

Supplementary file

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1 Modified Control Effective Flux (mCEF) algorithm

mCEF predicts the gene expression profile of genetically modified mutants where an enzyme coding gene is over-expressed (the relative enzyme activity ratio of a mutant versus wild type is > 1) or under-expressed ($0 < \text{enzyme activity ratio of mutant versus wild type} < 1$). Details of mCEF algorithm are described using a small metabolic network model.

The metabolic network model is shown in Figure S1. R6 is reversible reaction and the others are irreversible reactions. Ax, F and G are the external metabolites and the others are the internal metabolites.

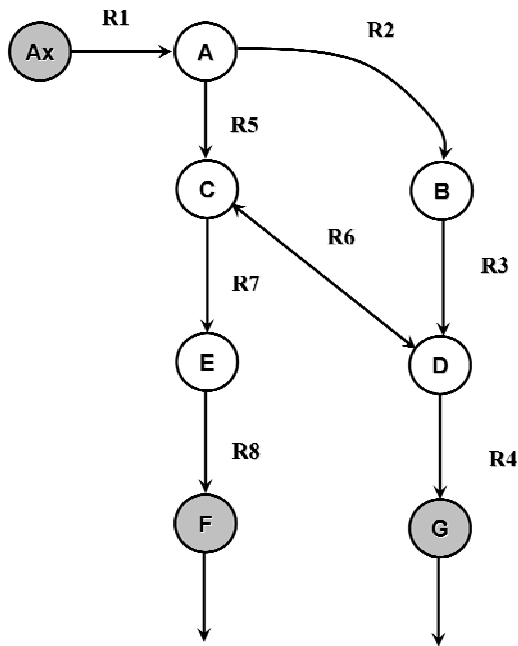


Figure S1. A small metabolic network map. The formation of F (R8: $E \rightarrow F$) is the cellular function. Ax, F and G are the external metabolites.

The formation of F from E is regarded as a biological function. In a mutant the gene responsible for the reaction $A \rightarrow C$ is 2-fold over-expressed, i.e., the relative enzyme activity parameter EAP of reaction 5 is set to 2.

There are four elementary modes (EMs) for the example network model. The fluxes could be decomposed onto EMs as follows:

$$\mathbf{v} = \mathbf{P} \cdot \boldsymbol{\lambda} = \begin{pmatrix} 1 & 6 & 6 & 3 \\ 0 & 3 & 3 & 0 \\ 0 & 1 & 1 & 0 \\ 0 & 0 & 2 & 1 \\ 1 & 0 & 0 & 3 \\ 0 & -2 & 0 & 1 \\ 1 & 6 & 0 & 0 \\ 1 & 6 & 0 & 0 \end{pmatrix} \cdot \begin{pmatrix} \lambda_1 \\ \lambda_2 \\ \lambda_3 \\ \lambda_4 \end{pmatrix}$$

where \mathbf{v} is the flux; \mathbf{P} is the EM matrix; $\boldsymbol{\lambda}$ is the EM coefficient (EMC) vector.

The elements in an EM are normalized by the element of the substrate uptake reaction in each EM.

Table S1 EMs normalized by the substrate uptake reaction

Reaction, i			Absolute element in the j -th EM $p_{i,j}$			
			1	2	3	4
1	R2	$2 A \rightarrow B$	$\frac{1}{2}$	0	0	$\frac{1}{2}$
2	R3	$3 B \rightarrow 4 D$	$\frac{1}{6}$	0	0	$\frac{1}{6}$
3	R4	$2 D \rightarrow G$	0	$\frac{1}{3}$	0	$\frac{1}{3}$
4	R5	$A \rightarrow C$	0	1	1	0
5	R6	$3 C \rightarrow 2 D$	$\frac{1}{3}$	$\frac{1}{3}$	0	0
6	R7	$C \rightarrow E$	1	0	1	0
7	R8	$E \rightarrow F$	1	0	1	0

The efficiency of each EM for the mutant is calculated by:

$$\varepsilon_{j,CEllobj}^m = \frac{p_{CEllobj,j} \cdot EA_j}{\sum_i (|p_{i,j}| \cdot \eta_i)}$$

$$\eta_i = \begin{cases} EAP_i & (\text{if reaction } i \text{ is modified}) \\ 1 & (\text{if reaction } i \text{ is not modified}) \end{cases}$$

Table S2 Parameter η_i for each reaction

Reaction, i			η_i
1	R2	$2 A \rightarrow B$	1
2	R3	$3 B \rightarrow 4 D$	1
3	R4	$2 D \rightarrow G$	1
4	R5	$A \rightarrow C$	2
5	R6	$3 C \rightarrow 2 D$	1
6	R7	$C \rightarrow E$	1
7	R8	$E \rightarrow F$	1

$$EA_j = \prod_{i=1}^n ge_{i,j}$$

$$ge_{i,j} = \begin{cases} EAP_i & (\text{if the } i\text{-th reaction is involved in the } j\text{-th EM}) \\ 1 & (\text{if the } i\text{-th reaction is not involved in the } j\text{-th EM}) \end{cases}$$

Table S3 Parameter EA_j for each EM

The j -th EM	EA_j
1	1
2	2
3	2
4	1

$$mCEF_i(mut) = \sum_{CEllobj} \frac{1}{P_{CEllobj}^{\max}} \frac{\sum_j (\varepsilon_{j,CEllobj}^m \cdot |p_{i,j}| \cdot \eta_i)}{\sum_j \varepsilon_{j,CEllobj}^m}$$

$$\varepsilon_{1,7} = \frac{1 \times 1}{\frac{1}{2} \times 1 + \frac{1}{6} \times 1 + 0 \times 1 + 0 \times 2 + \frac{1}{3} \times 1 + 1 \times 1 + 1 \times 1} = \frac{1}{3}$$

$$\varepsilon_{2,7} = \frac{0 \times 2}{0 \times 1 + 0 \times 1 + \frac{1}{3} \times 1 + 1 \times 2 + \frac{1}{3} \times 1 + 0 \times 1 + 0 \times 1} = 0$$

$$\varepsilon_{3,7} = \frac{1 \times 2}{0 \times 1 + 0 \times 1 + 0 \times 1 + 1 \times 2 + 0 \times 1 + 1 \times 1 + 1 \times 1} = \frac{1}{2}$$

$$\varepsilon_{4,7} = \frac{0 \times 1}{\frac{1}{2} \times 1 + \frac{1}{6} \times 1 + \frac{1}{3} \times 1 + 0 \times 2 + 0 \times 1 + 0 \times 1 + 0 \times 1} = 0$$

The mCEF for each reaction is calculated.

$$mCEF_1(mut) = \frac{1}{1} \cdot \frac{\frac{1}{3} \cdot (\frac{1}{2} \times 1) + 0 \cdot (0 \times 1) + \frac{1}{2} \cdot (0 \times 1) + 0 \cdot (\frac{1}{2} \times 1)}{(\frac{1}{3} + 0 + \frac{1}{2} + 0)} = \frac{1}{5}$$

$$mCEF_2(mut) = \frac{1}{1} \cdot \frac{\frac{1}{3} \cdot (\frac{1}{6} \times 1) + 0 \cdot (0 \times 1) + \frac{1}{2} \cdot (0 \times 1) + 0 \cdot (\frac{1}{6} \times 1)}{(\frac{1}{3} + 0 + \frac{1}{2} + 0)} = \frac{1}{15}$$

$$mCEF_3(mut) = \frac{1}{1} \cdot \frac{\frac{1}{3} \cdot (0 \times 1) + 0 \cdot (\frac{1}{3} \times 1) + \frac{1}{2} \cdot (0 \times 1) + 0 \cdot (\frac{1}{3} \times 1)}{(\frac{1}{3} + 0 + \frac{1}{2} + 0)} = 0$$

$$mCEF_4(mut) = \frac{1}{1} \cdot \frac{\frac{1}{3} \cdot (0 \times 2) + 0 \cdot (1 \times 2) + \frac{1}{2} \cdot (1 \times 2) + 0 \cdot (0 \times 2)}{(\frac{1}{3} + 0 + \frac{1}{2} + 0)} = \frac{6}{5}$$

$$mCEF_5(mut) = \frac{1}{1} \cdot \frac{\frac{1}{3} \cdot (\frac{1}{3} \times 1) + 0 \cdot (\frac{1}{3} \times 1) + \frac{1}{2} \cdot (0 \times 1) + 0 \cdot (0 \times 1)}{(\frac{1}{3} + 0 + \frac{1}{2} + 0)} = \frac{2}{15}$$

$$mCEF_6(mut) = \frac{1}{1} \cdot \frac{\frac{1}{3} \cdot (1 \times 1) + 0 \cdot (0 \times 1) + \frac{1}{2} \cdot (1 \times 1) + 0 \cdot (0 \times 1)}{(\frac{1}{3} + 0 + \frac{1}{2} + 0)} = 1$$

$$mCEF_7(mut) = \frac{1}{1} \cdot \frac{\frac{1}{3} \cdot (1 \times 1) + 0 \cdot (0 \times 1) + \frac{1}{2} \cdot (1 \times 1) + 0 \cdot (0 \times 1)}{(\frac{1}{3} + 0 + \frac{1}{2} + 0)} = 1$$

The mCEFs of an over-expressing mutant are calculated below.

$$\Theta_i(w, mut) = \frac{mCEF_i(mut)}{mCEF_i(w)}$$

Table S4 Values of mCEFs of an over-expressing mutant

Reaction i			mCEF _{i} (w)	mCEF _{i} (mut)	$\Theta_i(w, mut)$
1	R2	2 A \rightarrow B	$\frac{1}{4}$	$\frac{1}{5}$	$\frac{4}{5}$
2	R3	3 B \rightarrow 4 D	$\frac{1}{12}$	$\frac{1}{15}$	$\frac{4}{5}$
3	R4	2 D \rightarrow 3 F	0	0	1
4	R5	A \rightarrow C	$\frac{1}{2}$	$\frac{6}{5}$	$\frac{12}{5}$
5	R6	3 C \rightarrow 2 D	$\frac{1}{6}$	$\frac{2}{15}$	$\frac{6}{5}$
6	R7	C \rightarrow E	1	1	1
7	R8	E \rightarrow F	1	1	1

2. Metabolic networks models

Table S5 Metabolic network model of *Escherichia coli*

Gene	Gene description	Reaction
ACEA	Isocitrate lyase	ICIT ==> SUCC + GLX
ACEB	Malase synthase A	ACCOA + GLX ==> MAL + COA
ACK	Acetate kinase	ACTP + ADP <=> ATP + AC
ACN	Aconitase	CIT <=> ICIT
ACXT	Acetate transport	AC ==> ACXT
ADK	Adenylate kinase	ATP + AMP <=> 2 ADP
ATP	F0F1-ATPase	PI + 4 HEXT + ADP <=> ATP
ATPDRAIN	ATP drain	ATP ==> PI + ADP
CO ₂ XT	CO ₂ transport	CO ₂ <=> CO ₂ XT
CYO	Cytochrome oxidase bo3	2 QH ₂ + O ₂ ==> 2 Q + 4 HEXT
EDA	6-phosphogluconate dehydratase 2-keto-3-deoxy-6-phosphogluconate	KetoPGLuc ==> T3P1 + PYR
EDD	aldolase	D6PGC ==> KetoPGLuc
ENO	Enolase	P2G <=> PEP
FBA	Fructose-1,6-bisphosphatase aldolase	FDP <=> T3P1 + T3P2
FBP	Fructose-1,6-bisphosphatase	FDP ==> F6P + PI
FRD	Fumarate reductase	FADH + FUM ==> FAD + SUCC
FUM	Fumarase	FUM <=> MAL
GAP	Glyceraldehyde-3-phosphate dehydrogenase	PI + T3P1 + NAD <=> P13DG + NADH
GLCUP	uptake of glucose	GLCXT ==> GLC
GLT	Citrate synthase	ACCOA + OA ==> COA + CIT
GND	6-phosphoglyconate dehydrogenase	D6PGC + NADP ==> RL5P + CO ₂ + NADPH
GPM	Phosphoglycerate mutase	P3G <=> P2G
GROWTH	Growth	0.1 F6P + 1.5 P3G + 3.7 ACCOA + 0.2 G6P + 41.3 ATP + 0.5 PEP + 1.1 AKG + 0.4 E4P + 18.2 NADPH + 1.8 OA + 0.9 R5P + 0.1 T3P1 + 3.5 NAD + 2.8 PYR ==> 41.3 PI + 3.7 COA + 41.3 ADP + 3.5 NADH + 18.2 NADP + BIOMASS
ICD	Isocitrate dehydrogenase	ICIT + NADP <=> AKG + CO ₂ + NADPH
LPD	lipoamide dehydrogenase	COA + NAD + PYR ==> ACCOA + CO ₂ + NADH
MAEB	Malic enzyme (NADP)	MAL + NADP ==> CO ₂ + NADPH + PYR

Table S5 (*continued*)

Gene	Gene description	Reaction
MDH	Malase dehydrogenase	$\text{MAL} + \text{NAD} \rightleftharpoons \text{OA} + \text{NADH}$
NUO	NADH dehydrogenase	$\text{Q} + \text{NADH} \rightleftharpoons \text{QH2} + 2 \text{HEXT} + \text{NAD}$
O ₂ XT	Oxygen transport	$\text{O}_2 \rightleftharpoons \text{O}_2\text{XT}$
PCK	Phosphoenolpyruvate carboxykinase	$\text{ATP} + \text{OA} \rightleftharpoons \text{PEP} + \text{CO}_2 + \text{ADP}$
PFK	Phosphofructokinase	$\text{F6P} + \text{ATP} \rightleftharpoons \text{ADP} + \text{FDP}$
PGI	Phosphoglucoisomerase	$\text{G6P} \rightleftharpoons \text{F6P}$
PGK	Phosphoglycerate kinase	$\text{ADP} + \text{P13DG} \rightleftharpoons \text{P3G} + \text{ATP}$
PGL	6-phosphoglyconolactonase	$\text{D6PGL} \rightleftharpoons \text{D6PGC}$
PIXT	Inorganic phosphate transport	$\text{PI} \rightleftharpoons \text{PIXT}$
PNTA	Pyridine nucleotide transhydrogenase A	$\text{NADPH} + \text{NAD} \rightleftharpoons \text{NADH} + \text{NADP}$
PNTB	Pyridine nucleotide transhydrogenase B	$2 \text{HEXT} + \text{NADH} + \text{NADP} \rightleftharpoons \text{NADPH} + \text{NAD}$
PPC	Phosphoenolpyruvate carboxylase	$\text{PEP} + \text{CO}_2 \rightleftharpoons \text{PI} + \text{OA}$
PPS	Phosphoenolpyruvate synthase	$\text{ATP} + \text{PYR} \rightleftharpoons \text{PI} + \text{PEP} + \text{AMP}$
PTA	Phosphotransacetylase	$\text{ACCOA} + \text{PI} \rightleftharpoons \text{ACTP} + \text{COA}$
PTS	Glucose transport	$\text{PEP} + \text{GLC} \rightleftharpoons \text{G6P} + \text{PYR}$
PYK	Pyruvate kinase	$\text{PEP} + \text{ADP} \rightleftharpoons \text{ATP} + \text{PYR}$
RPE	Ribulose phosphate 3-epimerase	$\text{RL5P} \rightleftharpoons \text{X5P}$
RPI	Ribose-5-phosphate isomerase	$\text{RL5P} \rightleftharpoons \text{R5P}$
SDH	Succinate dehydrogenase	$\text{FAD} + \text{SUCC} \rightleftharpoons \text{FADH} + \text{FUM}$
SDH2	Succinate dehydrogenase complex	$\text{FADH} + \text{Q} \rightleftharpoons \text{FAD} + \text{QH2}$
SUCAB	2-Ketoglutarase dehydragenase	$\text{AKG} + \text{COA} + \text{NAD} \rightleftharpoons \text{CO}_2 + \text{SUCCOA} + \text{NADH}$
SUCCD	Succinyl-CoA synthetase	$\text{PI} + \text{ADP} + \text{SUCCOA} \rightleftharpoons \text{ATP} + \text{SUCC} + \text{COA}$
TALB	Transaldolase B	$\text{S7P} + \text{T3P1} \rightleftharpoons \text{F6P} + \text{E4P}$
TKTA	Transketolase I	$\text{X5P} + \text{R5P} \rightleftharpoons \text{S7P} + \text{T3P1}$
TKTB	Transketolase II	$\text{X5P} + \text{E4P} \rightleftharpoons \text{F6P} + \text{T3P1}$
TPI	Triosphosphate isomerase	$\text{T3P1} \rightleftharpoons \text{T3P2}$
ZWF	Glucose-6-phosphate 1-dehydrogenase	$\text{G6P} + \text{NADP} \rightleftharpoons \text{NADPH} + \text{D6PGL}$

Table S6 Metabolites in metabolic network model of *Escherichia coli*

Abbreviations	Full name	Abbreviations	Full name
AC	Acetate	KetoPGLuc	2-Keto-3-desoxy-6-phospho gluconate
ACCOA	Acetyl coenzyme A	MAL	malate
ACTP	Acetyl phosphate	NAD	Nicotinamide adenine dinucleotide
ACXT	external acetate	NADH	Nicotinamide adenine dinucleotide - reduced
ADP	Adenosine diphosphate	NADP	Nicotinamide adenine dinucleotide phosphate
AKG	alpha-Ketoglutarate	NADPH	Nicotinamide adenine dinucleotide phosphate-reduced
AMP	Adenosine 5'-monophosphate	O ₂	Oxygen
ATP	Adenosine triphosphate	O ₂ XT	External oxygen
BIOMASS	Biomass	OA	oxaloacetate
CIT	Citrate	P13DG	3-Phospho-D-glyceroyl phosphate
CO ₂	Carbon dioxide	P2G	2-Phosphoglycerate
CO ₂ XT	external carbon dioxide	P3G	3-Phosphoglycerate
COA	Coenzyme A	PEP	Phosphoenolpyruvate
D6PGC	6-Phospho-D-gluconate	PI	Phosphate
D6PGL	D-Glucono-1,5-lactone 6-phosphate	PIXT	external phosphate
E4P	Erythrose-4-phosphate	PYR	Pyruvate
F6P	Fructose-6-phosphate	Q	Ubiquinone-8
FAD	Flavin adenine dinucleotide	QH2	Ubiquinol-8
FADH	Flavin adenine dinucleotide-reduced	R5P	ribose-5-phosphate
FDP	D-Fructose 2,6-bisphosphate	RL5P	ribulose-5-phosphate
FUM	Fumarate	S7P	sedoheptulose-7-phosphate
G6P	Glucose-6-phosphate	SUCC	succinate
GLC	Glucose	SUCCOA	succinyl-coenzyme A
GLCXT	External glucose	T3P1	glyceraldehyde-3-phosphate
GLX	Glyoxylate	T3P2	dihydroxyacetate phosphate
HEXT	external hydrogen	X5P	xylulose-5-phosphate
ICIT	isocitrate		

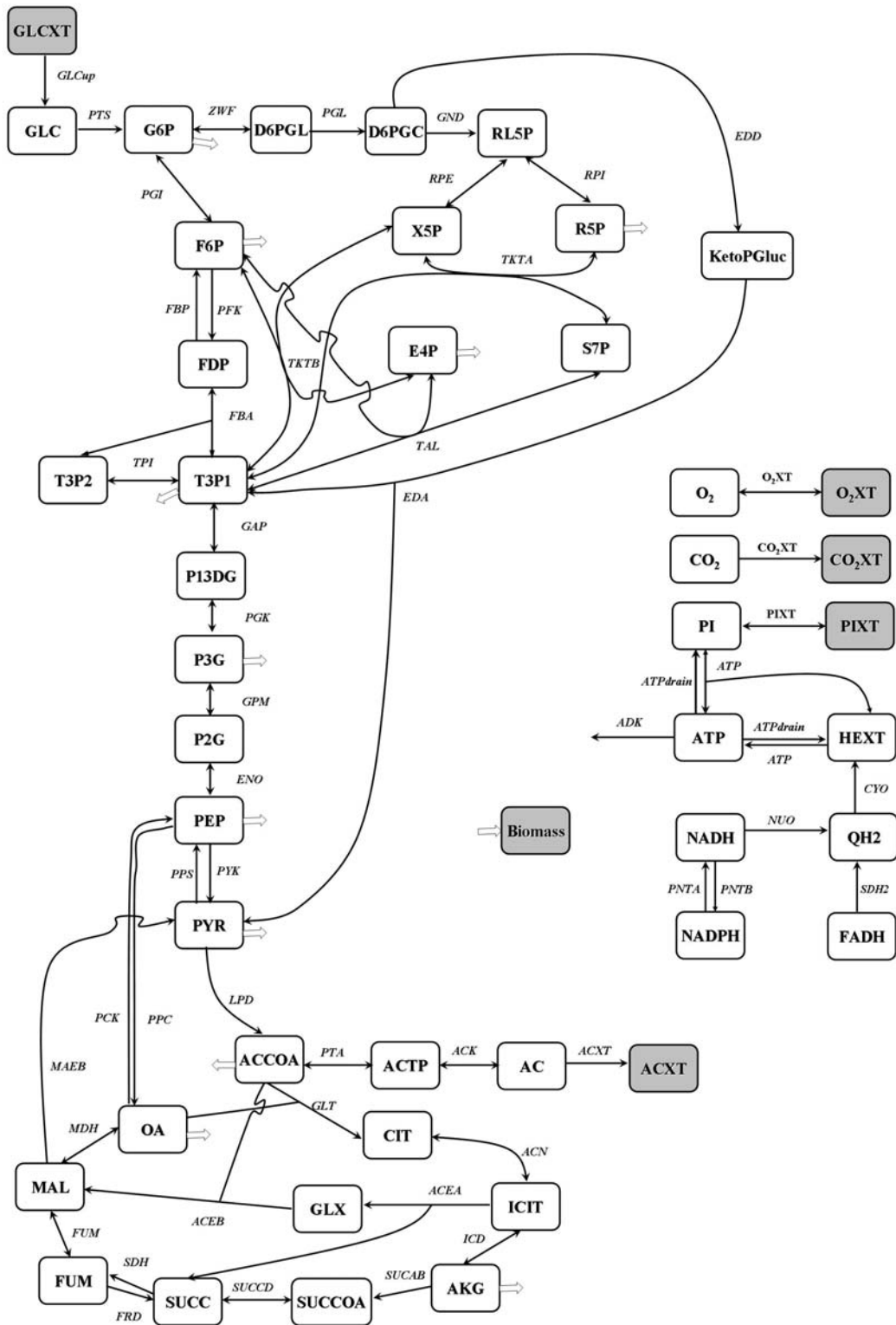


Figure S2 Central network model of *E. coli*. Grey ones are external metabolites.

Table S7 Metabolic network model for *Corynebacterium glutamicum*

Gene	Gene description	Reaction
asp	Asparagine transaminase	$OAA + GLUT \rightleftharpoons ASP + AKG$
glt, acn, icd	Citrate synthase, Aconitase, isocitrate dehydrogenase	$OAA + ACCOA + H_2O + NADP \rightleftharpoons AKG + CO_2 + NADPH + COA$
tkl II	Transketolase II	$E4P + XYL5P \rightleftharpoons GAP + FRU6P$
sucCD	SUCCoA synthase	$ADP + SUCCOA \rightleftharpoons ATP + COA + SUC$
gap, pgk	Phosphoglycerate kinase	$GAP + ADP + NAD \rightleftharpoons ATP + G3P + NADH$
tal	Transaldolase	$SED7P + GAP \rightleftharpoons E4P + FRU6P$
glutDH	Glutamate dehydrogenase	$AKG + NH_3 + NADPH \rightleftharpoons H_2O + NADP + GLUT$
ots	trehalose-6-phosphate synthase	$ATP + 2 GLC6P \rightleftharpoons TREHAL + ADP$
pgi	Phosphoglucoisomerase	$GLC6P \rightleftharpoons FRU6P$
rpi	Ribose-5-phosphate isomerase	$RIBU5P \rightleftharpoons RIB5P$
rpe	Ribulose phosphate 3-epimerase	$RIBU5P \rightleftharpoons XYL5P$
gpm, eno	Phosphoglycerate mutase, Enolase	$G3P \rightleftharpoons PEP + H_2O$
O ₂ ext	Oxygen uptake	$O_2 \rightleftharpoons O_2XT$
tkl I	Transketolase I	$RIB5P + XYL5P \rightleftharpoons SED7P + GAP$
sucCD	SUCC-CoA synthase	$FAD + H_2O + NAD + SUC \rightleftharpoons OAA + FADH + NADH$
LysexT	Lysine transport	$LYSI \rightleftharpoons LYSIXT$
pdh	Pyruvate dehydrogenase	$COA + NAD + PYR \rightleftharpoons ACCOA + CO_2 + NADH$
Treext	Trehalose transport	$TREHAL \rightleftharpoons TREHALXT$
sucAB	2-Ketoglutarate dehydragenase	$AKG + COA + NAD \rightleftharpoons CO_2 + SUCCOA + NADH$
pps, pyk	Phosphoenolpyruvate synthase, Pyruvate kinase	$PEP + ADP \rightleftharpoons ATP + PYR$
ilvBCDE	Acetolactate synthase I, Acetohydroxy acid isomeroreductase, Dihydroxy acid dehydratase, Branched chain amino acid aminotransferase	$NADPH + GLUT + 2 PYR \rightleftharpoons VAL + AKG + CO_2 + H_2O + NADP$
Growth	Biomass formation	$332 ACCOA + 40 VAL + 3000 ATP + 80 ASP + 126 RIB5P + 33 LYSI + 150 G3P + 52 PEP + 13 GAP + 100 NADPH + 54 ALA + 25 GLUM + 7 FRU6P + 21 GLC6P + 446 GLUT + 30 PYR \rightleftharpoons 364 AKG + 143 CO_2 + 332 COA + 3000 ADP + 100 NADP + 1000 BIOMASS$
ATP	ATP synthase	$4 ADP + 2 NADH + O_2 \rightleftharpoons 4 ATP + 2 H_2O + 2 NAD$

Table S7 (continued)

Gene	Gene description	Reaction
alaTr	Alanine transaminase	GLUT + PYR ==> AKG + ALA
ATPdrain	ATP drain	ATP ==> ADP
Lys	Diaminopimelate decarboxylase	MDAP ==> LYSI + CO ₂
FADHHDH	FADH dehydrogenase	2 FADH + 2 ADP + O ₂ ==> 2 ATP + 2 FAD + 2 H ₂ O
dapCEF	Succinyl diaminopimelate aminotransferase, Tetrahydrodipicolinate succinylase, Succinyl diaminopimelate desuccinylase	AKP + SUCCOA + H ₂ O + GLUT ==> MDAP + AKG + COA + SUC
fbp	Fructose-1,6-bisphosphatase	H ₂ O + FDP ==> FRU6P
pts	Phosphotransferase system	PEP + GLC ==> GLC6P + PYR
fba, tpi	Fructosebisphosphaphate aldolase, Triosphosphate isomerase	FDP ==> 2 GAP
CO ₂ ext	CO ₂ transport	CO ₂ ==> CO ₂ XT
ppc	Phosphoenolpyruvate carboxylase	PEP + CO ₂ ==> OAA
H ₂ Oext	Water transport	H ₂ O ==> H ₂ OXT
Thr, asd	Aspartate kinase, Aspartate semialdehyde	ATP + ASP + 2 NADPH + PYR ==> AKP + ADP + H ₂ O + 2 NADP
GLCup	Glucose uptake	GLCXT ==> GLC
Glum	Glutamine synthase	ATP + NH ₃ + GLUT ==> ADP + GLUM
pfk	Phosphofructokinase	ATP + FRU6P ==> ADP + FDP
NH ₃ ext	Ammonia transport	NH ₃ XT ==> NH ₃
Zwf, pgl, gnd	Glucose-6-phosphate 1-dehydrogenase, 6-phosphoglyconolactonase, 6-phosphoglycononate dehydrogenase	H ₂ O + 2 NADP + GLC6P ==> CO ₂ + 2 NADPH + RIBU5P

Table S8 Metabolites in metabolic network models for *Corynebacterium glutamicum*

Abbreviations	Full name	Abbreviations	Full name
OAA	Oxaloacetic acid	SUCCOA	Succinyl -coenzyme A
FADH	Flavin adenine dinucleotide (reduced)	H ₂ O	Water
ACCOA	Acetyl-coenzyme A	NADH	Nicotinamide adenine dinucleotide (reduced)
VAL	Valine	O ₂	Oxygen
ATP	Adenosine triphosphate	ALA	Alanine
ASP	Aspartate	NAD	Nicotinamide adenine dinucleotide (oxidized)
SED7P	Sedoheptulose-7-phosphate	GLUM	Glutamine
RIB5P	Ribose-5-phosphate	GLC	Glucose
LYSI	Lysine	NADP	Nicotinamide adenine dinucleotide phosphate (oxidized)
G3P	3-phosphoglycerate	FRU6P	Fructose-6-phosphate
AKP	2-Amino-6-ketopimelate	XYL5P	Xylulose-5-phosphate
PEP	Phosphoenolpyruvate	GLC6P	Glucose-6-phosphate
MDAP	meso-diaminopimelate	FDP	Fructose 2,6-bisphosphate
GAP	Glyceraldehyde-3-phosphate	GLUT	Glutamate
AKG	alpha-ketoglutarate	SUC	Succinate
E4P	Erythrose-4-phosphate	PYR	Pyruvate
TREHAL	Trehalose	LYSIXT	External lysine
NH ₃	Ammonia	O ₂ XT	External oxygen
FAD	Flavin adenine dinucleotide (oxidized)	CO ₂ XT	External carbon dioxide
CO ₂	Carbon dioxide	TREHALXT	External trehalose
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)	BIOMASS	Biomass
COA	Coenzyme A	H ₂ OXT	External Water
RIBU5P	Ribulose-5-phosphate	GLCXT	External glucose
ADP	Adenosine diphosphate	NH ₃ XT	External ammonia

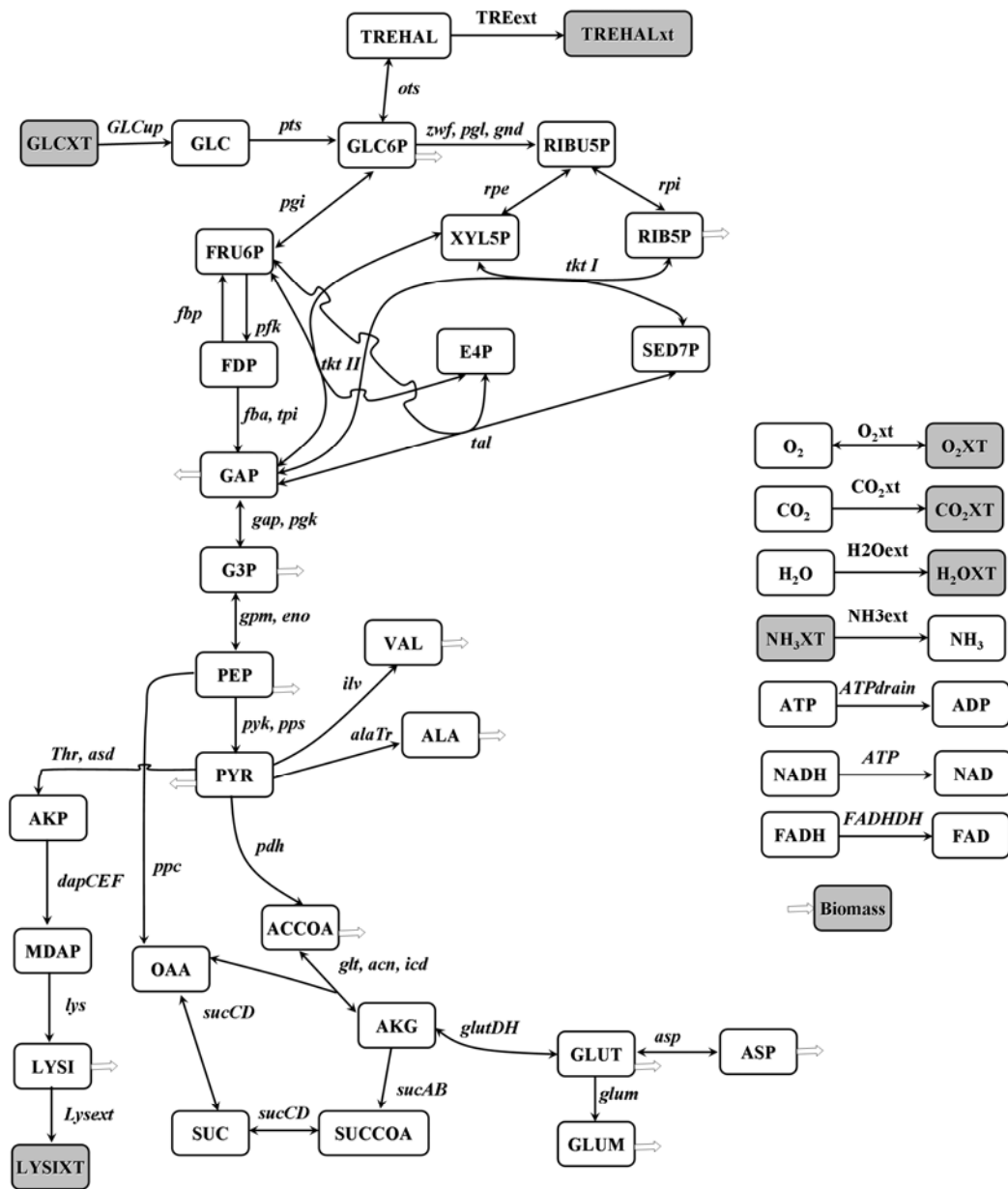


Figure S3 Central network model of *C. glutamicum*. Grey ones are external metabolites.

Table S9 Prediction errors for nine mutants of *E. coli* by FBA, MOMA and GMF

Mutant	FBA ^a	FBA ^b	MOMA	GMF
fbp	38.19	23.69	19.65	20.00
pps	35.31	20.66	10.78	9.97
rpe	32.65	34.84	11.64	9.20
tktA	38.44	40.82	15.17	14.12
tktB	21.01	23.25	4.78	4.68
fbp	26.93	28.85	20.20	19.43
pgi	26.97	24.80	19.94	24.56
pgl	21.97	23.68	21.04	21.97
gnd	28.61	32.30	17.22	14.39

a: the objective function in FBA is the maximum biomass formation;

b: the objective function in FBA is the maximum ATP per flux unit (Schuetz *et al.*, 2007);

The metabolic fluxes for mutants were cited from the reference (Ishii, N. *et al.*, 2007)

FBA is performed by linprog in Matlab.

$$\min \sum_i c_i v_i$$

$$\text{subject to } \mathbf{S} \cdot \mathbf{v} = 0 \quad v_{i,\min} \leq v_i \leq v_{i,\max} \quad (i = 1, \dots, n)$$

In the metabolic network models of *E. coli* and *C. glutamicum*, the reaction for biomass formation was presented. The coefficients of the objective function, c_i , are -1 for this biomass formation reaction while they are zero for other reactions.