Supporting Information

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SI Text

Theoretical Yield Calculation. To calculate the theoretical yield of homoalanine from glucose, MATLAB software with linear programming optimization was used. We first establish a set of mass balance equations describing all the relevant intracellular metabolites in terms of input and output flues. The input glucose flux is set to 1, so that the yield of isobutnaol is equal to the isobutanol flux (v_{iBOH}) divided by 1. To calculate the maximal theoretical yield, we carry out the following minimization:

$$\min(-v_{iBOH}) \quad \text{such that} \quad AV = B.$$

Here A is the stoichiometric matrix (Table S2), V is the vector of flux in each reaction included, and B is the vector that consists of negative glucose input and zeros (Table S3). The rows of A and B correspond to each metabolite, and the columns of A correspond to each reaction (or lumped reaction) considered (defined in Table S4).

To carry out this linear optimization problem, we used a MATLAB module "linprog", which uess the following formalism

$$\min_x f^T x \quad \text{such that} \quad A_{\text{eq}} x = b_{\text{eq}}.$$

To fit this formalism the f vector is defined in Table S4

After minimization, $V10$ is the maximum theoretical yield of isobutanol and the rest of the V (or x) vector is the flux distribution over the metabolic network.

In this calculation, there are two degrees of freedom: (*i*) whether NADH can be converted to NADPH by transhydrogenase, and (*ii*) the P/O ratio (number of ATP obtained by oxidizing NAD(P)H). Table S5 shows the results of the maximum theoretical yield for various combination of these two variations.

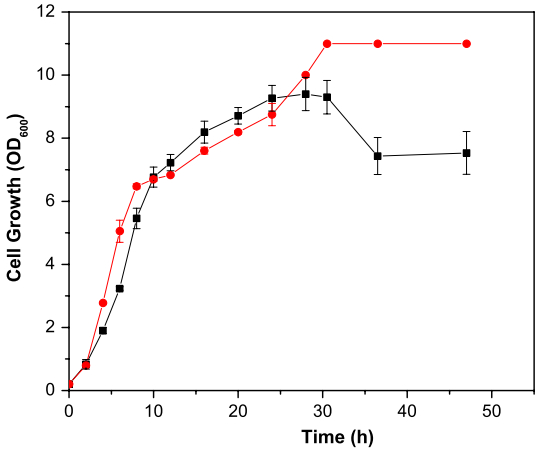

Fig. S1. Time courses for the growth of *E. coli* strains. OD_{600}, optical density at 600 nm. Cells were incubated in production medium at 33 °C. *Circles*, wild-type strain ATCC98082 with pZS_thrO; *diamonds*, best production strain ATCC98082 (*ΔrhtA*) with pZS_thrO and pZElac_ilvA$_{BS}$_GDH. Error bars: standard deviations from three independent experiments.

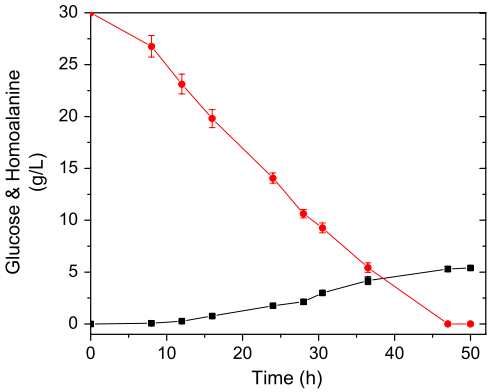

Fig. S2. Time courses for the production of L-homoalanine. *Diamonds*, L-homoalanine concentration; *circles*, residual glucose concentration. Error bars: standard deviations from three independent experiments.

Table S1. Synthetic oligonucleotides for plasmid construction

Name	Sequence
IlvEaccfwd	GCATACGGTACC ATGACCACGAAGAAAGCTGATTACATTTG
IlvExabrev	GCATACTCTAGA TTATTGATTAACTTGATCTAACCAGCCCCAT
Vdhsaaccfwd	GCATACGGTACC ATGACCGATGTATCCGACGGCGT
Vdhsaxbarev	GCATACTCTAGA TTAGCCCCGGCGGGCCTCCGCCATG
Vdhscaccfwd	GCATACGGTACC ATGACCGACGTAAACGGCGCACC
Vdhscxbarev	GCATACTCTAGA TTACGGCCGGGGACGGGCCTCCGCCATC
Vdhsfaccfwd	GCATACGGTACC ATGACCGACGCGTCCCACCCCAC
Vdhsfxbarev	GCATACTCTAGA TTAGACGGTGCGGGCCTCCGCCATG
GDHecaccfwd	GCATACGGTACC ATGGATCAGACATATTCTCTGGAGTCATTC
GDHecxabrev	GCATACTCTAGA TTAAATCACACCCTGCGCCAGC
GDH_k92lib	GCTCTGCCATCGGCCCGTACNNKGGCGGTATGCGCTTCCATCCG
GDH_k92lib_rev	CGGATGGAAGCGCATACCGCCMNNGTACGGGCCGATGGCAGAGC
GDH_T195lib	CAACAATACCGCCTGCGTCTTCNNKGGTAAGGGCCTTTCATTTGG
GDH_T195lib_rev	CCAAATGAAAGGCCCTTACCMNNGAAGACGCAGGCGGTATTGTTG
GDH_VSlib	GTAAAGCGGCTAATGCTGGTGGCNNKGCTACANNKGGCCTGGAAATGGCACAAAAC
GDH_VSlib_rev	GTTTTGTGCCATTTCCAGGCCMNNTGTAGCMNNGCCACCAGCATTAGCCGCTTTAC
GDHecsalfwd	GCATAC GTCGAC AAGAGGAGAAAGTTACC ATGGATCAGACATATTCTCTGGAGTCATTC
TdcBaccfwd	GCATACGGTACC ATGCATATTACATACGATCTGCCGGTTG
TdcBsalrev	GCATAC GTCGAC TTAAGCGTCAACGAAACCGGTGATTTG
IlvAecaccfwd	GCATACGGTACC ATGGCTGACTCGCAACCCCTG
IlvAecsalrev	GCATACGTCGAC CTAACCCGCCAAAAAGAACCTGA
IlvAbsaccfwd	GCATACGGTACC ATGAAACCGTTGCTTAAAGAAAACTCTCTC
IlvAbssalrev	GCATACGTCGAC TTAGATTAGCAAATGGAACAAGTCCTCATCC
GDHbamfwd	GCATAC GGATCCATGGATCAGACATATTCTCTGGAGTCATTC
GDHbamrev	GCATAC GGATCCTTAAATCACACCCTGCGCCAGC

Table S2. A matrix

matrix A	v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	V17	v18	v19	v20	v21	v22	V_{NA}	V_{NA}	V_{AT}	V_{CO}	vpp	Vna	Vna	Vna
Glucose	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G6P	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	−1	0	0	0	0	0	0	0	0	0	0
F6P	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
F16BP	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GA3P	0	0	0	2	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
PEP	−1	0	0	0	1	−1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Pyr	1	0	0	0	0	1	0	0	0	0	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	−1	0	0	0
6PG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0
R5P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	−3	0	0	0	0	0	0	0	0
OAA	0	0	0	0	0	0	1	−1	0	0	0	−1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
ASP	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
THR	0	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AceCoA	0	0	0	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CoASH	0	0	0	0	0	0	0	0	0	0	−1	1	0	−1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Citrate	0	0	0	0	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GA	0	0	0	0	0	0	0	−1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2KG	0	0	0	0	0	0	0	1	0	0	0	0	1	−1	0	0	0	0	−1	0	0	0	0	0	0	0	0	0	0	0
SucCoA	0	0	0	0	0	0	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Succinate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fumarate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0
Malate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0
CO2	0	0	0	0	0	0	−1	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	−1	0	0	0	0
NADH	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	−1	0	0	0	0	−1	−1	0
NADPH	0	0	0	0	0	0	0	0	−2	−1	0	0	1	0	0	0	0	0	−1	1	1	0	0	−1	0	0	0	1	0	−1
ATP	0	0	−1	0	1	1	0	0	−2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	−1	0	−1	0	3	3

Table S3. B matrix

Matrix B	"$-V_{in}$"
Glucose	−1.000
G6P	0.000
F6P	0.000
F16BP	0.000
GA3P	0.000
PEP	0.000
Pyr	0.000
6PG	0.000
R5P	0.000
OAA	0.000
ASP	0.000
THR	0.000
AceCoA	0.000
CoASH	0.000
Citrate	0.000
GA	0.000
2KG	0.000
SucCoA	0.000
Succinate	0.000
Fumarate	0.000
Malate	0.000
CO2	0.000
NADH	0.000
NADPH	0.000
ATP	0.000

Table S4. *f* vector and the definition of fluxes

Pathway	Flux	*f*
Glucose uptake (PTS)	v1	0.000
Glycolysis	v2	0.000
	v3	0.000
	v4	0.000
	v5	0.000
	v6	0.000
ppc	v7	0.000
V OAA → ASP	v8	0.000
v ASP → THR	v9	0.000
v THR → HA	v10	−1.000
v Pyr → AceCoA	v11	0.000
TCA	v12	0.000
	V13	0.000
	v14	0.000
	v15	0.000
	V16	0.000
	V17	0.000
	v18	0.000
v 2KG → Glu	v19	0.000
PPP	v20	0.000
	v21	0.000
	v22	0.000
	VNADH-out	0.000
	VNADPH-out	0.000
	VATP-out	0.000
	VCO2-out	0.000
	Vpps	0.000
	VNADH-NADPH	0.000
	VNADH-ATP	0.000
	VNADPH-ATP	0.000

Table S5. Summary of the theoretical yield for homoalanine production from glucose

Transhydrogenase (NADH → NADPH)	ATP/NAD(P)H	Theoretical yield	
		Molar yield (mol/mol)	Mass yield (g/g)
YES	1.5	1.188	0.68
	2.0	1.200	0.69
	3.0	**1.226**	**0.70**
NO	1.5 ~ 3.0	0.733	0.42

Assumption: 1) Energy production from UQH2 derived from succinate dehydrogenase in TCA cycle was not included. 2) Nonoxidative branch of Pentose Phosphate Pathway was assumed 3R5P → 2F6P + GA3P