Supplementary Information

Biosensor-assisted titratable CRISPRi high-throughput (BATCH) screening for over-production phenotypes

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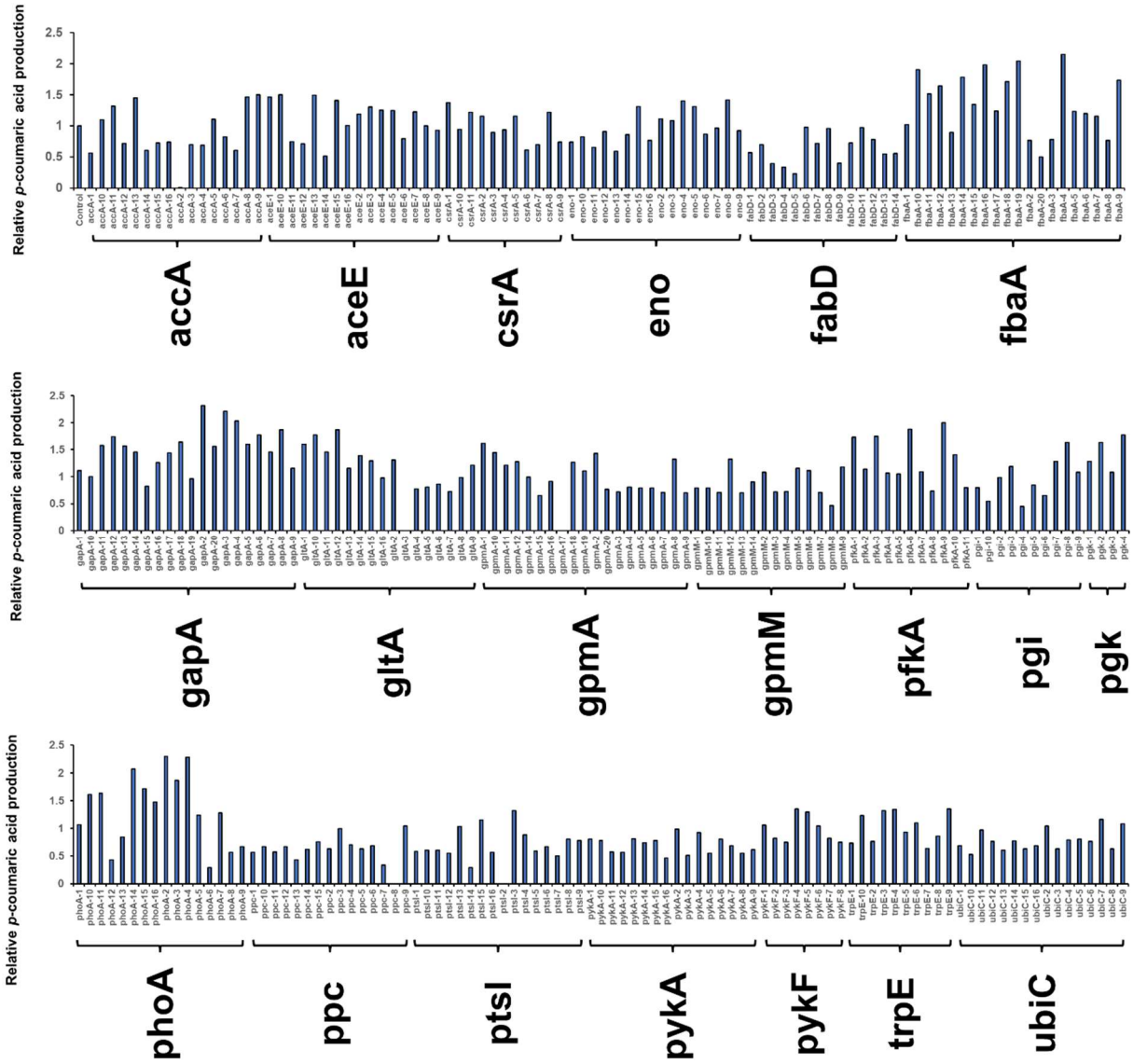

Figure S1. Relative *p*-coumaric acid titer of selected sgRNA variants in test tubes. The medium used for screening was M9Y with 20 g/L glycerol.

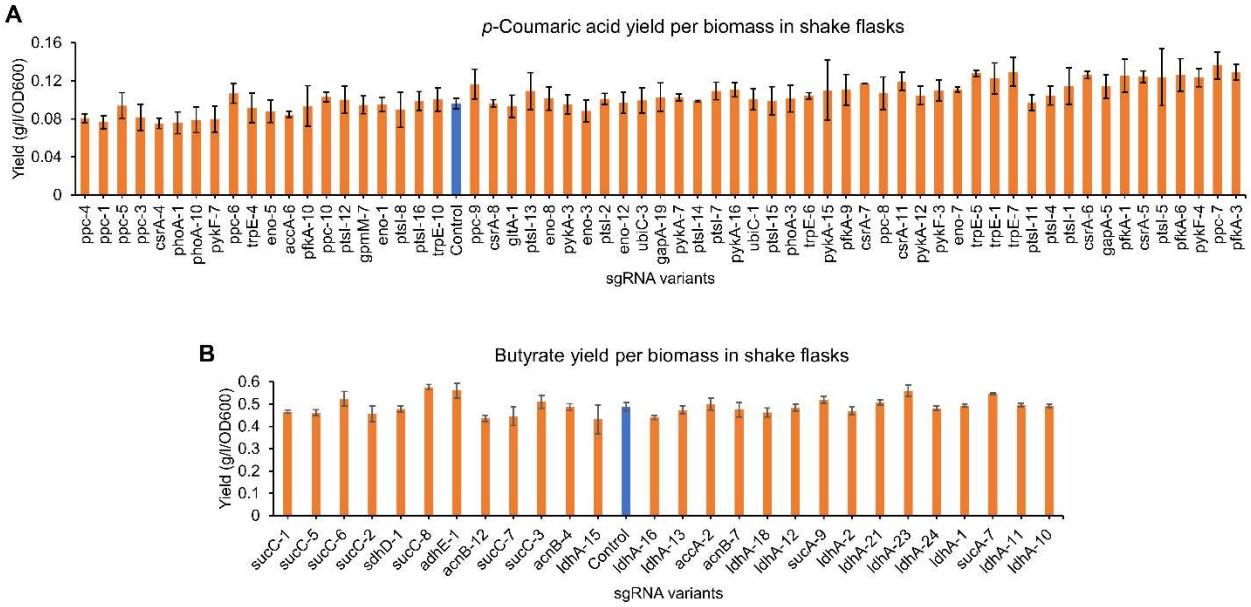

Figure S2. The overall yield (product titer/biomass (OD600)) profiles of *p*-coumaric acid (A) and butyrate (B) in shake flask experiments. The data used here corresponded to those from Figure 4C and 5E, respectively. All data represent the mean of three biologically independent samples and error bars show standard deviation.

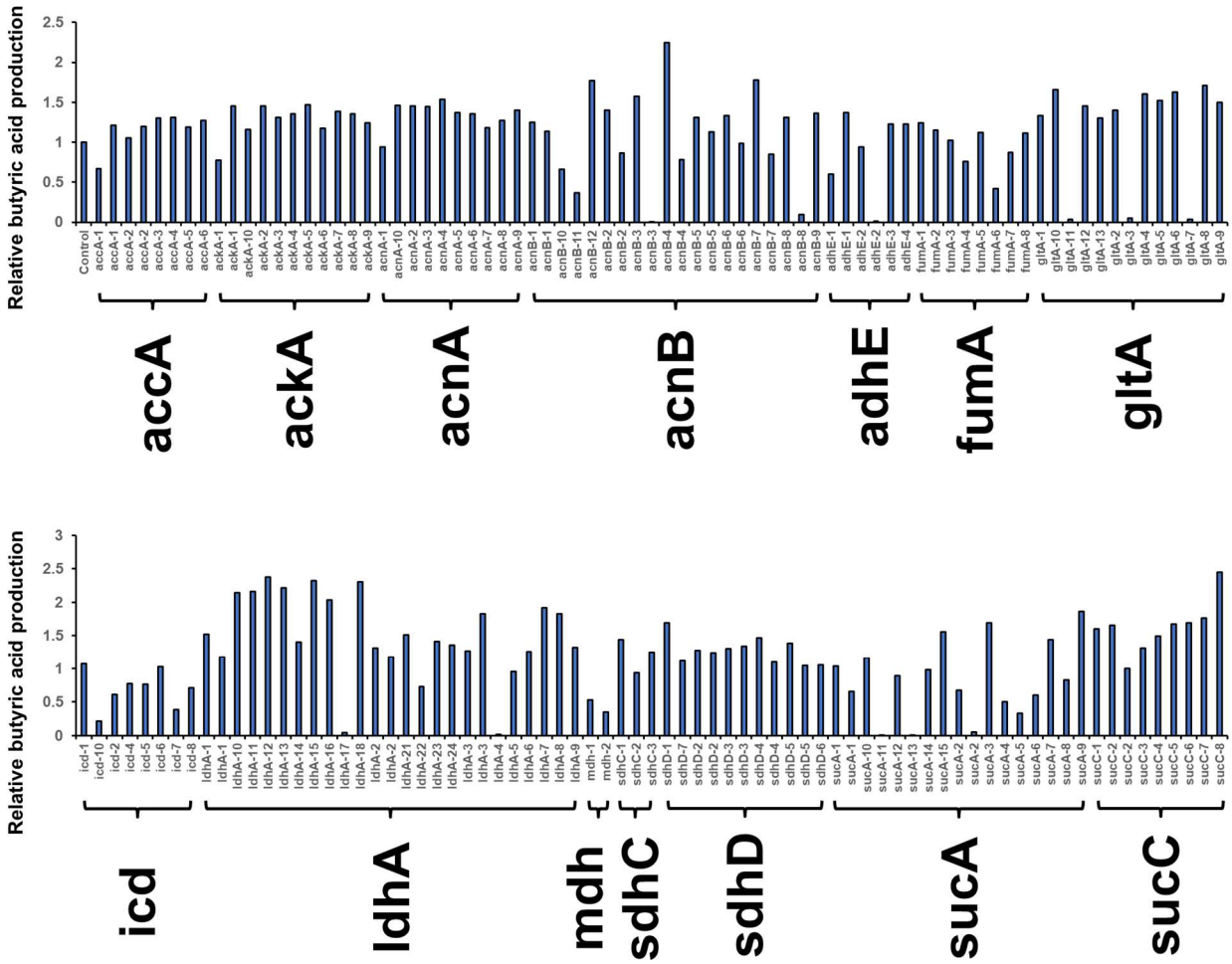

Figure S3. Relative butyric acid titer of selected sgRNA variants in test tubes. The medium used for screening was M9Y with 20 g/L glucose.

Table S1. Primers for constructing mismatched sgRNA arrays

Name	Sequence (5'-3') [a]
sgegfp-rbs-F (ApaLI)	ggaaaGTGCACATTTCTCCTCTTTNNTGAATTGTTTTAGAG CTAGAAATAGCAAGTT
sgegfp-cds2-F (ApaLI)	ggaaaGTGCACACTGTACATAACCNNCGGGCAGTTTTAGA GCTAGAAATAGCAAGTT
sgpgi-F(ApaLI)	ggaaaGTGCACATGCCAGGCAGCGNNCTGCGTGTTTTAGA GCTAGAAATAGCAAGTT
sgpfkA-F(ApaLI)	ggaaaGTGCACACGAATTGCGGCGNNCATGCCGTTTTAGA GCTAGAAATAGCAAGTT
sgfbaA-F(ApaLI)	ggaaaGTGCACATCATCACCAGTGNNTACGCCGTTTTAGA GCTAGAAATAGCAAGTT
sggapA-F(ApaLI)	ggaaaGTGCACAGGAAAACAATGCNNCCGATAGTTTTAG AGCTAGAAATAGCAAGTT
sgpgk-F(ApaLI)	ggaaaGTGCACATTTCCCAGCAAGNNCCAGATGTTTTAGA GCTAGAAATAGCAAGTT
sggpmA-F(ApaLI)	ggaaaGTGCACACGTCGTACCAACNNGTGAAAGTTTTAGA GCTAGAAATAGCAAGTT
sggpmM-F(ApaLI)	ggaaaGTGCACATCCAGAATCACCNNTACCATGTTTTAGA GCTAGAAATAGCAAGTT
sgeno-F(ApaLI)	ggaaaGTGCACAACCGATGATTTTNNCGATTTGTTTTAGA GCTAGAAATAGCAAGTT

sgpykA-F(ApaLI)	ggaaaGTGCACATTTTGTTCTGCGNNGCCTTCGTTTTAGAG CTAGAAATAGCAAGTT
sgpykF-F(ApaLI)	ggaaaGTGCACATCCGATGGTGCANNCAATTTGTTTTAGA GCTAGAAATAGCAAGTT
sgaceE-F(ApaLI)	ggaaaGTGCACACGATCGGATCCANNTCATTTGTTTTAGA GCTAGAAATAGCAAGTT
sggltA-F(ApaLI)	ggaaaGTGCACAAACAGCTGTATCNNCGTTGAGTTTTAGA GCTAGAAATAGCAAGTT
sgppc-F(ApaLI)	ggaaaGTGCACAACTGACATTACTNNGCAATGGTTTTAGA GCTAGAAATAGCAAGTT
sgaccA-F(ApaLI)	ggaaaGTGCACACAATCGGCTGTTNNAAATCAGTTTTAGA GCTAGAAATAGCAAGTT
sgfabD-F(ApaLI)	ggaaaGTGCACACGGTTTGAGAACNNTGTCCAGTTTTAGA GCTAGAAATAGCAAGTT
sgcsrA-F(ApaLI)	ggaaaGTGCACAGACCTCATCCCCNNTCATGAGTTTTAGA GCTAGAAATAGCAAGTT
sgmurA-F(ApaLI)	ggaaaGTGCACAACTTCGCCCTGGNNCTTCGTGTTTTAGA GCTAGAAATAGCAAGTT
sgphoA-F(ApaLI)	ggaaaGTGCACAGTCACAGGGGTANNCAGTAAGTTTTAGA GCTAGAAATAGCAAGTT
sgptsI-F(ApaLI)	ggaaaGTGCACATTTACCGAAAGCNNTACCCGGTTTTAGA GCTAGAAATAGCAAGTT

sgubiC-F(ApaLI)	ggaaaGTGCACACACGCAGTTGCGNNAACGCGGTTTTAGA GCTAGAAATAGCAAGTT
sgtrpE-F(ApaLI)	ggaaaGTGCACACAGGTTAGCAGTNNGAGAGTGTTTTAGA GCTAGAAATAGCAAGTT
sgldhA-F(ApaLI)	ggaaaGTGCACAGTACTGTTTTGTNNTATAAAGTTTTAGA GCTAGAAATAGCAAGTT
sgadhE-F(ApaLI)	ggaaaGTGCACAGAAACTGGCATANNCACGCTGTTTTAGA GCTAGAAATAGCAAGTT
sgackA-F(ApaLI)	ggaaaGTGCACAACCATTTACTGCNNCGATGAGTTTTAGA GCTAGAAATAGCAAGTT
sgacnA-F(ApaLI)	ggaaaGTGCACAGTCCTTACTGGCNNCTCGTAGTTTTAGA GCTAGAAATAGCAAGTT
sgacnB-F(ApaLI)	ggaaaGTGCACACACGCTCAGCTANNTGCTTAGTTTTAGA GCTAGAAATAGCAAGTT
sgicd-F(ApaLI)	ggaaaGTGCACAGTGATCTTCTTGNNTTGTGCGTTTTAGA GCTAGAAATAGCAAGTT
sgsucA-F(ApaLI)	ggaaaGTGCACAGAGGTAAGAAGANNCCAACCGTTTTAG AGCTAGAAATAGCAAGTT
sgsucC-F(ApaLI)	ggaaaGTGCACACGGTGCTGGTAANNCATAGCGTTTTAGA GCTAGAAATAGCAAGTT
sgsdhC-F(ApaLI)	ggaaaGTGCACAGTCTGTAGGTCCNNATTAACGTTTTAGA GCTAGAAATAGCAAGTT

sgsdhD-F(ApaLI)	ggaaaGTGCACAGCCATTGCGTCCNNATGCGGGTTTTAGAGCTAGAAATAGCAAGTT
sgfumA-F(ApaLI)	ggaaaGTGCACAAAAGGAGCCTGANNATGAAAGTTTTAGAGCTAGAAATAGCAAGTT
sgmdh-F(ApaLI)	ggaaaGTGCACACAATACCGCCAGNNGCGCCGGTTTTAGAGCTAGAAATAGCAAGTT
sgRNA-R(ApaLI)	ggaaagtgcAcagtatcttgttatccgctcacaat

[a] The sgRNA spacers were shown in red.

Table S2. DNA sequences of the screened sgRNA variants.

sgRNA variants	Spacer DNA sequence (fully matched base pair)
For *p*-coumaric acid screening	
csrA-5	GACCTCATCCCCTCTCATGA (AA)
csrA-6	GACCTCATCCCCTCTCATGA (AA)
csrA-7	GACCTCATCCCCATTCATGA (AA)
csrA-8	GACCTCATCCCCTGTCATGA (AA)
csrA-11	GACCTCATCCCCCGTCATGA (AA)
eno-1	ACCGATGATTTTTCCGATTT (TA)
eno-3	ACCGATGATTTTGGCGATTT (TA)
eno-5	ACCGATGATTTTTCCGATTT (TA)
eno-7	ACCGATGATTTTTCCGATTT (TA)
eno-8	ACCGATGATTTTTTCGATTT (TA)
eno-12	ACCGATGATTTTGGCGATTT (TA)
gapA-5	GGAAAACAATGCAGCCGATA (GA)
gapA-19	GGAAAACAATGCCGCCGATA (GA)
gltA-1	AACAGCTGTATCAACGTTGA (CC)
gpmM-7	TCCAGAATCACCTATACCAT (AG)
pfkA-3	CGAATTGCGGCGTTCATGCC (TT)
pfkA-6	CGAATTGCGGCGTGCATGCC (TT)
phoA-3	GTCACAGGGGTAAACAGTAA (AA)
ppc-7	ACTGACATTACTTGGCAATG (AC)
ppc-8	ACTGACATTACTTGGCAATG (AC)

ppc-9	ACTGACATTACTATGCAATG (AC)
ptsI-1	TTTACCGAAAGCATTACCCG (GA)
ptsI-4	TTTACCGAAAGCCTTACCCG (GA)
ptsI-5	TTTACCGAAAGCCGTACCCG (GA)
ptsI-7	TTTACCGAAAGCTTTACCCG (GA)
ptsI-11	TTTACCGAAAGCTGTACCCG (GA)
ptsI-14	TTTACCGAAAGCAGTACCCG (GA)
ptsI-15	TTTACCGAAAGCTTTACCCG (GA)
pykA-3	TTTTGTTCTGCGTGGCCTTC (AA)
pykA-7	TTTTGTTCTGCGGAGCCTTC (AA)
pykA-12	TTTTGTTCTGCGGAGCCTTC (AA)
pykA-15	TTTTGTTCTGCGGGGCCTTC (AA)
pykA-16	TTTTGTTCTGCGGCGCCTTC (AA)
pykF-3	TCCGATGGTGCAGACAATTT (AA)
pykF-4	TCCGATGGTGCAGGCAATTT (AA)
pykF-7	TCCGATGGTGCAATCAATTT (AA)
trpE-1	CAGGTTAGCAGTTCGAGAGT (TC)
trpE-5	CAGGTTAGCAGTGCGAGAGT (TC)
trpE-6	CAGGTTAGCAGTCGGAGAGT (TC)
trpE-7	CAGGTTAGCAGTCGGAGAGT (TC)
ubiC-1	CACGCAGTTGCGGCAACGCG (TT)
ubiC-3	CACGCAGTTGCGGCAACGCG (TT)

For butyrate screening

accA-2	CAATCGGCTGTTATAAATCA (CA)
acnB-4	CACGCTCAGCTAAATGCTTA (CG)
acnB-7	CACGCTCAGCTAGATGCTTA (CG)
acnB-12	CACGCTCAGCTATTTGCTTA (CG)
ldhA-2	GTACTGTTTTGTTATATAAA (GC)
ldhA-10	GTACTGTTTTGTCGTATAAA (GC)
ldhA-11	GTACTGTTTTGTCCTATAAA (GC)
ldhA-12	GTACTGTTTTGTGCTATAAA (GC)
ldhA-16	GTACTGTTTTGTAATATAAA (GC)
ldhA-18	GTACTGTTTTGTGTTATAAA (GC)
ldhA-21	GTACTGTTTTGTATTATAAA (GC)
ldhA-23	GTACTGTTTTGTTTTATAAA (GC)
ldhA-24	GTACTGTTTTGTTGTATAAA (GC)
sdhD-1	GCCATTGCGTCCGCATGCGG (TA)
sucA-7	GAGGTAAGAAGAATCCAACC (GT)
sucA-9	GAGGTAAGAAGATCCCAACC (GT)
sucC-1	CGGTGCTGGTAATGCATAGC (GC)
sucC-2	CGGTGCTGGTAACGCATAGC (GC)
sucC-3	CGGTGCTGGTAAATCATAGC (GC)
sucC-5	CGGTGCTGGTAAAGCATAGC (GC)
sucC-6	CGGTGCTGGTAAGGCATAGC (GC)
sucC-7	CGGTGCTGGTAAAACATAGC (GC)
sucC-8	CGGTGCTGGTAAGGCATAGC (GC)

Table S3. Sequences of hybrid promoters used in *p*-coumaric acid responsive biosensor system

promoter	DNA sequences [a]
P1	AACATGTAAATAGTTACATGATTTGACATAAATACCAC TGGCGGTGATACTGAGCACATCAGCAGGACGCACTGAC CGAATTCATTAAAGAGGAGAAA
P2	TAAATTATCTCTGGCGGTGTTGACAAACATGTAAATAGT TACATGATGATACTGAGCACATCAGCAGGACGCACTGA CCGAATTCATTAAAGAGGAGAAA
P7	AACATGTAAATAGTTACATGATTTGACAATGGTGTTAA AGTGAACATGTAGATACTGAGCACATCAGCAGGACGCA CTGACCGAATTCATTAAAGAGGAGAAA
P9	TAAATTATCTCTGGCGGTGTTGACATAAATACCACTGGC GGTGATACTGAACATGTAAATAGTTACATGATGAATTC ATTAAAGAGGAGAAA

[a] The PadR binding box 1 was labeled in blue while the PadR binding box 2 was labeled in brown. The RBS sequence was labeled in green.