Electronic Supplementary Material (ESI) for Integrative Biology.

Supplementary materials:

Model-guided identification of novel gene amplification targets for improving succinate production in *Escherichia coli* NZN111

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Table S1 Sources and characteristics of strains, plasmid, and primers in this study.

Strains or plasmid	Characteristics or description	Sources
NZN111	F^+ λ^- rpoS396(Am) rph-1 Δ*pflB*::Cam Δ*ldhA*::Kan	(Bunch, et al.,1997)
N-gapA	NZN111 pSE380-gapA	This study
N-pgk	NZN111 pSE380-pgk	This study
N-tpiA	NZN111 pSE380-tpiA	This study
N-ppc	NZN111 pSE380-ppc	This study
N-gapA-pgk	NZN111 pSE380-gapA-pgk	This study
N-gapA-tpiA	NZN111 pSE380-gapA-tpiA	This study
N-pgk-tpiA	NZN111 pSE380-pgk-tpiA	This study
N-gapA-pgk-tpiA	NZN111 pSE380-gapA-pgk-tpiA	This study
pSE380	Trc promoter $LacI^q$ Amp^r	Invitrogen™
Primers	Primer sequences (5' to 3')	
gapA-F	CACACAGGAAACAGACCATGGAATAGCTGGTGGAATATATGACTATCAAAGTAGGTATCAACG	This study
gapA-R	CGACGTGGTCACCAGCCATGGATCACAGTGTCATCTCAACTTATTT	This study
pgk-F	TCGTGGAATGCCTTCGAATTCCAACGAGAGGATTCACCATGTCTGTAA	This study
pgk-R	CCATGTGCAGGTGCTGAATTCGCCCTGTGATTTTTTACTTCTTAGC	This study
tpiA-F	CCGGGCCCTATATATGGATCCAAGCGTGGAGAATTAAAATGCGACATCCTTTAGTGATGGGTA	This study

tpiA-R	ATGATCATTGCAATTGGATCCTGTCAGACTTAAGCCTGTTTAGCCG	This study
ppc-F	TGGGCCGTGCACCTTAAGCTTGATGGGGTGTCTGGGGTAATATGAA	This study
ppc-R	TCCGCCAAAACAGCCAAGCTTCGAGGGTGTTAGAACAGAAGTATTT	This study

Note: The underlined bases are introduced to create restriction sites for DNA cloning

Reference

Bunch, P. K., Mat-Jan, F., Lee, N., Clark, D. P. 1997. The *ldhA* gene encoding the fermentative lactate dehydrogenase of *Escherichia coli*. *Microbiology*, **143**, 187-195.

Table S2 Reactions that appeared in the manuscript involves in rxns, rxnNames, rxnFormula, grRules, subSystems, which derived from GEM iJO1366 of *E. coli* (Orth et al., 2011).

rxns	rxnNames	rxnFormula	grRules	subSystems
GLCabcpp	D-glucose transport via ABC system (periplasm)	atp[c] + h2o[c] + glc-D[p] -> adp[c] + glc-D[c] + h[c] + pi[c]	(b2149 and b2150 and b2148)	Transport, Inner Membrane
GLCptspp	D-glucose transport via PEP:Pyr PTS (periplasm)	pep[c] + glc-D[p] -> g6p[c] + pyr[c]	((b2417 and b1621 and b2415 and b2416) or (b2417 and b1101 and b2415 and b2416) or (b1817 and b1818 and b1819 and b2415 and b2416))	Transport, Inner Membrane
GLCt2pp	D-glucose transport in via proton symport (periplasm)	glc-D[p] + h[p] -> glc-D[c] + h[c]	b2943	Transport, Inner Membrane
ENO	enolase	2pg[c] <=> h2o[c] + pep[c]	b2779	Glycolysis/Gluconeogenesis
F6PA	fructose 6-phosphate aldolase	f6p[c] <=> dha[c] + g3p[c]	(b0825 or b3946)	Glycolysis/Gluconeogenesis
FBA	fructose-bisphosphate aldolase	fdp[c] <=> dhap[c] + g3p[c]	(b2097 or b1773 or b2925)	Glycolysis/Gluconeogenesis
FBP	fructose-bisphosphatase	fdp[c] + h2o[c] -> f6p[c] + pi[c]	(b3925 or b4232 or b2930)	Glycolysis/Gluconeogenesis
G1PPpp	Glucose-1-phosphatase	g1p[p] + h2o[p] -> glc-D[p] + pi[p]	b1002	Glycolysis/Gluconeogenesis
G6PP	glucose-6-phosphate phosphatase	g6p[c] + h2o[c] -> glc-D[c] + pi[c]	b0822	Glycolysis/Gluconeogenesis
GAPD	glyceraldehyde-3-phosphate dehydrogenase	g3p[c] + nad[c] + pi[c] <=> 13dpg[c] + h[c] + nadh[c]	b1779	Glycolysis/Gluconeogenesis
HEX1	hexokinase (D-glucose:ATP)	atp[c] + glc-D[c] -> adp[c] + g6p[c] + h[c]	b2388	Glycolysis/Gluconeogenesis
PDH	pyruvate dehydrogenase	coa[c] + nad[c] + pyr[c] -> accoa[c] + co2[c] + nadh[c]	(b0114 and b0115 and b0116)	Glycolysis/Gluconeogenesis
PFK	phosphofructokinase	atp[c] + f6p[c] -> adp[c] + fdp[c] + h[c]	(b3916 or b1723)	Glycolysis/Gluconeogenesis
PGI	glucose-6-phosphate isomerase	g6p[c] <=> f6p[c]	b4025	Glycolysis/Gluconeogenesis
PGK	phosphoglycerate kinase	3pg[c] + atp[c] <=> 13dpg[c] + adp[c]	b2926	Glycolysis/Gluconeogenesis
PGM	phosphoglycerate mutase	2pg[c] <=> 3pg[c]	(b3612 or b4395 or b0755)	Glycolysis/Gluconeogenesis

PPS	phosphoenolpyruvate synthase	atp[c] + h2o[c] + pyr[c] -> amp[c] + 2 h[c] + pep[c] + pi[c]	b1702	Glycolysis/Gluconeogenesis
PYK	pyruvate kinase	adp[c] + h[c] + pep[c] -> atp[c] + pyr[c]	(b1854 or b1676)	Glycolysis/Gluconeogenesis
TPI	triose-phosphate isomerase	dhap[c] <=> g3p[c]	b3919	Glycolysis/Gluconeogenesis
FBA3	Sedoheptulose 1,7-bisphosphate D-glyceraldehyde-3-phosphate-lyase	s17bp[c] <=> dhap[c] + e4p[c]	b2925	Pentose Phosphate Pathway
G6PDH2r	glucose 6-phosphate dehydrogenase	g6p[c] + nadp[c] <=> 6pgl[c] + h[c] + nadph[c]	b1852	Pentose Phosphate Pathway
GND	phosphogluconate dehydrogenase	6pgc[c] + nadp[c] -> co2[c] + nadph[c] + ru5p-D[c]	b2029	Pentose Phosphate Pathway
PFK_3	phosphofructokinase (s7p)	atp[c] + s7p[c] -> adp[c] + h[c] + s17bp[c]	b3916	Pentose Phosphate Pathway
PGL	6-phosphogluconolactonase	6pgl[c] + h2o[c] -> 6pgc[c] + h[c]	b0767	Pentose Phosphate Pathway
RPE	ribulose 5-phosphate 3-epimerase	ru5p-D[c] <=> xu5p-D[c]	(b4301 or b3386)	Pentose Phosphate Pathway
RPI	ribose-5-phosphate isomerase	r5p[c] <=> ru5p-D[c]	(b2914 or b4090)	Pentose Phosphate Pathway
TALA	transaldolase	g3p[c] + s7p[c] <=> e4p[c] + f6p[c]	(b2464 or b0008)	Pentose Phosphate Pathway
TKT1	transketolase	r5p[c] + xu5p-D[c] <=> g3p[c] + s7p[c]	(b2935 or b2465)	Pentose Phosphate Pathway
TKT2	transketolase	e4p[c] + xu5p-D[c] <=> f6p[c] + g3p[c]	(b2935 or b2465)	Pentose Phosphate Pathway
ACALD	acetaldehyde dehydrogenase (acetylating)	acald[c] + coa[c] + nad[c] <=> accoa[c] + h[c] + nadh[c]	(b0351 or b1241)	Pyruvate Metabolism
ACKr	acetate kinase	ac[c] + atp[c] <=> actp[c] + adp[c]	(b3115 or b2296 or b1849)	Pyruvate Metabolism
ACS	acetyl-CoA synthetase	ac[c] + atp[c] + coa[c] -> accoa[c] + amp[c] + ppi[c]	b4069	Pyruvate Metabolism
ALCD2x	alcohol dehydrogenase (ethanol)	etoh[c] + nad[c] <=> acald[c] + h[c] + nadh[c]	(b1478 or b1241 or b0356)	Pyruvate Metabolism
FHL	Formate-hydrogen lyase	for[c] + h[c] -> co2[c] + h2[c]	((b4079 and (b2481 and b2482 and b2483 and b2484 and b2485 and b2486 and b2487 and b2488 and b2489 and b2490)) or (b4079 and (b2719 and b2720 and b2721 and b2722 and b2723 and b2724)))	Pyruvate Metabolism
LDH_D	D-lactate dehydrogenase	lac-D[c] + nad[c] <=> h[c] + nadh[c] + pyr[c]	(b2133 or b1380)	Pyruvate Metabolism
PFL	pyruvate formate lyase	coa[c] + pyr[c] -> accoa[c] + for[c]	((b0902 and b0903) or (b0902 and b3114)	Pyruvate Metabolism

			or (b3951 and b3952) or ((b0902 and b0903) and b2579))	
POR5	pyruvate synthase	coa[c] + 2 flxso[c] + pyr[c] <=> accoa[c] + co2[c] + 2 flxr[c] + h[c]	((b0684 and b1378) or (b2895 and b1378))	Pyruvate Metabolism
PTAr	phosphotransacetylase	accoa[c] + pi[c] <=> actp[c] + coa[c]	(b2297 or b2458)	Pyruvate Metabolism
ACONTa	aconitase (half-reaction A, Citrate hydro-lyase)	cit[c] <=> acon-C[c] + h2o[c]	(b0118 or b1276)	Citric Acid Cycle
ACONTb	aconitase (half-reaction B, Isocitrate hydro-lyase)	acon-C[c] + h2o[c] <=> icit[c]	(b0118 or b1276)	Citric Acid Cycle
AKGDH	2-Oxogluterate dehydrogenase	akg[c] + coa[c] + nad[c] -> co2[c] + nadh[c] + succoa[c]	(b0116 and b0726 and b0727)	Citric Acid Cycle
CITL	Citrate lyase	cit[c] -> ac[c] + oaa[c]	((b0615 and b0616 and b0617) and b0614)	Citric Acid Cycle
CS	citrate synthase	accoa[c] + h2o[c] + oaa[c] -> cit[c] + coa[c] + h[c]	b0720	Citric Acid Cycle
FRD2	fumarate reductase	fum[c] + mql8[c] -> mqn8[c] + succ[c]	(b4151 and b4152 and b4153 and b4154)	Citric Acid Cycle
FRD3	fumarate reductase	2dmmql8[c] + fum[c] -> 2dmmq8[c] + succ[c]	(b4151 and b4152 and b4153 and b4154)	Citric Acid Cycle
FUM	fumarase	fum[c] + h2o[c] <=> mal-L[c]	(b1612 or b4122 or b1611)	Citric Acid Cycle
ICDHyr	isocitrate dehydrogenase (NADP)	icit[c] + nadp[c] <=> akg[c] + co2[c] + nadph[c]	b1136	Citric Acid Cycle
MDH	malate dehydrogenase	mal-L[c] + nad[c] <=> h[c] + nadh[c] + oaa[c]	b3236	Citric Acid Cycle
MDH2	Malate dehydrogenase (ubiquinone 8 as acceptor)	mal-L[c] + q8[c] -> oaa[c] + q8h2[c]	b2210	Citric Acid Cycle
MDH3	Malate dehydrogenase (menaquinone 8 as acceptor)	mal-L[c] + mqn8[c] -> mql8[c] + oaa[c]	b2210	Citric Acid Cycle
SUCOAS	succinyl-CoA synthetase (ADP-forming)	atp[c] + coa[c] + succ[c] <=> adp[c] + pi[c] + succoa[c]	(b0728 and b0729)	Citric Acid Cycle
PPC	phosphoenolpyruvate carboxylase	co2[c] + h2o[c] + pep[c] -> h[c] + oaa[c] + pi[c]	b3956	Anaplerotic Reactions

ICL	Isocitrate lyase	icit[c] -> glx[c] + succ[c]	b4015	Anaplerotic Reactions
MALS	malate synthase	accoa[c] + glx[c] + h2o[c] -> coa[c] + h[c] + mal-L[c]	(b4014 or b2976)	Anaplerotic Reactions

Reference

Orth, J.D., Conrad, T.M., Na, J., Lerman, J.A., Nam, H., Feist, A.M., Palsson, B.O. 2011. A comprehensive genome-scale reconstruction of *Escherichia coli* metabolism--2011. *Mol. Syst. Biol.*, **7**, 535.