Supporting Information

Engineering the Reductive TCA Pathway to Dynamically Regulate the Biosynthesis of Adipic Acid in *Escherichia coli*

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Supplementary Table 1 Strains and plasmids used in this study

Name	Properties	Sources
Strains		
E. coli K12 MG1655	Wild type, for obtain the target gene	Lab preserved
E. coli JM109	Wild type, for plasmid construction	Lab preserved
E. coli DH5α	Wild type, for plasmid construction	Lab preserved
Corynebacterium crenatum	Wild type, for obtain the target gene	Lab preserved
Mad1415	MG1655Δ*sucD*Δ*ldhA*Δ*atoB* carrying pAD-14 and pAD-15	Lab preserved
Mad1415s	Mad1415 carrying pACM4G-F8s	This study
Mad1415-F8NAsf	Mad1415 carrying pACM4G-F8NAsf	This study
Mad1415- F8NAsp	Mad1415 carrying pACM4G-F8NAsp	This study
Mad1415- F8NAsm	Mad1415 carrying pACM4G-F8NAsm	This study
Mad1415- F8NAspf	Mad1415 carrying pACM4G-F8NAspf	This study
Mad1415- F8NAsmf	Mad1415 carrying pACM4G-F8NAsmf	This study
Mad1415- F8NAsmfp	Mad1415 carrying pACM4G-F8NAsmfp	This study
Mad1415- F8SXspf	Mad1415 carrying pACM4G-F8SXspf	This study
Mad1415- F8SAspf	Mad1415 carrying pACM4G-F8SAspf	This study
Mad1415- F8NAs4pf	Mad1415 carrying pACM4G-F8NAs4pf	This study
Mad1415- F8NAs7pf	Mad1415 carrying pACM4G-F8NAs7pf	This study
Mad1415- F8NA4spf	Mad1415 carrying pACM4G-F8NA4spf	This study
Mad1415- F8NA4s4pf	Mad1415 carrying pACM4G-F8NA4s4pf	This study
Mad1415- F8NA4s7pf	Mad1415 carrying pACM4G-F8NA4s7pf	This study
Mad1415- F8NA7spf	Mad1415 carrying pACM4G-F8NA7spf	This study
Mad1415- F8NA7s4pf	Mad1415 carrying pACM4G-F8NA7s4pf	This study
Mad1415- F8NA7s7pf	Mad1415 carrying pACM4G-F8NA7s7pf	This study
Plasmids		
pTrc99a	pBR322 ori, trc, Amp^R	Lab preserved
pHHD01K	p15 ori, tetR, Ptet, Kan^R	Lab preserved
pAD-12	pTrc99a harboring the optimized genes Tfu-2576 and Tfu-2577 from *Thermobifida fusca*, Amp^R	Lab preserved
pAD-13	pHHD01K harboring the optimized genes Tfu-0875, Tfu-2399, Tfu-0067 and Tfu-1647 from *Thermobifida fusca*, Kan^R	Lab preserved
pAD-14	pAD-12, pBR322 ori, PUTRlpp, Amp^R	Lab preserved
pAD-15	pAD-13, p15 ori, tetR, PUTRrpsT, Kan^R	Lab preserved
pACM4	p15A ori, T7, Chl^R	Lab preserved
pACM4G	p15A ori, T7, Gen^R	This study
pACM4G-F8*	pACM4G, p15A ori, P_{fnrF8*}, Gen^R	This study
pACM4G-F8	pACM4G, p15A ori, P_{fnrF8}, Gen^R	This study
pACM4G-F8*-GFP	pACM4G-F8* harboring the gene *sfGFP*, Gen^R	This study
pACM4G-F8-GFP	pACM4G-F8 harboring the gene *sfGFP*, Gen^R	This study
pACM4G-F8s	pACM4G-F8 harboring the gene *sucD*, Gen^R	This study
pACM4G-F8p	pACM4G-F8 harboring the gene *pyc*, Gen^R	This study
pACM4G-F8m	pACM4G-F8 harboring the gene *mdh*, Gen^R	This study
pACM4G-F8f	pACM4G-F8 harboring the gene *frdABCD*, Gen^R	This study

pACM4G-F8NAsp	pACM4G-F8 harboring the gene *sucD* and *pyc*, the monocistronic structure, Gen^R	This study
pACM4G-F8NAsf	pACM4G-F8 harboring the gene *sucD* and *frdABCD*, the monocistronic structure, Gen^R	This study
pACM4G-F8NAsm	pACM4G-F8 harboring the gene *sucD* and *mdh*, the monocistronic structure, Gen^R	This study
pACM4G-F8NAspf	pACM4G-F8 harboring the gene *sucD*, *pyc*, and *frdABCD*, the monocistronic structure, Gen^R	This study
pACM4G-F8NAsmf	pACM4G-F8 harboring the gene *sucD*, *mdh*, and *frdABCD*, the monocistronic structure, Gen^R	This study
pACM4G-F8NAsmfp	pACM4G-F8 harboring the gene *sucD*, *mdh*, *frdABCD*, and *pyc*, the monocistronic structure, Gen^R	This study
pACM4G-F8SXspf	pACM4G-F8 harboring the gene *sucD*, *pyc*, and *frdABCD*, the classical operon structure, Gen^R	This study
pACM4G-F8SAspf	pACM4G-F8 harboring the gene *sucD*, *pyc*, and *frdABCD*, the pseudo-operon structure, Gen^R	This study
pACM4G-U10F8-GFP	pACM4G-F8-GFP, p15A ori, $P_{fnrU10F8}$, Gen^R	This study
pACM4G-U15F8-GFP	pACM4G-F8-GFP, p15A ori, $P_{fnrU15F8}$, Gen^R	This study
pACM4G-U20F8-GFP	pACM4G-F8-GFP, p15A ori, $P_{fnrU20F8}$, Gen^R	This study
pACM4G-D3F8-GFP	pACM4G-F8-GFP, p15A ori, $P_{fnrD3F8}$, Gen^R	This study
pACM4G-D4F8-GFP	pACM4G-F8-GFP, p15A ori, $P_{fnrD4F8}$, Gen^R	This study
pACM4G-D7F8-GFP	pACM4G-F8-GFP, p15A ori, $P_{fnrD7F8}$, Gen^R	This study
pACM4G-F8NA4spf	pACM4G-F8NAspf, in which the promoter $P_{fnrD4F8}$ controled the expression of *sucD*, Gen^R	This study
pACM4G-F8NA7spf	pACM4G-F8NAspf, in which the promoter $P_{fnrD7F8}$ controled the expression of *sucD*, Gen^R	This study
pACM4G-F8NAs4pf	pACM4G-F8NAspf, in which the promoter $P_{fnrD4F8}$ controled the expression of *pyc*, Gen^R	This study
pACM4G-F8NAs7pf	pACM4G-F8NAspf, in which the promoter $P_{fnrD7F8}$ controled the expression of *pyc*, Gen^R	This study
pACM4G-F8NA4s4pf	pACM4G-F8NAspf, in which the promoter $P_{fnrD4F8}$ controled the expression of *sucD* and *pyc*, respectively, Gen^R	This study
pACM4G-F8NA4s7pf	pACM4G-F8NAspf, in which the promoter $P_{fnrD4F8}$ and $P_{fnrD7F8}$ controled the expression of *sucD* and *pyc*, respectively, Gen^R	This study
pACM4G-F8NA7s4pf	pACM4G-F8NAspf, in which the promoter $P_{fnrD7F8}$ and $P_{fnrD4F8}$ controled the expression of *sucD* and *pyc*, respectively, Gen^R	This study
pACM4G-F8NA7s7pf	pACM4G-F8NAspf, in which the promoter $P_{fnrD7F8}$ controled the expression of *sucD* and *pyc*, respectively, Gen^R	This study

Supplementary Table 2 Primers used in this study

Primer	Sequence (5' to 3')*	Restriction sites
sucD-F	GGGAATTCCATATGATGTCCATTTTAATCGATAAAAACACCAAGG	*Nde* I
sucD-R	CCGCTCGAGTTATTTCAGAACAGTTTTCAGTGCTTCACCG	*Xho* I
frdABCD-F	GGGAATTCCATATGGTGCAAACCTTTCAAGCCGATCT	*Nde* I
frdABCD -R	CCGCTCGAGTTAGATTGTAACGACACCAATCAGCGT	*Xho* I
pyc-F	GGGAATTCCATATGGTGTCGACTCACACATCTTCAACG	*Nde* I
pyc -R	CCGCTCGAGTTAGGAAACGACGACGATCAAGTCGC	*Xho* I
mdh-F	GGGAATTCCATATGATGAAAGTCGCAGTCCTCGG	*Nde* I
mdh -R	CCGCTCGAGTTACTTATTAACGAACTCTTCGCCCAGG	*Xho* I
GFP-F	GGGAATTCCATATGATGCGTATAGGTGAAGAACTGTTCACC	*Nde* I
GFP-R	CCGCTCGAGCTTTGTACAGTTCGTCCATACCGTG	*Xho* I
A-f/p/m-F	GACCTAGGATCGAGATCGATTTTGATTTACATCAATTACGG	*Avr* II
f/p/m-R	CCCAAGCTTTTTTTTTAAGGCAGTTATTGGTGCCCTTAAAC	*Hind* III
X-f/p/m-F	GCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATAC	*Xba* I
X-f/p/m-R	CCCAAGCTTTTTTTTTAAGGCAGTTATTGGTGCCCTTAAAC	*Hind* III
ffs-F	ATTGAGAGCGTTGAGAACCAACG	
ffs-R	ATAGCCTTCGGGAATAGCGGC	
Z-ffs-F	ATTCCCGAAGGCTATCCTAGGATCGAGATCGATTTTGATT	
Z-ffs-R	CTCAACGCTCTCAATTCTAGAAATATTTTATCTGATTAATAAGATGATCTTCT	
FNR-F	TCAGGCAACGTTACGCGTATG	
FNR-R	ATGATCCCGGAAAAGCGAATTATACG	
Z-FNR-F	CTTTTCCGGGATCATATTGAGAGCGTTGAGAACCAACG	
Z-FNR-R	CGTAACGTTGCCTGATCTAGAAATATTTTATCTGATTAATAAGATGATCTTCT	

*: Underlined letters are restriction enzyme cut sites.

Supplementary Table 3 Synthesized oligonucleotides

	Sequence (5' to 3')*
P_{fnrF8}	tTTGATttacATCAAttacggctaggtcagtcctcggtattatgctagtta
P_{fnrF8*}	taataagaggtgggattacggctaggtcagtcctcggtattatgctagtta
$P_{fnrU10F8}$	tTTGATttacATCAAatgcctgatcttacggctaggtcagtcctcggtattatgctagtta
$P_{fnrU15F8}$	tTTGATttacATCAAatgcctgatcctagcttacggctaggtcagtcctcggtattatgctagtta
$P_{fnrU20F8}$	tTTGATttacATCAAatgcctgatcctagctcaggttacggctaggtcagtcctcggtattatgctagtta
$P_{fnrD3F8}$	tTTGATttacATCAAcggctaggtcagtcctcggtattatgctagtta
$P_{fnrD4F8}$	tTTGATttacATCAAggctaggtcagtcctcggtattatgctagtta
$P_{fnrD7F8}$	tTTGATttacATCAAtaggtcagtcctcggtattatgctagtta

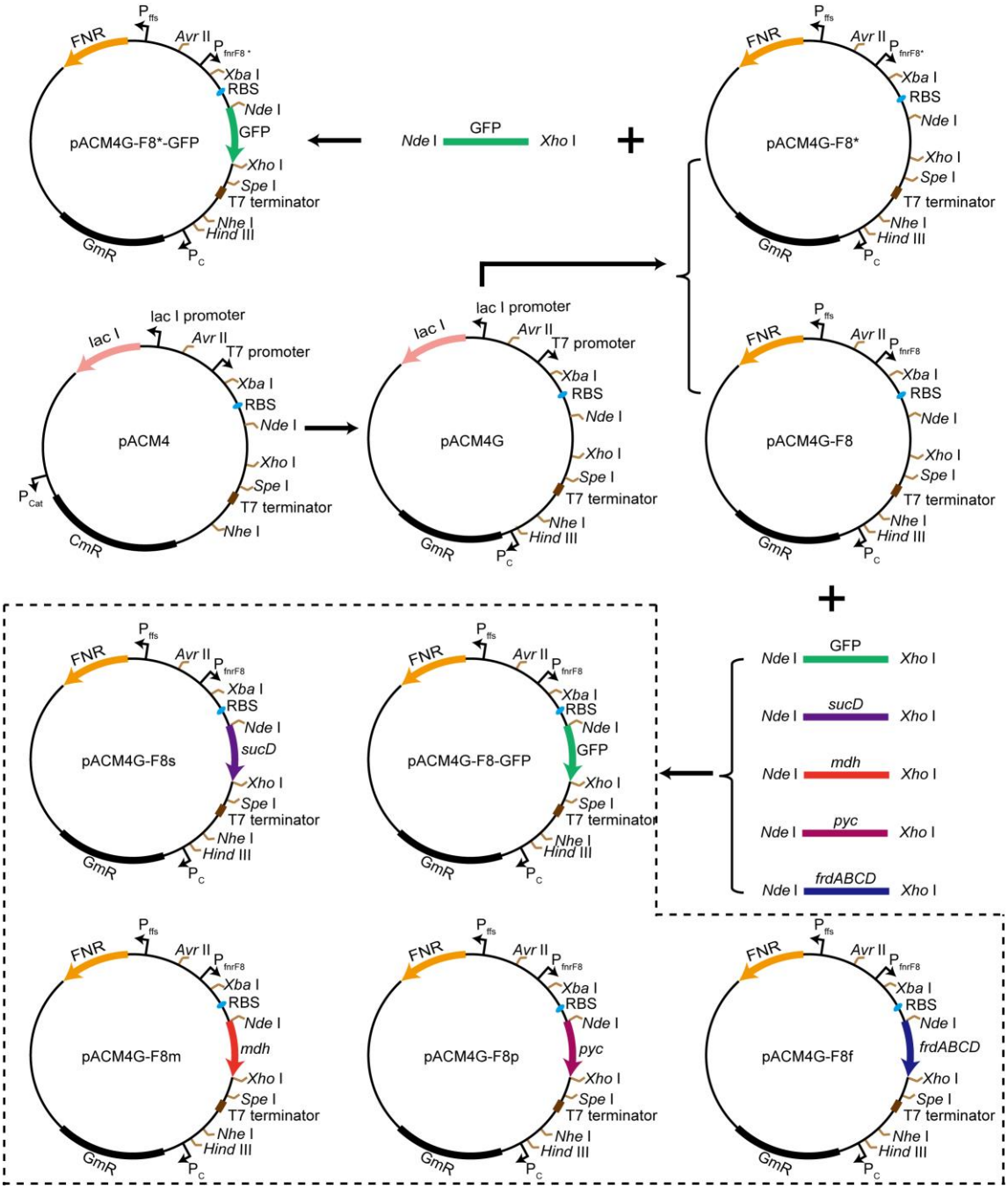
*: Uppercase letters are FNR binding site, green lowercase bold letters are -35 region, red lowercase bold letters are -35 region.

Supplementary Table 4 The stoichiometry of producing adipic acid from glucose

1. Glucose + 2ADP + 2NAD++2Pi → 2Pyruvate + 2ATP + 2NADH + $2H^+$ + $2H_2O$
2. Pyruvate + CoA + NAD^+ → NADH + H^+ + Acetyl-CoA + CO_2
3. Pyruvate + CO_2 + ATP + H_2O → Oxaloacetate + ADP + Pi
4. Oxaloacetate + NADH + H^+ → Malate + NAD^+
5. Malate → Fumarate + H_2O
6. Fumarate + $FADH_2$ → Succinic acid + FAD
7. Succinic acid + GTP + CoA → Succinyl-CoA + Pi + GDP
8. Acetyl-CoA + Succinyl-CoA + NADH + $FADH_2$ + Pi + ADP + H^+ → 2 CoA + NAD^+ + FAD + ATP + Adipic acid + H_2O
*Glucose + 2ADP + Pi + NAD^+ + $2FADH_2$ + GTP → Adipic acid + GDP +$3H_2O$ + 2ATP + NADH + 2 FAD + H^+ Yield: (146/180) X 100% = 81.11%

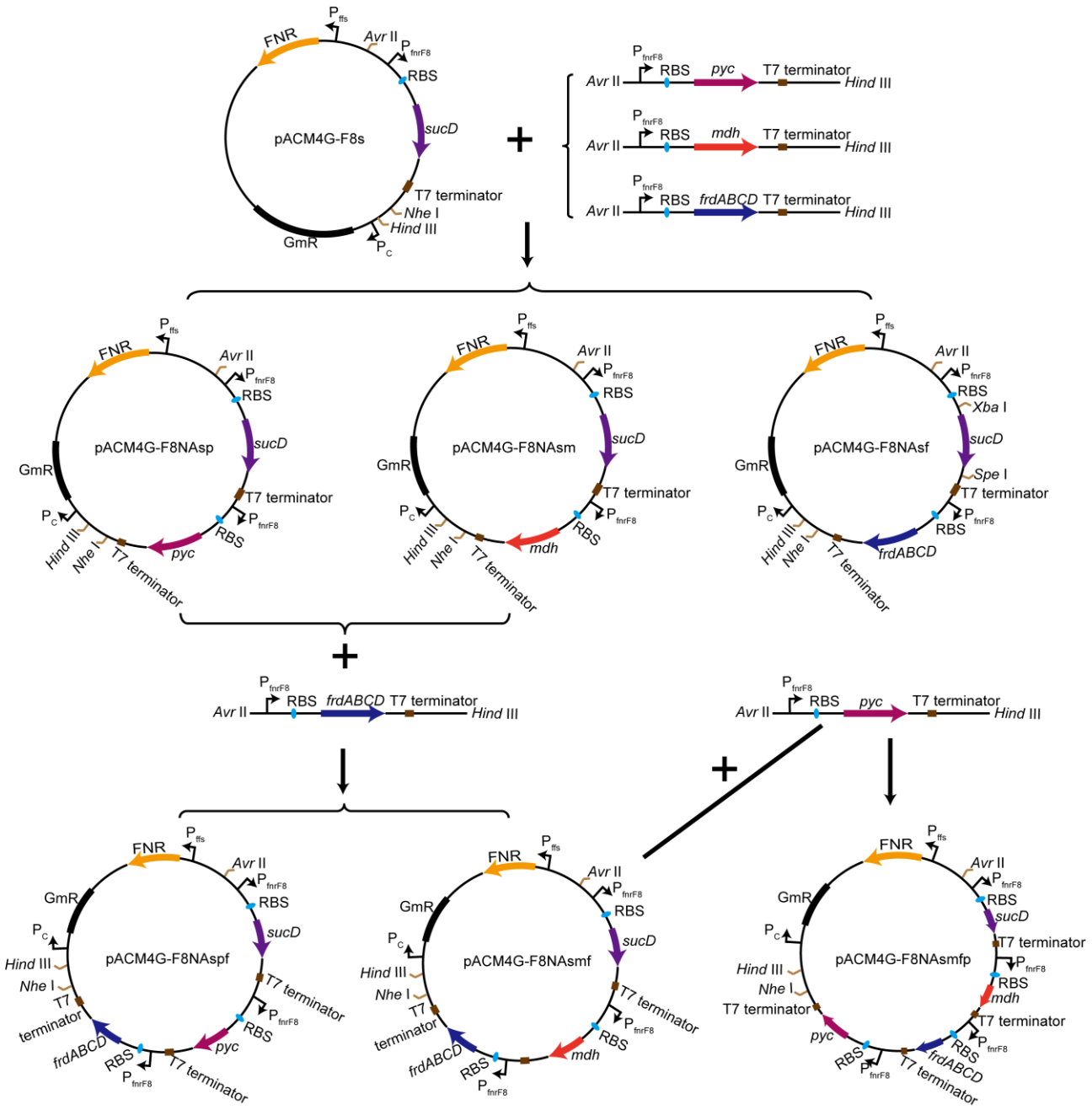
1-8: Single reactions in order from glucose to adipic acid.

*: Total reaction from glucose to adipic acid.



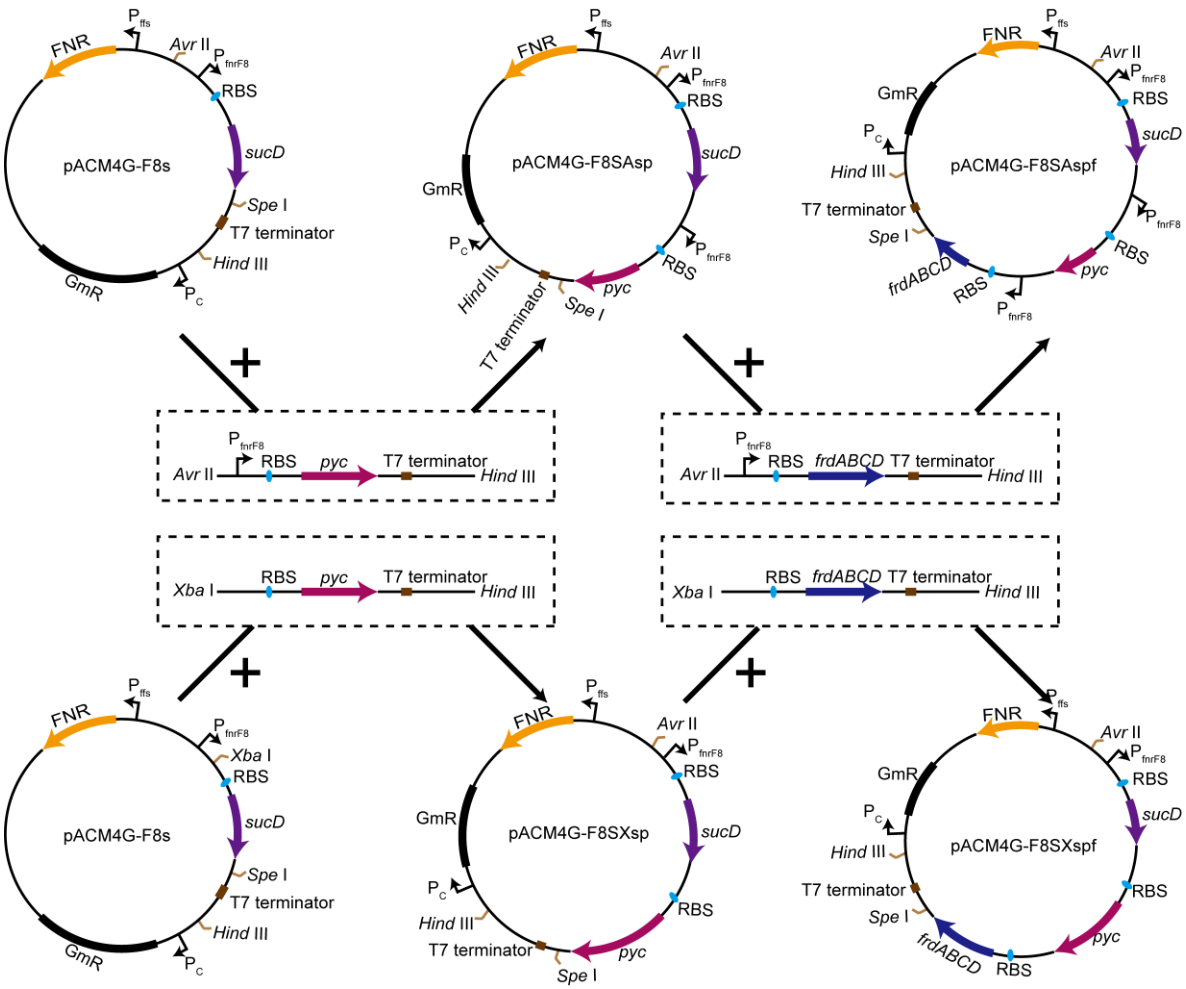
Supplementary Figure 1.The plasmid schematic diagrams and construction processes of oxygen responsive biosensor plasmids and basic plasmids used to tandem other genes.

The plasmids were used to generate data in Figure 3C. pACM4-F8 is the modified backbone plasmid and contains a multiple cloning site (MCS) for gene insertion. pACM4G-F8-GFP and pACM4G-F8*-GFP are the sensor plasmids for the P_{fnrF8} and P_{fnrF8*} promoters, respectively, used to generate sensor response data in Figure 3C. pACM4G-F8s, pACM4G-F8m, pACM4G-F8p, and pACM4G-F8f are used as the basis for subsequent plasmid construction.



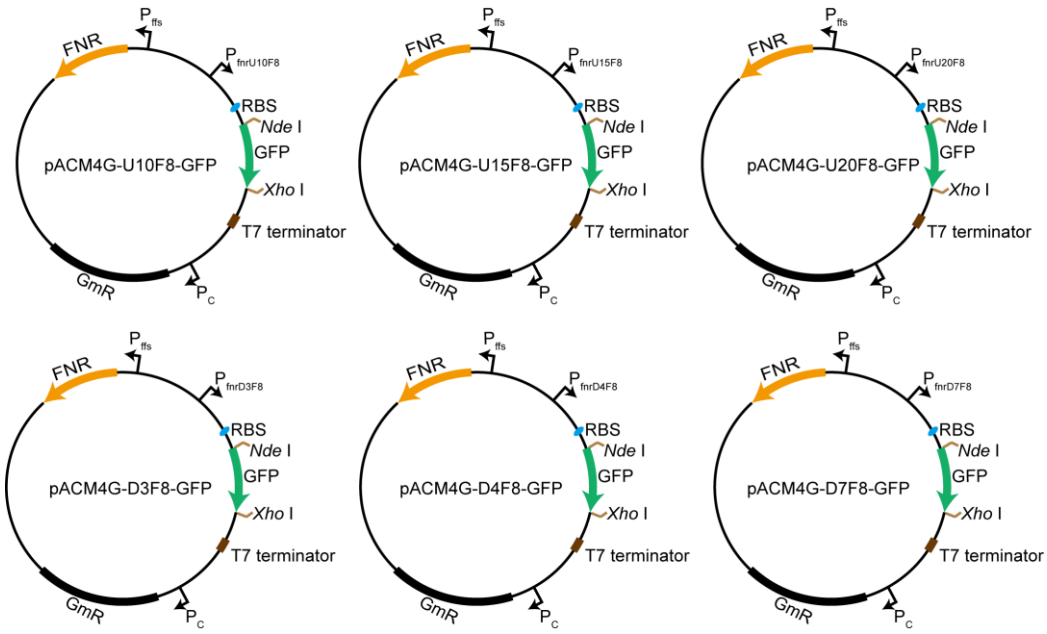
Supplementary Figure 2. The plasmid schematic diagrams and construction processes of multigene combinatorial plasmids with monocistronic structure.

The plasmids were used to generate data in Figure 4. pACM4G-F8NAsf, pACM4G-F8NAsm, pACM4G-F8NAsp, pACM4G-F8NAsmf, pACM4G-F8NAspf, and pACM4G-F8NAsmfp hold the monocistronic structure by utilizing homo-tail enzymes pairs *Avr* II and *Nhe* I.



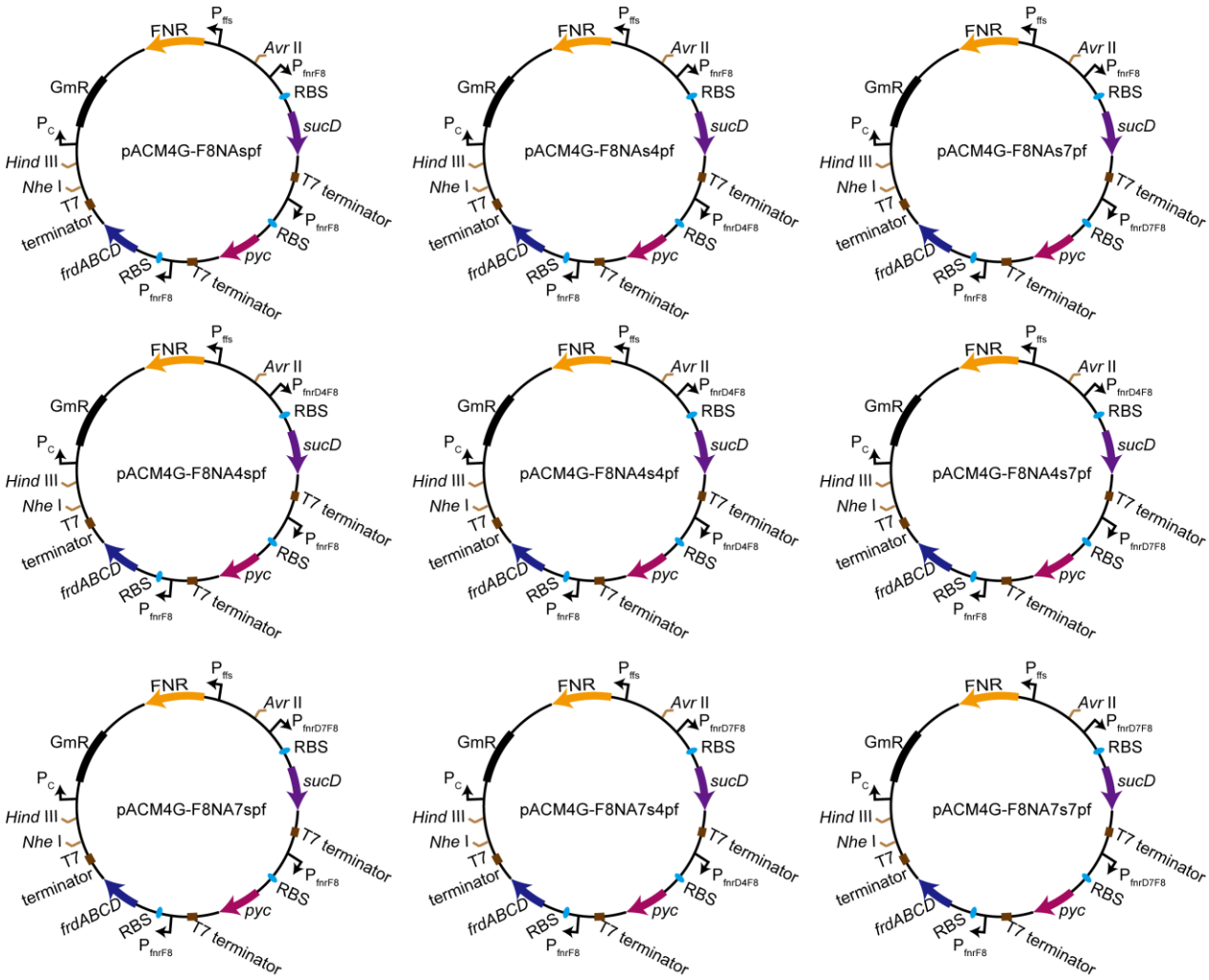
Supplementary Figure 3. The plasmid schematic diagrams and construction processes of plasmids harboring gene *sucD-pyc-frdABCD* with pseudo-operon structure and classical operon structure, respectively.

The plasmids were used to generate data in Figure 5. pACM4G-F8SAsp and pACM4G-F8SAspf hold pseudo-operon structure by utilizing homo-tail enzymes pairs *Avr* II and *Spe* I. pACM4G-F8SXsp and pACM4G-F8SXspf hold classical operon structure by utilizing homo-tail enzymes pairs *Xba* I and *Spe* I.



Supplementary Figure 4. The plasmid schematic diagrams of plasmids with different strength anaerobic promoter.

The plasmids were used to generate data in Figure 6B. pACM4G-U10F8-GFP, pACM4G-U10F8-GFP, and pACM4G-U10F8-GFP harbor promoter $P_{fnrU10F8}$, $P_{fnrU15F8}$, and $P_{fnrU20F8}$, respectively, which represent increase the distance of 10 bp, 15 bp, 20 bp between the FNR binding site and the -35 region. pACM4G-D3F8-GFP, pACM4G-D4F8-GFP, and pACM4G-D7F8-GFP harbor promoter $P_{fnrD3F8}$, $P_{fnrD4F8}$, and $P_{fnrD7F8}$, respectively, which represent decrease the distance of 3 bp, 4 bp, 7 bp between the FNR binding site and the -35 region.



Supplementary Figure 5. The plasmid schematic diagrams of plasmids used to coordinate genes expression.

The plasmids were used to generate data in Figure 6C.

Supplementary Notes

The various medium were prepared as following:

Lysogeny broth (LB): 10 g/L NaCl, 10 g/L tryptone, and 5 g/L yeast extract. For agar plates.

Super optimal broth (SOB): 20 g/L tryptone, 5 g/L yeast extract, 2.47 g/L $MgSO_4 \cdot 7H_2O$, 5 g/L NaCl, 0.186 g/L KCl, and supplemented with various concentrations of glucose.

***C. crenatum* broth:** 30 g/L glucose, 10 g/L yeast extract, 20 g/L (NH4)2 SO4, 1 g/L KH2PO4, 0.5 g/L MgSO4 7H2O, 1.5 g/L urea; pH 7.0.