

Supplementary Information

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Figure S3. Electropherogram of colony PCR results when constructing pACYC177 biosensor plasmids.

Table S1. Strains, Kits, Chemical Reagents and other Materials used in this study

Material	Purchased From	Cart No.
Trelief 5 α	Tsingke	TSC-C01
BL21(DE3)	Biosharp Life Science	BL1287A
<i>V.natriegens</i> ATCC14048	Mingzhou Biology	BMZ146848
Phanta Max Super-Fidelity DNA Polymerase	Vazyme	P505
2 \times Rapid Taq Master Mix	Vazyme	P201
FastPure Gel DNA Extraction Mini Kit	Vazyme	DC301
RapidLyse Plasmid Mini Kit	Vazyme	DC211
ClonExpress II One Step Cloning Kit	Vazyme	C112
Ultra GelRed (10,000 \times)	Vazyme	GR501
100 bp DNA Ladder	Vazyme	MD104
DL5000 DNA Marker	Vazyme	MD102
SanPrep Column Plasmid Mini-Preps Kit	Sangon Biotech	B518191
50X TAE Buffer	Sangon Biotech	B548101
IPTG	Sangon Biotech	A100487
Carbenicillin disodium	Sangon Biotech	A429319
Kanamycin sulfate	Sangon Biotech	A430277
Chloramphenicol	Sangon Biotech	A429048
Magnesium chloride hexahydrate	Sangon Biotech	A601336
Potassium acetate	Sangon Biotech	A601169
Magnesium sulfate anhydrous	Sangon Biotech	A601988
Sucrose	Sangon Biotech	A502792
1 kb DNA Ladder (Dye Plus)	TaKaRa	3426A
QuickCut™ <i>Dpn</i> I	TaKaRa	1609
QuickCut™ <i>Xho</i> I	TaKaRa	1635
QuickCut™ <i>Kpn</i> I	TaKaRa	1618
QuickCut™ <i>Pst</i> I	TaKaRa	1624
Seamless Cloning Kit	Beyotime	D7010
Manganese Chloride Tetrahydrate	Aladdin	M109463

TE buffer	Aladdin	T301525
Dimethyl sulfoxide	Aladdin	D103272
PIPES Buffered Solution	Aladdin	P301883
D-Sorbitol solution	Aladdin	S104833
Naringenin	Aladdin	N164488
L-Tyrosine	Aladdin	T103976
Naringenin chalcone	Aladdin	N414406
Agar	BIOFROXX	1182
Yeast Extract	OXOID	LP0021
Peptone	OXOID	LP0042B
Sodium Chloride	Sangon Biotech	A100241
Agarose	BIOFROXX	1110
Pure Water	Wahaha	/
Gene Pulser Cuvette 0.1cm	Biorad	1652089
2216E Liquid Medium	Hopebio	HB0132-1
Brain Heart Infusion Broth (BHI)	Hopebio	HB8297-5
Ethanol, anhydrate	Hushi	10009218
Glycerol	Hushi	10010618

Table S2. Genes' Name and Sequences

Gene or Component	Sequence (5'-3')
4CL	ATGGCGCCACAAGAACAAGCAGTTTCTCAGGTGAT GGAGAAACAGAGCAACAACAACAGTGACGTC ATTTTCCGATCAAAGTTACCGGATATTTACATCCCGA ACCACCTATCTCTCCACGACTACATCTTCCAAAACA TCTCCGAATTCGCCACTAAGCCTTGCCTAATCAACG GACCAACCGGCCACGTGTACACTTACTCCGACGTC CACGTCATCTCCCGCCAAATCGCCGCCAATTTTCAC AAACTCGGCGTTAACCAAAACGACGTCGTCATGCT CCTCCTCCCAAACGTGTCCTCGAATTCGTCCTCTCTTT CCTCGCCGCCTCCTTCCGCGGCGCAACCGCCACCG CCGCAAACCCTTTCTTCACTCCGGCGGAGATAGCTA AACAAGCCAAAGCCTCCAACACCAAACCTCATAATC ACCGAAGCTCGTTACGTCGACAAAATCAAACCACT TCAAAACGACGACGGAGTAGTCATCGTCTGCATCG ACGACAACGAATCCGTGCCAATCCCTGAAGGCTGC CTCCGCTTCACCGAGTTGACTCAGTCGACAACCGA GGCATCAGAAGTCATCGACTCGGTGGAGATTTAC CGGACGACGTGGTGGCACTACCTTACTCCTCTGGC ACGACGGGATTACCAAAAAGGAGTGATGCTGACTCA CAAGGGACTAGTCACGAGCGTTGCTCAGCAAGTCG ACGGCGAGAACCCGAATCTTTATTTCCACAGCGATG ACGTCATACTCTGTGTTTTGCCCATGTTTCATATCTA CGCTTTGAACTCGATCATGTTGTGTGGTCTTAGAGT TGGTGCGGCGATTCTGATAATGCCGAAGTTTGAGAT CAATCTGCTATTGGAGCTGATCCAGAGGTGTAAAGT GACGGTGGCTCCGATGGTTCCGCCGATTGTGTTGGC CATTGCGAAGTCTTCGGAGACGGAGAAGTATGATTT GAGCTCGATAAGAGTGGTGAAATCTGGTGCTGCTC CTCTTGGTAAAGAACTTGAAGATGCCGTTAATGCCA AGTTTCCTAATGCCAAACTCGGTCAGGGATACGGA ATGACGGAAGCAGGTCCAGTGCTCGCAATGTCGTT AGGTTTTGCAAAGGAACCTTTTCCGGTTAAGTCAG GAGCTTGTGGTACTGTTGTAAGAAATGCTGAGATGA AAATAGTTGATCCAGACACCGGAGATTCTCTTTCGA GGAATCAACCCGGTGAGATTTGTATTCGTGGTCACC AGATCATGAAAGGTTACCTCAACAATCCGGCAGCTA CAGCAGAGACCATTGATAAAGACGGTTGGCTTCATA CTGGAGATATTGGATTGATCGATGACGATGACGAGC TTTTCATCGTTGATCGATTGAAAGAACTTATCAAGT ATAAAGGTTTTTCAGGTAGCTCCGGCTGAGCTAGAG GCTTTGCTCATCGGTCATCCTGACATTACTGATGTTG CTGTTGTCGCAATGAAAGAAGAAGCAGCTGGTGAA

	G TTCCTGTTGCATTTGTGGTGAAATCGAAGGATTTCG GAGTTATCAGAAGATGATGTGAAGCAATTCGTGTCTG AAACAGGTTGTGTTTTACAAGAGAATCAACAAAGT GTTCTTCACTGAATCCATTCTAAAGCTCCATCAGG GAAGATATTGAGGAAAGATCTGAGGGCAAACACTAG CAAATGGATTGTGA
TAL	ATGCTACAACCATCTGCCCAAGGAAGCTACAGCAA GGCCGTGCTGTCGACATTTTCGATCCCTGGATGATCT CACCTGTGATGGTTCCAAAATCATTCTCGATGGCCA ATCGCTCAATGTGAGCTCAGTGGTCGCCGCAGCATG CCATCAAGTGCCAGCGTCAATCAGCAAGGACCCGC AGCTTCATGCGCGGCTGCGAGAAAGCGTCGAGCTG CTTGCCCGCAAGCTGGCTGAAGGCGAAATCGCCTA TGGGGTCAACACCGGGTTTGGTGGCAGCGCTGACA CCCGCACCGATGACTATTCCACCCTGCAGCAGGCA CTGGTCCAGCATCTGGCGTCCGGTGTGTTGCTGCCC ACCGACCGCAGCAGTAGTCGACCATCGTCGCGGTA CCCGCATGGTTTGAGAAGCCATAGCATGCCCACAG CCGTGGTCAAGGCTGCCATGCTCGTCCGATGTAACT CACTCTTGCGAGGGCATTCCGCCGTCCGCCCCGAA GTCATTGAGCACATCCTGGCTTTCCTACGCAGTGGT CTAATCCCGGTGGTGCCTGTTTCGGGGGAGCATCTCG GCATCCGGCGACCTGTCCCCGCTCTCGTATATCGCC AACGCTCTCGAGGGGAACCCCGACATTGCTATCCA GAATGAGGCCACTGGGGACGTCATCCGCGCCGACG AGGCGTTGCAGCAGTTGGGCCTTGCGCCTCTCAGG TTCGGGCCCCAAGGAGGGCCTGGGCCTGCTCAACGG CACGGCCTTTAGCGCTGGCGCCGCCAGCTTGGTCC TCTTCGAAGCCAACCAGCTCGTGCTCCTTTCCAGG TACTGACAGCGATGGGAACGGAAGCGCTGGCGGGC TCGACCGGGAACCTACCATCCCTTCATTGCGGGCGTC CGTCCCCACCGCGGGCAAATCGAGGCGGCCGGA CATCTTCCACTTACTGCGGGACTCGCGCATGGCCAC CAGCCCTGGCGCCGATGGCGCAAGCCACAGTAGTA GCTTGGCGCAGGACCGGTATGCGCTTCGCACCGCA TCCCAATGGATAGGCCACAGATCGAAGACATGTCT CTTGCTTCGGAACAGGTCCACTGCGAATTGAACTC CACCACAGACAATCCCTTGCTGGATCCGGGGCTCGG GCCACATGCACCATGGAGGGAACTTCCAGGCCACT TCGATTACCAAGTCCCATGGAGAAGACCATGAGCGC GATGCAGATGCTTGGCCGCATGATCTTTTCCAGTG TACCGAGCTCATCAACCCGGCCTTGAACAATGGGC TGCCACCAAACCTCTCCTTCGACGACCCCAAGTCTGT CGTTCACCATGAAAGGAATCGACATCAACATGTCG

	GCATACATGGCCGAATTAAGCTACCTGAATCACCAC GTCAGCAACCACGTCCAGTCCGCCGAGATGCACAA CCAGGGCCTCAACTCCCTCGCCCTGGTCGCCAGCC GCTACGCGGCCGAGACGGTCGAGGTCTTGTCTTG ATGGCCGCGGCATATCTCTATGCACTGTGTCAGGCG CTCGATCTCCGGGCCTGCCACCTGGAGTTCCTCCGC GACGCTCGCAGCACGGTCGATAGCCTCACCGCCGA GTTATGCCTGTCATTCTCCCCCTGTTGTCCGAGTCC GACCAGCGTCAGATCCAGGATTCCACATGGGAGCA GCTCCTGCACCACTGGAACCGAAGCAGCACCAGCG ACCTCCACGACCGCAGTCGCAATGCGGCGAGTCAC ACGATGGGTGCCCTCGTCGAGCTGCTGCCGATGCA GCTGAACGAAGCGACTGCTCTGCCCACTCGCATCG CCCCCAGCAGCAGTGGCTCGACGAGGTGTCTGCC ACGTTGGCTGGCAGCTACGATGCAACCCGAGCCAA GTTCCAATCCAATCCGACCACCCCTTCTATCTCTG CAGTGCATCCCGCAGGATGTACGAATTCGTGCGCA AGGGGCTGGGGGTGCCGCTTCACCGCGGGATTGTT GACCACCCGACCTATCCTTCTGTAACCGAGGAGCA CGCTGGCAAGGAGCTGATCGGGTCGCAGGTGAGCA AGATCTACATGGCATTGCGTGGAGGTGCGTTCCGTG ACGTGCTACAAGATTGTTGGTATAGTTCGGATTCTG TGTGGGGTTTTGCTGGCGAGGAACTGGTTTTGGCTT CCAAGTTGTGA
CHI (codon optimized)	ATGGCGGATTTTAAATTTGAACCAATGCGTAGTCTC ATATATGTTGACTGCGTCAGCGAAGACTATCGACCT AAACTCCAACGCTGGATTTACAAAGTGCATATTCCT GATTCGATCTCTCAGTTCGAGCCTTATGTGACAAAA TACGCGTTTTATCCCTCTTCCCAATCCCACCCCAAG GAGATAGGTTTGGTTACGCCCCGTATGCAACTTACAG AACACCACTGGTTAGTCTCTGACCTTGATCCACGAT TAGAAATCAAAGCGATTGCCGAAACTTTCCCATGG ACGTACTAGTGTGGCAAGGGCAAATACCTGCCGCA GCCCACACGGATGCTCAGATCGATAGTGATGGAGAT GCGGGCAATGCCGCGCGAAAATCCAATAATGCTGA GGGAAATCCATTTATTTTCGCGTTTCTCCGATGTGG TGGGAAAAGGATTTGAAGGGCAAAGGTAGAACAAT AGAAGATGGCGCAAACCTATCGGTTTAACATGACAAT TGGCTTTCCAGAGGGGGTGGATAAGGCAGAAGGTG AGAAATGGCTGTTGAAAAAGTGGTACCGATCTTA CAGGCAGCTCCGGAATGTACTCGTGTGTTGGCGAG CGCGGTGAAAAAAGACATTAATGGATGCGTGATGG ATTGGGTTCTCGAAATATGGTTTGAAAACCAATCGG GCTGGTACAAGGTTATGGTTGATGATATGAAAGCAC

	TAGAAAAACCCTCATGGGCTCAGCAGGACGCATTC CCCTTTCTGAAGCCCTATCATAATGTGTGCTCGGCT GCCGTTGCAGATTATACCCCATCTAACAACCTTAGCT AACTACCGGGGGTACATCACCATGCGT
CHS (codon optimized)	ATGGTACGGAAGCGTTACGTACATTTAACCGAAGAA TTCATCCAAAGAAACCCTAATATATGTGATAACACTT CTCCGTCTTTGGATGTGCGACAGGACTTGCTAGTAG TGGAAGTTCCCAAACCTGGGGCAAGAAGCGGCTACT AAAGCTATCAAAGAGTGGGGCCAACCTAAGAGCAA CATAACACACCTGATTTTTTTGTACCAACTCTTGTGT GGAGATGCCGGGGTCTGATTATCGACTCGCTAACCT CCTGGGTCTAAACCCCTACGTAAAACGCTATATGAT GTATCAGCAGGGGTGCTACGCTGGCGGGATGGTTCT TAGACTTGCTAAAGACTTGGCCGAGAACACCAAGG GAGCCCGTGTCTTAGTCGTATGCTCTGAAATTACTG CCATAGCATTTACGGTCCAAACGAAAACACTTCG GTTGATTACCTAGTCGGTCAGGCAATTTTTGGTGAT GGAGCAGGTGCAGTCATAGTGGGCAGCGATCCAGA TCTCAAGAGAGAACTGCCGCTGTTTGAAATGGTATC GGCAACGCAGACCTTCGTGCCCCGACTCAGTGGGCG CGATCGGGGGTAGGTTGTCAGAGGCTGGGCTTATGT TCTACCTTGGTAAGACTGTTCCCGGTTTGATAAGCG AAAATATCGAGAAAGCTTTGATTCAAGCATTTAGTC CCTTAGGGATAACTGATTGGAACCTCAATCTTTTGGA TTGTACACCCTGGCGGTCCTGCTATTCTTGATCAGG TTGAACTTAAACTCGGCTTAGAAAAGGACAAGATG CGAGCTAGCCGCCACGTGTTGAGCGAATATGGCAAT ATGTCCCATGTATCCGTCTTATTCATTATCGATGAGAT GAGGAAGAAATCAGTGACGGATGGAGCCGCGACTA CGGGTGAAGGACTGGATTGGGGTGTGCTTTTTGGC TTTGGTCCCGGCTAACCATCGAGACGATTGTTCTT CATAGTATACCCACGACCCTGCCAGTTTCTTCCACT ATTGGCAGTCAGGTC
OMT3	ATGAAGAATTCATCAACGGATGAAGATTTATCCATAT TCGCGATGCAAGTGGCTACTTCTTCCATAGTCCCCA GAGCTCTGAAAGCTGTCATAGAACTAGACCTGCTG GAGATGATGAAGAAGGCCGGCCGTCCCCTTTCCAC GTCAGAAATGGCCGCCCAGATTCAGGCCACCAACC CGGAGGCCGCCCTCATGATTGACAGGATCCTTCGA GTTCTCATCGCCAGCAACATTCTAGAATGCACCACT GCTGCCTCTCACGGTGGCGCTGAGCGGCTTTATTCT TTGGCTCCGGTTTGCAAGTTCTTCACCAAGAATGAT GATGGTGTTTCTTGGGCTCCATTGTTTCTCATGATCC AAGACAGAGTCTTCACAGAAGCCTGGGATCATGTA

	AAGGATGCAATAGTTGAAGGAGGAATCCCGTTCAA TATAGCCCATGGAATGAGTGGGTTCGAATACCCGGC AACCGACCCGAGATATAACAAGATTTTCAACCAAG CCATGTCTGATGAATCCACCATGTTTATGCATAAAAT TCTTGAATTATATGATGGATTTGACGGTTTGAAATCT GTTGTGGATGTTGGTGGTGGGAATTGGAGCTTCACTA AAGATGATTATAACCAAGTATCCATCTATTCAGGCCA TCAATTTGATTTGCCCCATGTCATCCAAAATGCTCC ATCTCATCCTGGGTGGAGCACAGAAGTGGAGACA TGTTTGTAGTGTGCCTACAGGAGATGCCATTTTGT TGAAGTGGATTATCCATAATTGGAGCGATGGGCATT GCTTAAAACTCCTGAAAACTGCTACGAAGCACTT CCAGAAAAGGGAAAAGTGATAATCGCAGATCGGAT TCTTCCGGAAACAGAGAATTATAAGGAGGCATCGG CGTCGGTAGACTTGGCCGGCGACGCGCTCATGTTA ACGTTGTTACCCGGTGGGAAGGAGAGGGCAGAAG CAGAATTTCAAGCTTTGGCAAAAGCATCTGGTTTCA AACATTTCCGTAAAGTTTGTGTGCTTTCAGCACTT GGATCATGGAACCTCTACAAATAA
T7 RNA polymerase	ATGAACACTATCAACATTGCGAAGAACGATTTCTCT GACATCGAACTCGCGGCAATCCCGTTCAACACCCT GGCAGATCACTACGGTGAACGTCTGGCCCGTGAAC AGCTGGCTCTGGAACACGAAAGCTATGAAATGGGT GAGGCGCGTTTCCGTAAAGATGTTCGAACGTCAGTTA AAAGCGGGTGAAGTTGCAGACAACGCCGCGGCGA AGCCGCTGATCACCACCCTGCTGCCGAAAATGATC GCTCGCATCAACGATTGGTTCGAAGAAGTGAAAGC GAAACGTGGCAAACGTCCGACTGCCTTCCAGTTCC TGCAAGAAATTAAACCGGAGGCAGTGGCGTATATC ACTATCAAACTACCCTGGCGTGCCTGACCTCTGCG GATAACACGACCGTTCAGGCGGTAGCGTCCGCAAT CGGTCGTGCGATCGAAGATGAAGCCCGCTTCGGCC GTATCCGTGACCTTGAAGCAAAACACTTTAAAAAA AACGTTGAAGAACAGCTGAACAAACGCGTTGGTCA CGTATACAAAAAGGCGTTTATGCAGGTTGTGGAAG CTGATATGCTGTCCAAAGGCCTGCTGGGCGGCGAA GCGTGGAGCTCTTGGCATAAAGAAGATTCTATTCAC GTGGGCGTTTCGCTGCATTGAAATGCTGATCGAATCG ACTGGTATGGTTTCTCTGCACCGTCAGAACGCTGGT GTTGTTGGCCAGGATTCTGAAACTATCGAACTGGCC CCGGAATATGCGGAAGCGATCGCTACTCGCGCAGG TGCGCTGGCCGGTATTTCCCCGATGTTCCAGCCGTG CGTTGTACCGCCTAAACCGTGGACCGGCATCACCG GCGGTGGTTATTGGGCTAACGGTCGCCGTCCGCTGG

	CACTCGTTCGTACCCACAGCAAAAAGGCACTGATG CGTTACGAAGATGTGTACATGCCGGAGGTTTACAAA GCCATCAACATTGCTCAGAATACCGCCTGGAAGATT AATAAAAAAGTGCTCGCGGTGGCGAACGTTATTAC CAAGTGGAACACTGTCCGGTGGAAGATATCCCGG CTATCGAGCGCGAGGAAGTCCGATGAAACCGGAA GATATCGATATGAACCCGGAAGCTCTGACCGCGTGG AAACGTGCTGCTGCTGCCGTATATCGCAAAGACAA GGCGCGTAAGTCTCGCCGTATCTCTCTGGAGTTTAT GCTGGAACAGGCGAACAATTCGCCAACACAAA GCCATCTGGTTCCCGTACAACATGGATTGGCGTGGC CGTGTCTATGCAGTTTCTATGTTCAACCCGCAAGGG AATGATATGACCAAAGGCCTGCTGACTCTGGCAAA AGGTAAGCCGATCGGCAAAGAAGGTTATTACTGGC TGAAAATCCATGGCGCGAATTGCGCGGGTGTAAGATA AGGTACCGTTCCCGGAACGTATTAAGTTCATTGAAG AGAACCACGAGAATATCATGGCATGCGCGAAAAGC CCGCTGGAAAACACTTGGTGGGCTGAGCAGGACTC CCCGTTCTGCTTCCTGGCGTTCTGCTTCGAGTACGC AGGTGTTCAACATCATGGCCTGAGCTACAAGTCTC CCTGCCTCTGGCATTTCGACGGTTCTTGCAGCGGTAT CCAGCACTTTTCCGCTATGTTACGTGATGAAGTAGG TGGCCGTGCTGTCAATCTGCTGCCGTCCGAAACCGT TCAAGATATCTATGGTATCGTAGCGAAAAAGGTTAA CGAGATTCTGCAAGCAGATGCTATCAACGGTACCGA CAACGAGGTAGTTACCGTGACCGATGAAAACACCG GTGAAATTTCCGAAAAAGTAAAAGTGGGGACCAAG GCACTGGCTGGCCAGTGGCTGGCCTACGGCGTGAC TCGCAGCGTCACCAAACGTAGCGTGATGACGCTCG CTTATGGCAGCAAAGAGTTTGGCTTTCGCCAGCAA GTTCTGGAGGATACTATCCAGCCAGCCATTGACTCG GGCAAGGGTCTGATGTTTACCCAGCCGAACCAGGC CGCTGGATATATGGCAAAAGTATCTGGGAATCTGT AAGCGTCACCGTTGTGGCAGCGGTTGAAGCTATGA ATTGGTTGAAAAGCGCCGCGAAAGTCTGGCAGCG GAAGTGAAAGATAAAAAAGTGGCGAAATCCTGCG TAAACGCTGCGCGGTTCACTGGGTCACTCCGGATG GCTTCCCAGTTTGGCAGGAATACAAAAAACCTATCC AGACCCGCCTGAATCTGATGTTCTGGGCCAATTCC GTCTGCAACCGACCATCAACACCAACAAAGACAGC GAAATTGACGCCCAACAAACAGGAGTCCGGCATTGC CCCTAACTTCGTTCACTCTCAGGACGGCTCTCATCT GCGCAAAAGTGTGCTTTGGGCTCATGAAAAGTACG GCATCGAATCCTTTGCGCTGATCCACGACTCCTTCG
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	GTACCATCCCGGCGGACGCGGCCAACCTGTTCAAA GCTGTTTCGTGAAACTATGGTAGACACGTACGAAAG CTGCGACGTTCTGGCAGACTTCTACGATCAGTTCGC TGACCAGCTGCACGAAAGCCAGCTGGACAAGATGC CAGCTCTGCCGGCTAAAGGTAATCTGAACCTGCGC GACATTCTGGAATCTGACTTCGCTTTCGCCTGA
TtgR	ATGGTTAGACGAACAAAAGAGGAGGCCCAGGAGA CCCGCGCGCAAATTATAGAAGCTGCGGAAAAGGCA TTTTACAAACGTGGCGTTGCGCGAACCACCCTTGC GGACATCGCCGAGCTGGCCGGAGTCACCCGAGGTG CTATCTACTGGCACTTCTCGAACAAAGCTGAGTTAG TGCAAGCGCTGCTGGACTCATTACACGAGACTCAT GACCACCTGGCAAGGGCTTCTGAATCTGAAGATGA GCTCGACCCGTTTCGGCTGTATGCGCAAATTACTATT ACAGGTCTTCAACGAACTTGTACTGGACGCCCCGTA CACGACGTATTAACGAAATTCTCCATCATAAGTGCG AATTTACCGACGATATGTGCGAAATTCGTCAACAAC GACAGGGTGCTGTGTTGGACTGTCACGAAGGTGTT GCGTTGGCATTGGCTAATGCTGTTTCGTCGGGAGCAA CTGCCGACTGATCTTGATATCGAACGTGCTGCCGTG GCGATTTTTGCTTACGTCGACGGTCTTATCGGTGCG TGGCTACTTCTGCCCCGATTCGTTTGACTTACTGCGC GATGTGGAAAAATGGGTTGACACAGGTTTGGATAT GCTTCGGCTGTCCCCGGCCCTCCGAAAA
GFP	ATGCGTAAAGGAGAAGAAGCTTTTCACTGGAGTTGT CCCAATTCTTGTGTAATTAGATGGTGATGTTAATGGG CACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGA TGCAACATACGGAAAACCTACCCTTAAATTTATTTG CACTACTGGAAAACCTACCTGTTCCATGGCCAACACT TGTCACTACTTTTCGGTTATGGTGTTCAATGCTTTGCG AGATACCCAGATCATATGAAACAGCATGACTTTTTTC AAGAGTGCCATGCCCCGAAGGTTATGTACAGGAAAG AACTATATTTTCAAAGATGACGGGAACTACAAGAC ACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTG TTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAG AAGATGGAAACATTCTTGGACACAAATTGGAATAC AACTATAACTCACACAATGTATACATCATGGCAGAC AAACAAAAGAATGGAATCAAAGTTAACTTCAAAT TAGACACAACATTGAAGATGGAAGCGTTCAACTAG CAGACCATTATCAACAAAATACTCCAATTGGCGATG GCCCTGTCCTTTTACCAGACAACCATTACCTGTCCA CACAATCTGCCCTTTCGAAAGATCCCAACGAAAAG AGAGATCACATGGTCCTTCTTGAGTTTGTAAACAGCT GCTGGGATTACACATGGCATGGATGAACTATACAAA

	TAA
Promoter J23102	TTGACAGCTAGCTCAGTCCTAGGTACTGTGCTAGC
Promoter J23106	TTTACGGCTAGCTCAGTCCTAGGTATAGTGCTAGC
Promoter J23107	TTTACGGCTAGCTCAGCCCTAGGTATTATGCTAGC
Promoter ttg2	CAGCAGTATTTACAAACAACCATGAATGTAAGTATA ATCC
Promoter ttg1	CAGCAGTATTTACAAACAACCATGAATGTAAGTATA TTCC
RBS B003m	AGAGTCACACAGGACTACTA
RBS B0032m	AGAGTCACACAGGAAAGTACTA
RBS for V.natriegens 1	GGCAGCAGCAGCCAAAGGAGATATA
RBS for V.natriegens 2	AGAGTCACACAGGAAAGTACTA
Plac and LacO	GAGGATCGAGATCGAAATTAATGTGAGTTAGCTCAC TCATTAGGCACCCCAGGCTTTACACTTTATGCTTCC GGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACA ATTTC

Table S3. Primers' Name and Sequences

Primer	Primer's Usage	Sequence (5'-3')
TAL-f-XhoI	insert the TAL gene into the pETDuet-4CL plasmid	ATCGCTGACGTCGGTACCCTCGAGG GCAGCAGCAGCCAAAGGAG
TAL-r-XhoI	insert the TAL gene into the pETDuet-4CL plasmid	GCAGCGGTTTCTTTACCAGACTCGA GTCACAACCTTGAAGCCAAAACCA GTTCC
pETDuet-f-Plac	Linearize the vector pETDuet-4CL-TAL	TCGATCTCGATCCTCTACGCC
pETDuet-r-MCS1del	Linearize the vector pETDuet-4CL-TAL	TATTGTACACGGCCGCATAATC
pETDuet-r-linear	Linearize the vector pETDuet-4CL-TAL	CAGCACATGGACTCGTCTACTA
T7pol-r-Plac-new	Linearize the vector pMB1-T7pol	TTTCGATCTCGATCCTCTAGAGACCC TGATATTGACGGC
T7pol-f-Plac	Linearize the vector pMB1-T7pol	GTGAGCGGATAACAATTCATGAAC ACTATCAACATTGCGAAGAAC
cap-plac-up	Anneal to form short pieces	GAGGATCGAGATCGAAATTAATGTG AGTTAGCTCACTCATTAGGCACCCC AGGCTTTACACTTTATGCTTCCGGCT CGTATGTTGTGTGGAATTGTGAGCG GATAACAATTTC
cap-plac-bottom	Anneal to form short pieces	GAAATTGTTATCCGCTCACAATTCCA CACAACATACGAGCCGGAAGCATAA AGTGTAAGCCTGGGGTGCCTAATG AGTGAGCTAACTCACATTAATTTTCG ATCTCGATCCTC
pMB1-Plac-T7pol-f	Linearize the vector pMB1-plac-T7pol	AGAGGATCGAGATCGAAATTAATGT GAGTTAG
pMB1-Plac-T7pol-r	Linearize the vector pMB1-plac-T7pol	TGCGGCCGTGTACAATACAGGAAAC AGCTATGACCATGC
pETDuet-r-MCS1del	Linearize the vector pETDuet-4CL-TAL	TATTGTACACGGCCGCATAATC
4CL-TAL-r	Linearize the vector pETDuet-4CL-TAL	GTAGACGAGTCCATGTGCTG
pACYCDuet-I-S-MCS1-r	Linearize the vector pACYCDuet-OMT3	CGGCCGATATCCAATTGAGATCTG
pACYCD	Linearize the vector	GTCGGTACCCTCGAGTCTGGTAAAG

uet-I-S-MCS1-f	pACYCDuet-OMT3	
singlet-CHI-f	insert the CHI gene into the pACYCDuet-OMT3 plasmid	CTCAATTGGATATCGGCCGATGGCG GATTTTAAATTTGAACCAATGCG
singlet-CHI-r	insert the CHI gene into the pACYCDuet-OMT3 plasmid	TACCAGACTCGAGGGTACCGACTTA ACGCATGGTGATGTACCCC
singlet-CHS-r	insert the CHS gene into the pACYCDuet-OMT3-CHI plasmid	CAGCGGTTTCTTTACCAGACTCGAG TTAGACCTGACTGCCAATAGTG
singlet-CHS-f	insert the CHS gene into the pACYCDuet-OMT3-CHI plasmid	CATGCGTTAAGTCGGTACCCTCGAG AGAGTCACACAGGAAAGTAC
OMT3-f-PstI	insert the OMT3 gene into the pACYCDuet-1 plasmid	AATTCGAGCTCGGCGCGCCTGCAGA TGAAGAATTCATCAACGGATGAAG
OMT3-r-PstI	insert the OMT3 gene into the pACYCDuet-1 plasmid	GCCGCAAGCTTGTGACCTGCAGTT ATTTGTAGAGTTCCATGATCCAAGTG
pACYC177-r-MCS2	Linearize the vector pACYC177	GACAGTTTTATTGTTTCATG
pACYC177-f-MCS2	Linearize the vector pACYC177	CATAAACAGTAATACAAGGG
Pttg2-J23106-linker-up	Anneal to form short pieces	TGAACAATAAACTGTCCAGCAGTA TTTACAAACAACCATGAATGTAAGT ATAATCCTGTGAAATTCCACAAGAG TCACACAGGAAAGTACTACATAAAC AGTAATACAAGGGG
Pttg2-J23106-linker-bottom	Anneal to form short pieces	CCCCTTGTATTACTGTTTATGTAGTA CTTTCCTGTGTGACTCTTGTGGAATT TCACAGGATTATACTTACATTCATGG TTGTTTGTAATACTGCTGGACAGTT TTATTGTTCA
pACYC177-Ttgr-MCS3-f	Linearize the vector pACYC177-Pttg2	TTCAACAGATCGGGAAGGG
pACYC177-Ttgr-MCS3-r	Linearize the vector pACYC177-Pttg2	CTTGTCGGTAAAGATGCGG
J23106-rbs-ttgr-linker-r	insert the J23106-TtgR gene into the pACYC177-Pttg2-GFP plasmid	CTTCCCGATCTGTTGAATTATTTTCG GAGGGCCGG

J23106-rbs-ttgr-linker-f	insert the J23106-TtgR gene into the pACYC177-Pttg2-GFP plasmid	GCATCTTTACCGACAAGTTTACGGC TAGCTCAGTCCT
cTtgr pro-f	Linearize the vector pACYC177-Pttg2-GFP-J23106-TtgR	GATATACCATGGGCAGC
cTtgr pro-r	Linearize the vector pACYC177-Pttg2-GFP-J23106-TtgR	CTTGTCGGTAAAGATGC
J23102-rbs-linker-up	Anneal to form short pieces	GGGTAAAAGCTAACCGCATCTTTAC CGACAAGTTGACAGCTAGCTCAGTC CTAGGTACTGTGCTAGCTGTGAAAT TCCACAAGAGTCACACAGGACTACT ATAAGGAGATATACCATGGGC
J23102-rbs-linker-bottom	Anneal to form short pieces	GCCCATGGTATATCTCCTTATAGTAG TCCTGTGTGACTCTTGTGGAATTTC ACAGCTAGCACAGTACCTAGGACTG AGCTAGCTGTCAACTTGTCGGTAAA GATGCGGTTAGCTTTTACCC
J23107-rbs-linker-up	Anneal to form short pieces	GGGTAAAAGCTAACCGCATCTTTAC CGACAAGTTTACGGCTAGCTCAGCC CTAGGTATTATGCTAGCTGTGAAATT CCACAAGAGTCACACAGGACTACTA TAAGGAGATATACCATGGGC
J23107-rbs-linker-bottom	Anneal to form short pieces	GCCCATGGTATATCTCCTTATAGTAG TCCTGTGTGACTCTTGTGGAATTTC ACAGCTAGCATAATACCTAGGGCTG AGCTAGCCGTAAACTTGTCGGTAAA GATGCGGTTAGCTTTTACCC
cPttg-linear-f	Linearize the vector pACYC177-Pttg2-GFP-J23106-TtgR	GTGTATGCGTAAAGGAGAAG
cPttg-linear-r	Linearize the vector pACYC177-Pttg2-GFP-J23106-TtgR	CTGCTGGACAGTTTTATTG
petduet-t7-4cl-tal-colpcr-r	Colony PCR for pETDuet-TAL-4CL	CGACGATGGTCGACTACTG
petduet-t7-4cl-tal-colpcr-f	Colony PCR for pETDuet-TAL-4CL	GAACCAGGCCGCTGGATATATG
L66A-TtgR-f	single point mutation	GCTGGACTCAGCACACGAGACTCAT GACCACCTGGCAAGG

L66A-TtgR-r	single point mutation	GTCTCGTGTGCTGAGTCCAGCAGCG CTTGCACTAACTCAG
G176R-TtgR-f	single point mutation	CGGTCTTATCCGCCGGTGGCTACTTC TGCCCGATTTCGTTTG
G176R-TtgR-r	single point mutation	CCACCGGCGGATAAGACCGTCGACG TAAGCAAAAATCGCCAC
N110A-TtgR-f	single point mutation	CGACGTATTGCCGAAATTCTCCATCA TAAGTGCGAATTTACCGACG
N110A-TtgR-r	single point mutation	CGACGTATTGCCGAAATTCTCCATCA TAAGTGCGAATTTACCGACG
177colpcr-f	Colony PCR for pACYC177-J23106- TtgR-Pttg2-GFP	CAAAGCCACGTTGTGTCTC
177colpcr-r	Colony PCR for pACYC177-J23106- TtgR-Pttg2-GFP	CCGTCTCAATAAACCGAACC
chi-chs-colpcr-f	Colony PCR for pACYCDuet-OMT-CHI- CHS	CTGCGCTAGTAGACGAGTCCATGTG
chi-chs-colpcr-r	Colony PCR for pACYCDuet-OMT-CHI- CHS	GATATACCATGGGCAGCAGCCATC
CHS-F153V-f	single point mutation	GGCAATTGTAGGTGATGGAGCAGGT GCAGTCATAG
CHS-F153V-r	single point mutation	CCATCACCTACAATTGCCTGACCGA CTAGGTAATCAACCG
CHS-A82M-f	single point mutation	GATTATCGACTCATGAACCTCCTGG GTCTAAACCCCTACG
CHS-A82M-r	single point mutation	GAGGTTTCATGAGTCGATAATCAGAC CCCGGCATCTCCAC
mutantCH S-A82M-f	single point mutation	CAGGTTAATGAATTTGCTCGGTCTG AACCCTTACGTG
mutantCH S-A82M-r	single point mutation	GCAAATTCATTAACCTGTAATCAGA ACCGGGCATTTC

Table S4. Plasmids' Components and Function

Plasmid	Components	Function
pETDuet-T7RNAP-4CL-TAL	Plac-T7RNAP, PT7-4CL-TAL	To express T7 RNAP and 4CL, TAL for biosynthesis of flavonoids.
pACYCDuet-OMT3-CHI-CHS	PT7-OMT3, PT7-CHI-CHS	To express OMT3, CHI and CHS for biosynthesis for flavonoids.
pACYC177-Pttg2-GFP-J23106-TtgR	PJ23106-TtgR, Pttg2-GFP	To building biosensors for product detection
pACYC177-Pttg2-GFP-J23102-TtgR	PJ23102-TtgR, Pttg2-GFP	To building biosensors for product detection
pACYC177-Pttg2-GFP-J23107-TtgR	PJ23107-TtgR, Pttg2-GFP	To building biosensors for product detection
pACYC177-Pttg1-GFP-J23106-TtgR	PJ23106-TtgR, Pttg1-GFP	To building biosensors for product detection
pACYC177-Pttg1-GFP-J23107-TtgR	PJ23107-TtgR, Pttg1-GFP	To building biosensors for product detection
pETDuet-Plac-4CL-CHSmutants	Plac-4CL, Plac-CHSmutants-CHI	To express 4CL, CHS and CHI for detect the effect of directed evolution

S5 Protocols for Molecular Cloning and Bacteria Culture

PCR: We use Vazyme's Phanta Max kit for amplifying the genes or fragments. The protocol was followed and the PCR product was purified using Vazyme's FastPure Kit.

Restriction Enzyme Digestion: For digesting plasmids or fragments, we add 4 μ g plasmids or fragments, 3 μ L FastDigest Buffer in a 30 μ L system. The reaction liquid was then incubated under 37°C for 30min then heated under 85°C for 20min.

Point mutation: To mutate certain site in a gene in cloning vector (pUC19 or pUC57), we used primers with point mutation to linearize the plasmid and introduced the mutation to it. The linearized plasmid contains 15-20bp of homologous sequences (which contains the mutated site). It is recircled using Vazyme's CE II kit.

Homologous Recombination: We used Vazyme's CE II kit for single fragment insertion and Beyotime's Seamless Clone kit for multi-fragments insertion. The reaction liquid was mixed by the instruction then incubated under 37°C for 30min.

Plasmid transformation for *E. Coli*: Unfreeze Trelief 5 α Competent Cell on ice for about 15min. Then add no more than 10 μ L recombination product or 1 μ g plasmid to 100 μ L cell. Incubated on ice for 30min, then heat shock under 42°C for 60s. Then after recover on ice for 3min, add 1mL LB to the competent cell, incubated under 37°C, 200rpm. Then spread the cell on LB agar plate with appropriate antibiotic.

Plasmid extraction and sequencing: Pick up a single colony of *E. Coli* Trelief 5 α containing the plasmid from the agar plate and add into LB liquid medium with appropriate antibiotic. Then culture under the condition of 37°C, 250rpm for 12-16h.

Then use Sangon's SanPrep kit (only pACYC plasmids) or Vazyme's RapidLyse kit for plasmid extraction. The plasmid was then sent to Sangon Biotech for sequencing (Sanger Sequencing).

Colony PCR of bacteria: Pick up a single colony on the plate and add into the PCR reaction liquid (RapidTaq) as template. PCR Procedure: 95°C 10min – (95°C 30s – 55°C 15s – 72°C 60s) 30 cycles – 72°C 5min.

Culturing *V. natriegens* ATCC14048: *V. natriegens* ATCC14048 grows well in 2216E and LB medium. V2 salts can be added to LB to make LBv2 medium. To make competent cell and perform transformation, only use BHIV2 medium.

Preparation of antibiotic solution: For carbenicillin storage solution, dissolve 500mg carbenicillin disodium powder in water and volume to 10mL. For kanamycin storage solution, dissolve 300mg kanamycin sulfate powder in water and volume to 10mL. For chloramphenicol storage solution, dissolve 250mg chloramphenicol powder in anhydrate ethanol and volume to 10mL. All the storage solution was filtered with 0.22µm filter membrane and stored under -20°C. Add the storage solution to the medium under the ratio of 1:1000 when antibiotic is used.

Preparation of medium: LB medium: dissolve 5g yeast extract, 10g peptone, 10g sodium chloride in water and volume to 1L. To make solid medium, add another 20g agar. Then the medium is sterilized under 121°C for 20min. To make 2216E or BHI medium, follow the instructions. To make BHIV2 or BHIV2 sucrose medium, sterilize BHI medium, v2 salt solution and sucrose solution separately and mix together after cooling down to room temperature.

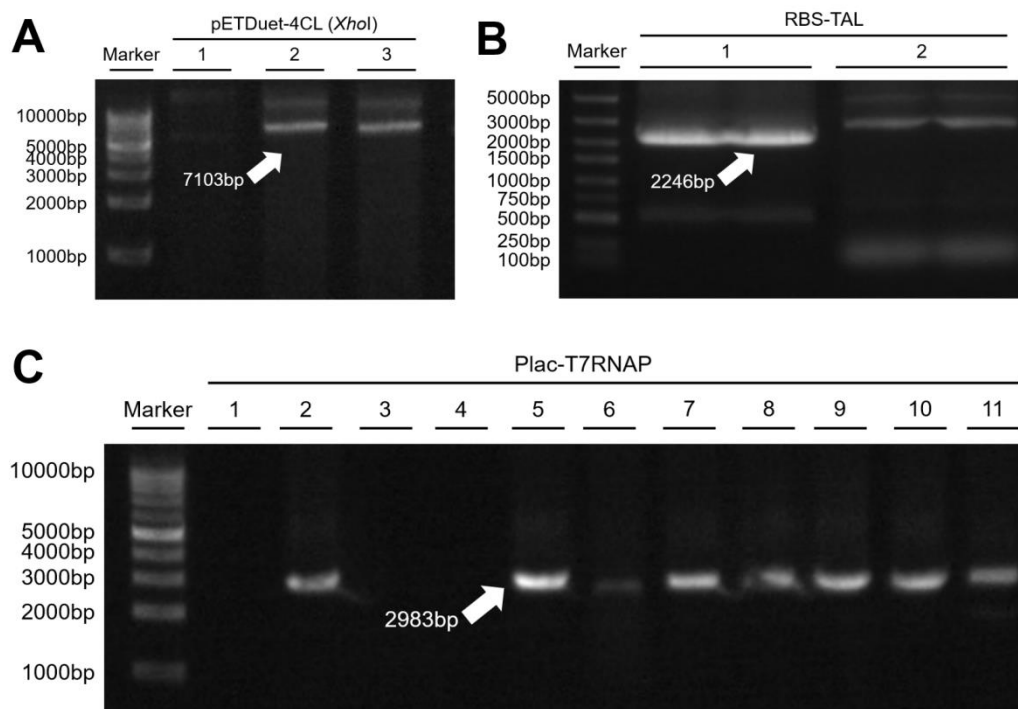


Figure S1. Electropherogram of fragments used when constructing pETDuet-T7RNAP-4CL-TAL. A. Digestion product of plasmid pETDuet-4CL; B. Amplified RBS-TAL fragments; C. Amplified Plac-T7RNAP fragments.

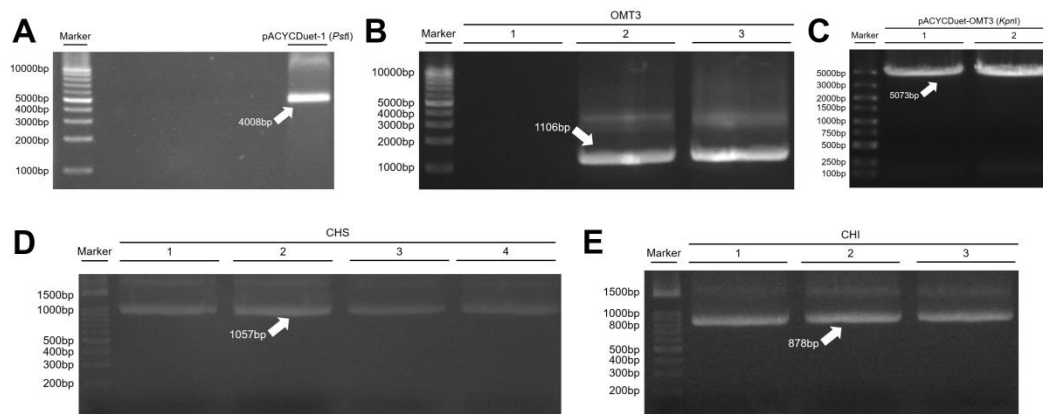


Figure S2. Electropherogram of fragments used when constructing pACYCDuet-OMT3-CHI-CHS. A. Digestion product of plasmid pACYCDuet-1; B. Amplified OMT3 fragments; C. Digestion product of plasmid pACYCDuet-OMT3; D, E. Amplified CHS or CHI fragments.

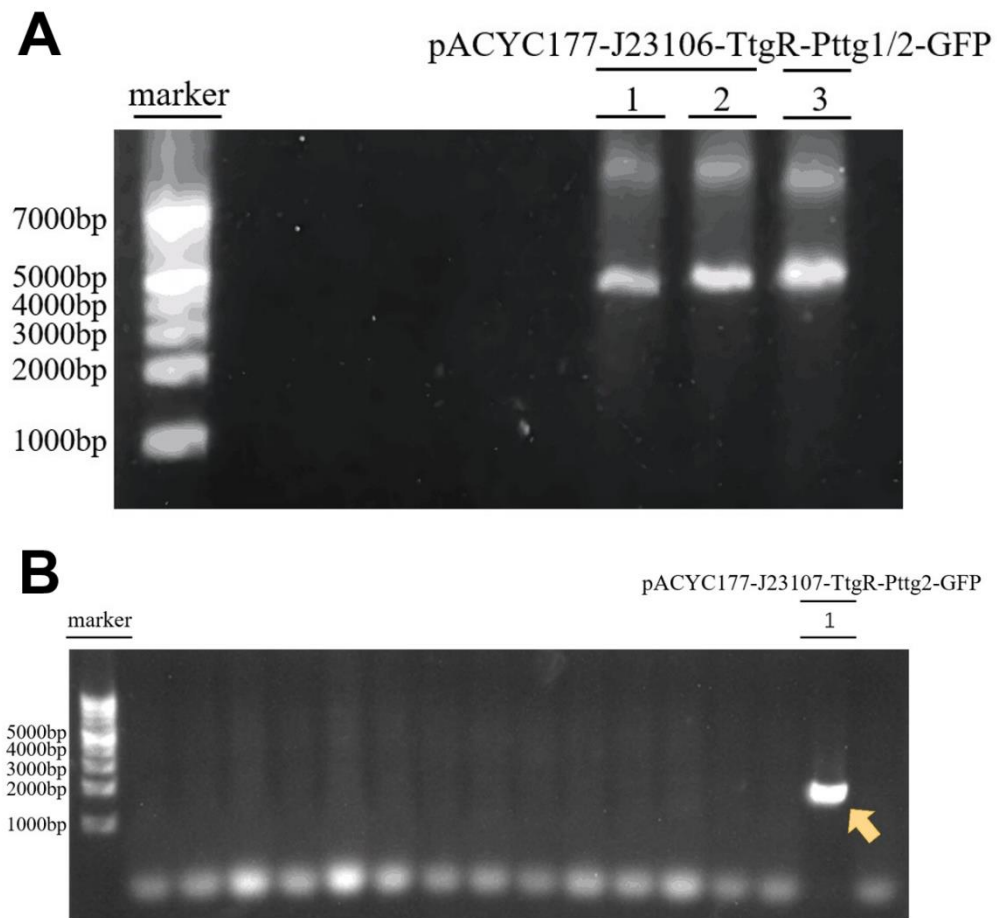


Figure S3. Electropherogram of colony PCR results when constructing pACYC177 biosensor plasmids. A. Colony PCR result of *E. coli* clones containing pACYC177-J23106-TtgR-Pttg1-GFP or pACYC177-J23106-TtgR-Pttg2-GFP; B. Colony PCR result of *E. coli* clone containing pACYC177-J23107-TtgR-Pttg2-GFP.