CRISPathBrick: Modular Combinatorial Assembly of Type II-A CRISPR Arrays for dCas9-Mediated Multiplex Transcriptional Repression in *E. coli*

Supporting Information

Supporting Figures.

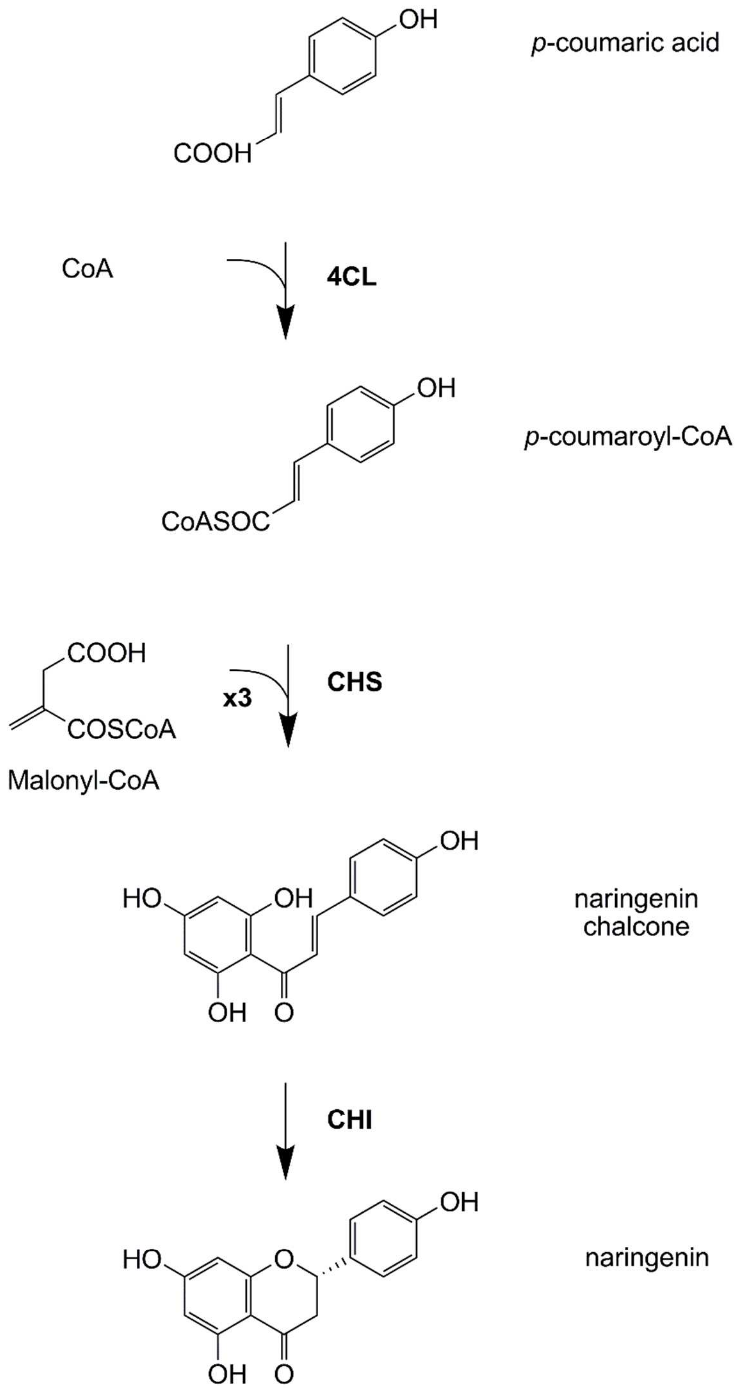

Figure S1. Naringenin biosynthetic pathway from *p*-coumaric acid.

Supporting Tables.

Table S1. Plasmids used in this study

ID	Properties/Genotype	Spacers in CRISPR Array	Reference
pdCas9	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	non-targeting with two BsaI sites from Marraffini et al.	[1]
pCRISPathBrick	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	1xBsaI	This Study
pETM6	ColE1(Amp^R), ePathBrick feature	N/A	[2]
pETM6-mCherry	ColE1(Amp^R), ePathBrick feature, mCherry	N/A	[3]
pCRISPReporter	ColE1(Amp^R), CRISPReporter feature	N/A	This Study
pCRISPReporter-mCherry	ColE1(Amp^R), CRISPReporter feature, codon optimized mCherry	N/A	This Study
pCRISPReporter-FadR_mCherry_fusion	ColE1(Amp^R), CRISPReporter feature, BL21 P_{fadR} and 40 amino acids of FadR N-terminus translationally fused to codon-optimized mCherry through flexible $(GGGGS)_3$ linker	N/A	This Study
pCRISPReporter-PGK_mCherry_fusion	ColE1(Amp^R), CRISPReporter feature with BL21 P_{epd} and P_{pgk} promoters and 40 amino acids of FadR N-terminus translationally fused to codon-optimized mCherry through flexible $(GGGGS)_3$ linker	N/A	This Study
pCRISPathBrick-T7	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	T7_KO **(A in Figure 3)**	This Study
pCRISPathBrick-T7_opp	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	T7_opp_KO **(B in Figure 3)**	This Study
pCRISPathBrick-mCherry	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	mCherry_KO **(C in Figure 3)**	This Study
pCRISPathBrick-mCherry_opp	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	mCherry_opp_KO **(D in Figure 3)**	This Study
pCRISPathBrick-T7-mCherry	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	T7_KO; mCherry_KO **(A and C in Figure 3)**	This Study
pCRISPathBrick-CysH	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	CysH_KO	This Study
pCRISPathBrick-TrpCBA	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	TrpCBA_KO	This Study
pCRISPathBrick-AroF_TyrA	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	AroF_TyrA_KO	This Study
pCRISPathBrick-HisBHAFI	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	HisBHAFI_KO	This Study
pCRISPathBrick-CysH-TrpCBA	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	CysH_KO; TrpCBA_KO	This Study
pCRISPathBrick-CysH-AroF_TyrA	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	CysH_KO; AroF_TyrA_KO	This Study
pCRISPathBrick-CysH-HisBHAFI	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	CysH_KO; HisBHAFI_KO	This Study
pCRISPathBrick-KpsMT	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	KpsMT_KO	This Study
pCRISPathBrick-FadR	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	FadR_KO	This Study
pCRISPathBrick-PGK	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	FadR_KO	This Study
pCRISPathBrick-SucABCD	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	SucABCD_KO	This Study
pCRISPathBrick-SucABCD-FumC	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	SucABCD_KO; FumC_KO	This Study
pCRISPathBrick-SucABCD-FumC-ScpC	pACYC184(Cm^R), tracrRNA, *cas9*(D10A, H840A)	SucABCD_KO; FumC_KO; ScpC_OK	This Study
pUC57-*Vv*4CL	pUC57(Amp^R), *Vv*4CL	N/A	This Study
pUC57-*Cm*CHS	pUC57(Amp^R), *Cm*CHS	N/A	This Study
pUC57-*Cm*CHI	pUC57(Amp^R), *Cm*CHI	N/A	This Study
pETM6-*Vv*4CL	ColE1(Amp^R), ePathBrick feature, *Vv*4CL	N/A	This Study
pETM6-*Cm*CHS	ColE1(Amp^R), ePathBrick feature, *Cm*CHS	N/A	This Study
pETM6-*Cm*CHI	ColE1(Amp^R), ePathBrick feature, *Cm*CHI	N/A	This Study
pETM6-*Vv*4CL-m-*Cm*CHS	ColE1(Amp^R), ePathBrick feature, *Vv*4CL, *Cm*CHS	N/A	This Study
pETM6-*Vv*4CL-m-*Cm*CHS-m-*Cm*CHI	ColE1(Amp^R), ePathBrick feature, *Vv*4CL, *Cm*CHS, and *Cm*CHI	N/A	This Study
pTKRED	pSC101(Spc^R), *recA*, I-SceI, λ-red genes	N/A	[4]
pTKS/CS	p15a(Tet^R)	N/A	[4]
pTKIP	pMB1(Amp^R, Kan^R)	N/A	[4]
pTKIP-mCherry	pMB1(Amp^R, Kan^R), *mCherry*	N/A	This Study

Table S2. Strains used in this study

ID	Genotype	Source
E. coli DH5α™	F- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(rK- mK+), λ–	Novagen
E. coli BL21 Star™ (DE3)	F^- *omp*T gal dcm lon *hsd*$S_B(r_B^-\ m_B^-)$ *galdcmrne*131 λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])	Invitrogen

E. coli BLΔ*suc*C	BL21 Star™ (DE3) Δ*sucC*::*FRT*	[5]
E. coli K-12 MG1655	F- λ- ilvG- rfb-50 rph-1	CSCG
E. coli K-12 MG1655 (DE3)	MG1655 (DE3)	This Study
E. coli K-12 MG1655 JE1 (DE3)	MG1655 (DE3) Δ*atpI*::[Kan^R P_{T7lac} *mCherry*]	This Study
E. coli K5 (strain ATCC 23506, serovar O10:K5:H4)	unmodified	ATCC
E. coli Nissle 1917 (serovar O6:K5:H1)	unmodified	[6]

Table S3. PCR Primers used in this study

ID	Nucelotide Sequence (5'-->3')
CRISPReporter_ApaI_F	GGAAAGGGCCCGCTGGGAGTTCGTAG
CRISPReporter_SalI_R	CCCTTGTCGACGCTTGGATTCTGC
fadR_prom_PacI_F	CGCTTTAATTAATCGGGGGACTGGCCCAAAAAATTGC
fadR_prom_XhoI_R	GGAGGACTCGAGGCCAATTAATTCTGAAAGTTCACG
pgk_prom_PacI_F	CGCTTTAATTAAACTGAAGAAGGCCGGTATCAC
pgk_prom_XhoI_R	GGGAACTCGAGGAGGTAATCTTTAACCAGACG
For_pETM6_w/ApaI	CGCGCGGGCCCATGCGTCCGGCGTAGCCTA
Rev_pETM6_w/SalI	CGCCAATCCGGATATAGTCGAC
AtpI-pTKSCSfor	CAAAAAGCGGTCAAATTATACGGTGCGCCCCCGTGATTTCAAACAATAAGTACGGCCCCAAGGTCCAAACGGTGA
AtpI-pTKSCSrev	ATAACGTGGCTTTTTTTGGTAAGCAGAAAATAAGTCATTAGTGAAAATATTTGGCTTCAGGGATGAGGCGCCATC
ChkTetAFOR	CAGGTTATCTTTGCTCCTTG
atpI/gidB_ver_R	CTTCGTCAGGTGCAACATGAGC
ChkNEOfor	CTGAATGAACTGCAGGACGA
atpI/gidB_ver_F	CAGTAACTGAACGAGCAGAAG
mCherryREV	CGGATGCTTAACGTACGCTTTCG

Table S4. CRISPathBrick oligonucleotides used in this study

ID	Nucleotide Sequence (5'→3')	[Proto]spacer Sequence (5'→3')	PAM (5'→3')
1xBsaI_F	AAACTGAGACCTGTCTCGGAAGCTCATAGGACTCG	N/A	N/A
1xBsaI_R	AAAACGAGTCCTATGAGCTTCCGAGACAGGTCTCA		
T7_KO_F	AAACCGATCCCGCGAAATTAATACGACTCACTATGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA	CGATCCCGCGAAATTAATACGACTCACTAT	AGG
T7_KO_R	GTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACATAGTGAGTCGTATTAATTTCGCGGGATCG		
T7_opp_KO_F	AAACTTCCCCTATAGTGAGTCGTATTAATTTCGCGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA	TTCCCCTATAGTGAGTCGTATTAATTTCGC	GGG
T7_opp_KO_R	GTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACGCGAAATTAATACGACTCACTATAGGGGAA		
mCherry_KO_F	AAACATATGGTTTCAAAAGGCGAAGAAGACAACAGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA	ATATGGTTTCAAAAGGCGAAGAAGACAACA	TGG
mCherry_KO_R	GTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACTGTTGTCTTCTTCGCCTTTTGAAACCATAT		
mCherry_opp_KO_F	AAACAATTCGTGACCATTGACGCTGCCTTCCATGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA	AATTCGTGACCATTGACGCTGCCTTCCATG	TGG
mCherry_opp_KO_R	GTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACCATGGAAGGCAGCGTCAATGGTCACGAATT		
CysH_KO_F	AAACGCAGTTCGTTCAGGGCGTTTAGATCGAGTTGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA	GCAGTTCGTTCAGGGCGTTTAGATCGAGTT	TGG
CysH_KO_R	GTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACAACTCGATCTAAACGCCCTGAACGAACTGC		
TrpCBA_KO_F	AAACGATCTTCATGGCCATGCAGGCGCATTAACAGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA	GATCTTCATGGCCATGCAGGCGCATTAACA	TGG
TrpCBA_KO_R	GTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACTGTTAATGCGCCTGCATGGCCATGAAGATC		
AroF_TyrA_KO_F	AAACATGCTCGTTTGCGATAGTTGATCCTCAGCGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA	ATGCTCGTTTGCGATAGTTGATCCTCAGCG	AGG

AroF_TyrA_KO_R	GTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACCGCTGAGGATCAACTATCGCAAACGAGCAT		
HisBHAFI_KO_F	AAACGACGGTAATTCGCAGGCAGCCGCTTAAAGAGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA	GACGGTAATTCGCAGGCAGCCGCTTAAAGA	GGG
HisBHAFI_KO_R	GTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACTCTTTAAGCGGCTGCCTGCGAATTACCGTC		
KpsMT_KO_F	AAACTTATTTGAAATTCCCTTTCTGATTATCTCCGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA	TTATTTGAAATTCCCTTTCTGATTATCTCC	AGG
KpsMT_KO_R	GTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACGGAGATAATCAGAAAGGGAATTTCAAATAA		
FadR_KO_F	AAACTGAGATTTCCATAACACAGCAAAACAAAGTGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA	TGAGATTTCCATAACACAGCAAAACAAAGT	TGG
FadR_KO_R	GTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACACTTTGTTTTGCTGTGTTATGGAAATCTCA		
SucABCD_KO_F	AAACAGGAACCTTTAAAAACTGTCTTAGTGTCAGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA	AGGAACCTTTAAAAACTGTCTTAGTGTCAG	GGG
SucABCD_KO_R	GTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACCTGACACTAAGACAGTTTTTAAAGGTTCCT		
FumC_KO_F	AAACCTTATTATTTACCATTTGATAACAAATGTTGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA	CTTATTATTTACCATTTGATAACAAATGTT	TGG
FumC_KO_R	GTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACAACATTTGTTATCAAATGGTAAATAATAAG		
ScpC_KO_F	AAACTATGCTGGATAATTTCTGCCGCTTCATTGGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA	TATGCTGGATAATTTCTGCCGCTTCATTGG	CGG
ScpC_KO_R	GTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACCCAATGAAGCGGCAGAAATTATCCAGCATA		

Table S5. Synthesized DNA used in this study

ID	Nucelotide Sequence (5'→3')	Purpose
CRISPReporter_gBlock	GGAAAGGGCCCGCTGGGAGTTCGTAGACGGACCTAGGTCTAGATTCAGCCAAAAAACTTAAGACCGCCGGTCT TGTCCACTACCTTGCAGTAATGCGGTGGACAGGATCGGCGGTTTTCTTTTCTCTTCTCAACATTACTCGCATC CATTCTCGGCGATCGCGCGGCCGCTTTAATTAAGAATTCAAGCTTAGATCTCAATTGCTCGAGGGCGGTGGCG GTAGTGGCGGTGGCGGTTCCGGCGGTGGCGGTTCACATATGGCACTGAAGGTCCTCAATCGCACTGGAAACAT CAAGGTCGGGTACCGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGGAG GCTGTCTCGTCTCGTCTCACTAGTGCTAGCAACAAACGCAGAATCCAAGCGTCGACAAGGG	gBlock from IDT cloned into pETM6 with ApaI-SalI
*Vv*4CL	ATGATTAGTATTGAAACGCAAAACCCGGATGTTAGCAACCTGGACACCTCGCACTCTATTCCGAAAATGGCAA ACCGTATTGATGACCATGTGTTTCGTTCTAAACTGCCGGAAATTCCGATCAGTAACCATCTGCCGCTGCACAC GTATTGCTTCGAAAATTACTCGCAGTTTGCAGACCGTCCGTGTCTGATTGTTGGCTCGACGAACAAAACCTAT AGCTTCGCTGAAACCCATCTGATCTCTCGCAAAGTGGGCGCAGGTTTTGCTCACCTGGGTCTGAAACAGGGCG ATGTGGTTATGATTCTGCTGCAAAATTGCGCGGAATTTGCCTTCAGCTTTCTGGGTGCGTCTATGGTTGGCGC CGTCACCACGACCGCAAACCCGTTCTACACGTCCGCGGAAATCTTCAAACAGCTGAACGCATCAAAAGCTAAA ATCGTCGTGACCCAGGCGCAATATGTGGATAAACTGCGCGACTACCCGGATGGTCAAGTTGCCAAAATTGGCG AAGGTTTCACGGTCATTACCATCGATGACCCGCCGGAAAACTGTATGCATTTTAGTGTTGTCTCCGAAGCGAA CGAAAGCGAACTGCCGGAAGTCTCAATTAATTCGGATGACCCGGTGGCCCTGCCGTTTAGCTCTGGTACGACC GGCCTGCCGAAAGGCGTGGTTCTGACGCACAAATCACTGATCACCTCGGTCGCCCAGCAAGTGGATGGTGAAA ACCCGAATCTGCATCTGACCCCGGATGACGTCGTGCTGTGCGTGCTGCCGCTGTTCCACATTTATAGCCTGAA CTCTGTTCTGCTGTGTAGTCTGCGTGCAGGTGCAGCAGTGCTGCTGATGCAGAAATTTGAAATTGGTACCCTG CTGGAACTGATCCAACGTTACCGCGTGAGCGTTGCAGCTGTTGTCCCGCCGCTGGTTCTGGCACTGGCTAAAA ATCCGATGGTGGAATCGTTTGATCTGAGTTCCATCCGTGTGGTTCTGAGCGGTGCAGCACCGCTGGGCAAAGA ACTGGAAGCAGCTCTGCGTTCCCGCGTTCCGCAGGCAGTCCTGGGCCAAGGTTATGGCATGACGGAAGCAGGC CCGGTGCTGTCAATGTGCCTGGGTTTCGCTAAACAGCCGTTTCCGACGAAATCAGGTTCGTGTGGCACCGTCG TGCGTAACGCGGAACTGAAAGTTGTGGATCCGGAAACCGGTTGCTCCCTGGGCCGTAATCAGCCGGGTGAAAT TTGTATCCGCGGCCAGCAAATTATGAAAGGTTATCTGAATGATCCGGAAGCGACGGCCTCTACCATTGACGTT GATGGCTGGCTGCATACCGGTGACATCGGCTACGTGGATGACGATGAAGAAGTGTTCATTGTTGATCGCGTCA AAGAACTGATCAAATTCAAAGGTTTTCAGGTTCCGCCGGCAGAACTGGAAGCTCTGCTGGTGTCTCACCCGTC CATTGCCGATGCGGCCGTGGTTCCGCAAAAAGACGATGTTGCTGGCGAAGTCCCGGTGGCGTTCGTCGTGCGT TCTAACGGTTTTGAACTGACCGAAGAAGCAGTGAAAGAATTCATCAGTAAACAGGTTGTCTTTTATAAACGCC TGCATAAAGTGTACTTTGTTCACGCGATTCCGAAAAGCCCGTCTGGCAAAATCCTGCGTAAAGATCTGCGCGC GAAACTGGCCGAAAAAACCCCGGAACCGAAC	*Vitis vinifera* 4CL from GenScript, codon-optimized for *E. coli*
*Cm*CHS	ATGGCTACGGTCCAAGAAATCCGCAACGCTCAACGCGCAGATGGTCCGGCGACGGTCCTGGCAATCGGCACGG CAACCCCGGCTCATAGCGTGAACCAGGCAGATTATCCGGACTATTACTTTCGTATTACCAAATCTGAACACAT GACGGAACTGAAAGAAAAATTCAAACGTATGTGCGATAAAAGTATGATTAAAAAACGCTACATGTACCTGACC GAAGAAATCCTGAAAGAAAACCCGAATATGTGTGCCTACATGGCACCGAGCCTGGATGCGCGCCAGGACATTG TGGTTGTCGAAGTTCCGAAACTGGGTAAAGAAGCGGCCACCAAAGCCATCAAAGAATGGGGCCAACCGAAATC AAAAATTACGCACCTGATCTTTTGCACCACGTCGGGTGTGGATATGCCGGGTGCAGACTATCAGCTGACCAAA CTGCTGGGTCTGCGTCCGAGCGTTAAACGCTTTATGATGTACCAGCAAGGCTGCTTCGCAGGCGGTACGGTCC TGCGTCTGGCTAAAGATCTGGCGGAAAACAATAAAGGTGCTCGCGTTCTGGTGGTTTGTAGTGAAATTACCGC TGTCACGTTTCGTGGTCCGGCGGATACCCATCTGGACTCCCTGGTTGGCCAGGCCCTGTTCGGCGATGGTGCA GCTGCGGTTATCGTCGGCGCAGATCCGGACACGAGTGTGGAACGTCCGCTGTATCAGCTGGTTTCAACCTCGC AAACGATTCTGCCGGATTCCGACGGTGCGATCGATGGCCATCTGCGCGAAGTGGGTCTGACCTTTCACCTGCT GAAAGACGTTCCGGGCCTGATTTCAAAAAACATCGAAAAAAGCCTGTCTGAAGCCTTTGCACCGGTTGGTATT TCGGATTGGAGCTCTATTTTCTGGATCGCACATCCGGGCGGTCCGGCAATCCTGGACCAGGTGGAAAGCAAAC TGGGTCTGAAAGAAGAAAAACTGAAAGCTACCCGTCAAGTCCTGTCTGAATACGGCAATATGAGTTCCGCGTG TGTGCTGTTCATTCTGGATGAAATGCGCAAAAAATCTGCCGAAGAAGCTAAAGCGACCACGGGCGAAGGTCTG GATTGGGGCGTGCTGTTTGGTTTCGGTCCGGGTCTGACCGTCGAAACGGTCGTGCTGCACAGTGTGCCGATCA AAGCG	*Citrus maxima* CHS from GenScript, codon-optimized for *E. coli*
*Cm*CHI	ATGAATCCGTCGCCGTCTGTTACCGAACTGCAAGTGGAAAATGTCACCTTTACGCCGAGTCTGCAACCGCCGG GCTCTACCAAATCGCATTTTCTGGGCGGTGCAGGTGAACGTGGCCTGGAAATCGAAGGCAAATTTGTTAAATT CACCGCTATTGGTGTCTATCTGGAAGAAAACGCCGTGCCGCTGCTGGCAGGCAAATGGAAAGGCAAAACCGCC GGTGAACTGACGGAATCTGTCGAATTTTTCCGCGATGTGGTTACCGGCCCGTTTGAAAAATTCATGAAAGTGA CCATGATCCTGCCGCTGACGGGTGCGCAGTATTCAGAAAAAGTTGCTGAAAATTGCATGGCGATTTGGAAATT TTTCGGCATCTACACCGATGCAGAAGCTAAAGCGATTGAAAAATTTACGGAAGTGTTCAAAGACGAAATTTTT CCGCCGGGCAGCTCTATCCTGTTCACCCAAAGTTCCGGTTCGCTGACGATTTCATTTTCGAAAGATGGCAGCA TCCCGAAAGACGGTGTCGCGGTGATTGAAAACAATCTGCTGAGCGAAGCCGTTCTGGAATCTATGATCGGTAA AAACGGCGTCAGTCCGGCGGCCAAAAAATCCCTGGCCGAACGTCTGTCAGCACTGCTGAATGTTGCTTCCGAC AAAATGAAA	*Citrus maxima* CHI from GenScript, codon-optimized for *E. coli*

Supporting Methods.

Genome integration of T7-mCherry cassette

The gene encoding mCherry was integrated onto the genome of *E. coli* K-12 MG1655 (DE3) using the methods described by Kuhlman and Cox.[4] The system uses 3 plasmids to integrate DNA site-specifically into the genome of *E. coli*. The helper plasmid, pTKRED, encodes the genes for λ-Red recombinase and the endonuclease *I-SceI*, under control of the P_{lac} and P_{araBAD} promoters, respectively, as well as a spectinomycin resistance marker and a temperature-sensitive origin of replication. The "landing-pad," plasmid, pTKS/CS contains the gene for a tetracycline resistance marker, TetA, flanked by *I-SceI* restriction sites and "landing-pad," regions of 25 randomized nucleotides used for homologous recombination. Finally, ampicillin resistant integration plasmid, pTKIP, contains a multiple cloning site flanked by a kanamycin resistance marker, all flanked by the same landing-pad regions and *I-SceI* restriction sites.

The gene for codon-optimized mCherry, under transcriptional control of promoter P_{T7lac} and T7 terminator, was amplified out of pETM6-mCherry[3] and cloned into the integration vector pTKIP-neo using primers For_pETM6_w/ApaI and Rev_pETM6_w/SalI to form plasmid pTKIP-mCherry as verified by restriction digestion. Next, the TetA landing pad from pTKS/CS was integrated into the *atpI* locus on the genome of *E. coli* MG1655 (DE3) using standard recombineering protocols. Specifically, the strain was transformed with pTKRED and plated onto LB plates containing 100 µg/mL spectinomycin at 30°C. Next, the TetA landing-pad was amplified from pTKS/CS using primers AtpI-pTKSCSfor and AtpI-pTKSCSrev, which includes 40bp of DNA homologous to the *atpI* region being replaced on the genome. The PCR product was then treated with the restriction enzyme *DpnI* to digest any methylated parental DNA. The strain containing pTKRED was grown at 30°C to an OD of 0.2, where expression of λ-Red recombinase was induced with 2mM IPTG for 2 hours. These cells were then made electrocompetent using standard protocols and electroporated with 10µL of the linear landing-pad DNA and plated onto LB plates containing 100 µg/mL spectinomycin and 10 µg/mL tetracycline at 30°C. Integration of the landing-pad into the *atpI* locus to create strain K-12 MG1655 (DE3) Δ*atpI*::[Tet^R] was verified by colony PCR using primers ChkTetAFOR and atpI/gidB_ver_R, which create an amplicon spanning the junction between integrated DNA and adjacent chromosomal DNA.

Next, the landing-pad strain, K-12 MG1655 (DE3) Δ*atpI*::[Tet^R], which still carries pTKRED, was transformed with pTKIP-mCherry and plated onto LB plates containing 100 µg/mL spectinomycin, 10 µg/mL tetracycline, and 25 µg/mL kanamycin and incubated at 30°C overnight. Colonies from this plate were then picked, inoculated into 2mL supplemented EZ Rich Defined Medium (Teknova) [0.5%(v/v) glycerol, 100 µg/mL spectinomycin, 25 µg/mL kanamycin, 2mM IPTG to induce λ-Red recombinase, and 0.4% arabinose to induce *I-SceI*], and incubated at 30°C overnight to swap the previously integrated TetA landing pad with the T7-mCherry-kanamycin cassette, creating strain K-12 MG1655 JE1 (DE3) (genotype Δ*atpI*::[Kan^R P_{T7lac} mCherry]). Once the cultures were saturated with cells, they were diluted 10^5x and 100 µL was plated onto LB containing kanamycin (25 µg/mL). Individual colonies were then plated onto three separate plates, one containing kanamycin (25 µg/mL) to select for positive integration, one containing tetracycline (10 µg/mL) to screen against presence of the landing pad, and one containing ampicillin to screen against pTKIP-mCherry. Colonies that passed the screen—those that only grew on the kanamycin plate—were then verified by colony PCR using primers sets atpI/gidB_ver_R with

ChkNEOfor and atpI/gidB_ver_F with mCherryREV, producing amplicons which span the junctions between the kanamycin resistance gene and adjacent chromosomal DNA on both sides of the integration. The PCR products from both were then sequenced to confirm the accuracy of the integration junctions. Finally, a colony meeting the stringent criteria described here was further confirmed with IPTG-inducible expression of mCherry.

Metabolite Quantification

Naringenin production was analyzed using an Agilent 1200 series HPLC equipped with a ZORBAX SB-18 column (5 μm, 4.6 x 150 mm, Agilent) and a diode array detector as previously described.[7] Mobile phase A was acetonitrile B was water (both containing 0.1% formic acid) at a flow rate of 1 mL/min. The following gradient was used: 10-40% A (0-10 min) and 40-60% A (10-15 min). Naringenin was monitored at 280 nm and quantified by peak area integration against a standard curve generated with naringenin analytical standard (Sigma).

Heparosan polysaccharide was analyzed in crude supernatant from K5 and Nissle 1917 cultures using an established methodology known as disaccharide analysis[8] with modifications. Supernatant was added to buffer containing internal standard, followed by addition of heparin lyase III (EC 4.2.2.8 from *Flavobacterium heparinum*, expressed and purified in-house)[9] and incubation at 33˚C overnight to achieve complete depolymerization of heparosan into disaccharides. Digested samples were analyzed by HILIC-MS/MS using a Thermo Scientific triple quadrupole mass spectrometer equipped with an electrospray ionization source operating in negative ion mode, coupled to an Agilent 1200 HPLC and autosampler. A Luna HILIC column (2.0 x 30 mm, 200 Å, Phenomenex) was used to separate heparosan disaccharides from other components in the media. Mobile phase A was 5 mM ammonium acetate in LC-MS grade water. Mobile phase B was LC-MS grade acetonitrile with 5 mM ammonium acetate. Rapid separation was achieved using the following HPLC program: 30% A (0-12 s), 30% A (12-84 s), 30-50% A (84-90 s), and 50% A (90-120 s) at a flowrate of 250 μL/min. Heparosan disaccharides were quantified by peak area integration (transition m/z 378.1→259.2) against a standard curve prepared from heparosan purified in-house (purity >90%) as reported previously.[10] Samples were diluted as necessary to fall within the linear range of the assay.

Supporting References.

(1) Bikard, D., Jiang, W., Samai, P., Hochschild, a., Zhang, F., and Marraffini, L. a. (2013) Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas system. *Nucleic Acids Res.* 1–9.

(2) Xu, P., Vansiri, A., Bhan, N., and Koffas, M. a. G. (2012) ePathBrick: A Synthetic Biology Platform for Engineering Metabolic Pathways in E. coli. *ACS Synth. Biol.* 120504094454000.

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