACTAGAGC
02.o10	CGACTGAGCCTTTCGTTTTATTTGATGCCTGGCTCTAGTAAGAACGACATTAACATTAT
02.o11	TCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTC
02.o12	CCAGTGTGACTCTAGTAGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAGGC
02.o13	CTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATAC
02.o14	CTTGCGTAAACCTGTACGATCCTACAGGTCTCTAGTATATAAACGCAGAAAGGCCCA
02.o15	AGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTTATAGTCGAATAAATACTAGTAGCGGC
02.o16	CCTGCAGGCGGCCGCTACTAGTATTTATTCGACTATAA
mtrA	1208bp
mtrA-BB01	624 bp
01.o01	GCAGAATTCGCGGCCGCTTCTAGAGAAAGAGGAGAAATACTAGAGATGAAGAACTGCCTAAAAATG
01.o02	GGTAAGTGCCGGCAGTAGGTTTTTCATTTTTAGGCAGTTCTTCATCTCTAGTATTT
01.o03	AAACCTACTGCCGGCACTTACCATCACAATGGCAATGTCCGCAGTTATGGCATTAGTC
01.o04	CCCACTTCGACGCATAAGCGTTTGGTGTGACGACTAATGCCATAACTGCGGACAT
01.o05	ACGCTTATGCGTCGAAGTGGGATGAGAAAATGACGCCAGAGCAAGTCGAAGCC
01.o06	GGGAGTAGTTGCCTTCGGCAAACTTCTTATCTAAGGTGGCTTCGACTTGCTCTGGC
01.o07	TTTGCCGAAGGCAACTACTCCCCTAAAGGCGCCGATTCTTGCTTGATGTGCCATAAGAAATCC
01.o08	GACACCTTTGAAAAGGTCCATGACTTTTTCGGATTTCTTATGGCACATCAAGCAAGAAT
01.o09	AGTCATGGACCTTTTCAAAGGTGTCCACGGTGCGATTGACTCCTCTAAGAGTCCAATGG
01.o10	GCCGTGGCATGCCTCACATTGCAGGCCAGCCATTGGACTCTTAGAGGAGTCAAT
01.o11	ATGTGAGGCATGCCACGGCCCACTGGGTCAGCACAACAAAGGCGGCAACGAGCC

01.o12	GTCGGCACTTAAGGTTGATTGCTTACCAAAAGTGATCATCGGCTCGTTGCCGCCTTTGT
01.o13	AAGCAATCAACCTTAAGTGCCGACAAGCAAAACAGCGTATGTATGAGCTGTCACCAAGA
01.o14	GGTGACCGCCATTCCAAGACATACGCTTATCGTCTTGGTGACAGCTCATACATACG
01.o15	TGTCTTGGAATGGCGGTCACCATGACAATGCCGATGTTGCTTGTGCTTCTTGTCACCAA
01.o16	TCCGTGTTTTTAGATAACACAGGATCTTTTGCGACGTGTACTTGGTGACAAGAAGCACAAGCAAC
01.o17	AAAAGATCCTGTGTTATCTAAAAACACGGAAATGGAAGTCTGTACTAGCTGCCATACAAAG
01.o18	TCCGCTTTTTGCTTTGTATGGCAGCTAGTACAGACTT
mtrA-BB02	624 bp
02.o01	TGGAAGTCTGTACTAGCTGCCATACAAAGCAAAAAGCGGATATGAATAAACGCTCAAGTC
02.o02	GCTACAGGTCATTTGTGCCCATTTGAGTGGGTGACTTGAGCGTTTATTCATATCCGCT
02.o03	AAATGGGCACAAATGACCTGTAGCGACTGTCACAATCCCCATGGGAGCATGACAG
02.o04	CAGGTATCATTCACGCTAGGCTTGTTAAGATCGGAATCTGTCATGCTCCCATGGGGAT
02.o05	CAAGCCTAGCGTGAATGATACCTGTTATTCCTGTCACGCCGAAAAACGCGGCC
02.o06	CACAATTCTCAGTGACGGGTGCATGCTCCCAAAGTTTTGGGCCGCGTTTTTCGGCGTG
02.o07	ATGCACCCGTCACTGAGAATTGTGTCACTTGCCACAATCCTCACGGTAGTGTGAATGA
02.o08	GCTGTGGCGCACGGGTTTTCAGCATACCGTCATTCACACTACCGTGAGGATTG
02.o09	AAAACCCGTGCGCCACAGCTATGTCAGCAATGTCACGCCAGCGATGGCCACGCC
02.o10	CGACATTTGAACCTAATCCAGTGTTACCTAAGTAGGCGTTGCTGGCGTGGCCATCGCTGGCG
02.o11	GTAACACTGGATTAGGTTCAAATGTCGGTGACAATGCCTTTACTGGTGGAAGAAGCTG
02.o12	GATGGTTAGAACCATGAACCTGACTATGGCAATTTAAGCAGCTTCTTCCACCAGTAAAGGC
02.o13	TAGTCAGGTTCATGGTTCTAACCATCCATCTGGCAAGCTATTACAGCGCTAATACTAGAGC
02.o14	GTCTTTCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCTCTAGTATTAGCGCTGTAATAGCT
02.o15	AATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAAC
02.o16	GGTGAGCCAGTGTGACTCTAGTAGAGAGCGTTCACCGACAAACAACAGATAAAACGAA
02.o17	TACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATACTAGTAGCGGC
02.o18	CCTGCAGGCGGCCGCTACTAGTATATAAACGCAGAAA

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