Supporting Information to

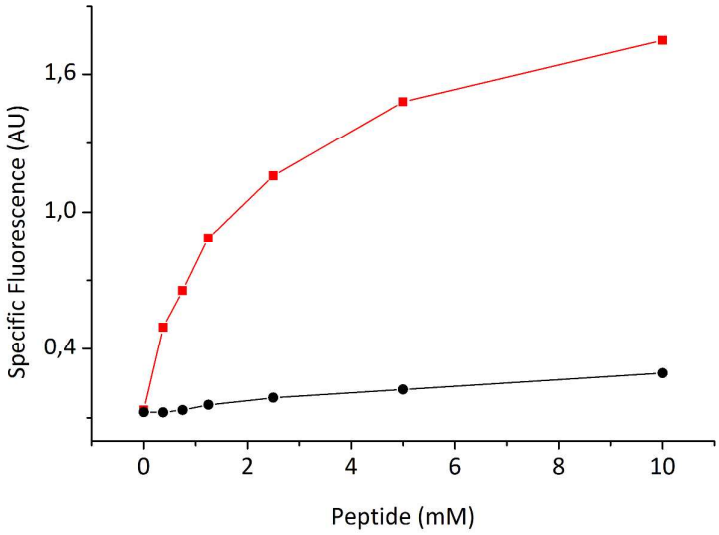
Taking control over control: Use of product sensing in single cells to remove flux control at key enzymes in biosynthesis pathways

by Schendzielorz et al.

Supplementary Video S1

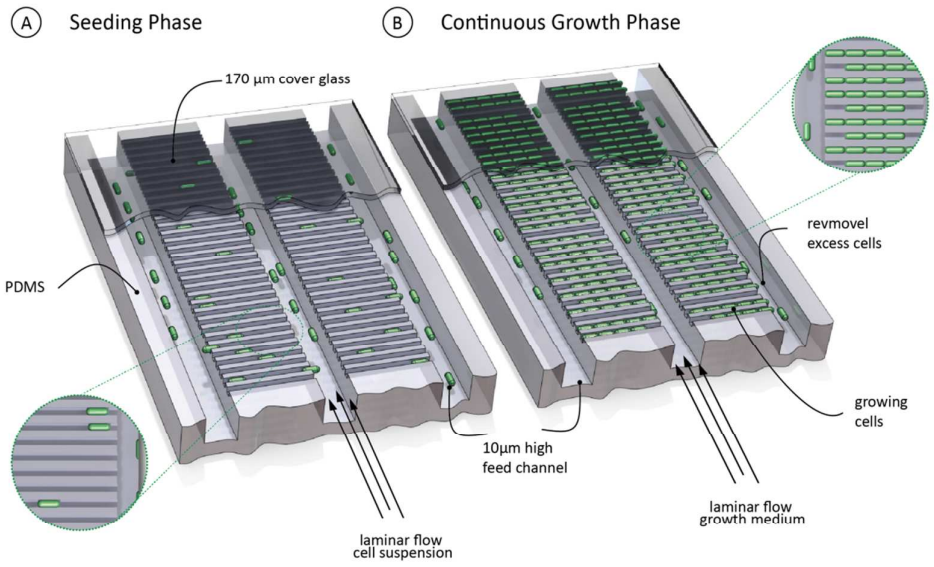
Supplementary Figure S1

Exogenous-Arg-Ala-dose response (red squares) and Ala-Ala-dose response (black circles) of *C. glutamicum* carrying pSenLys-Spc.



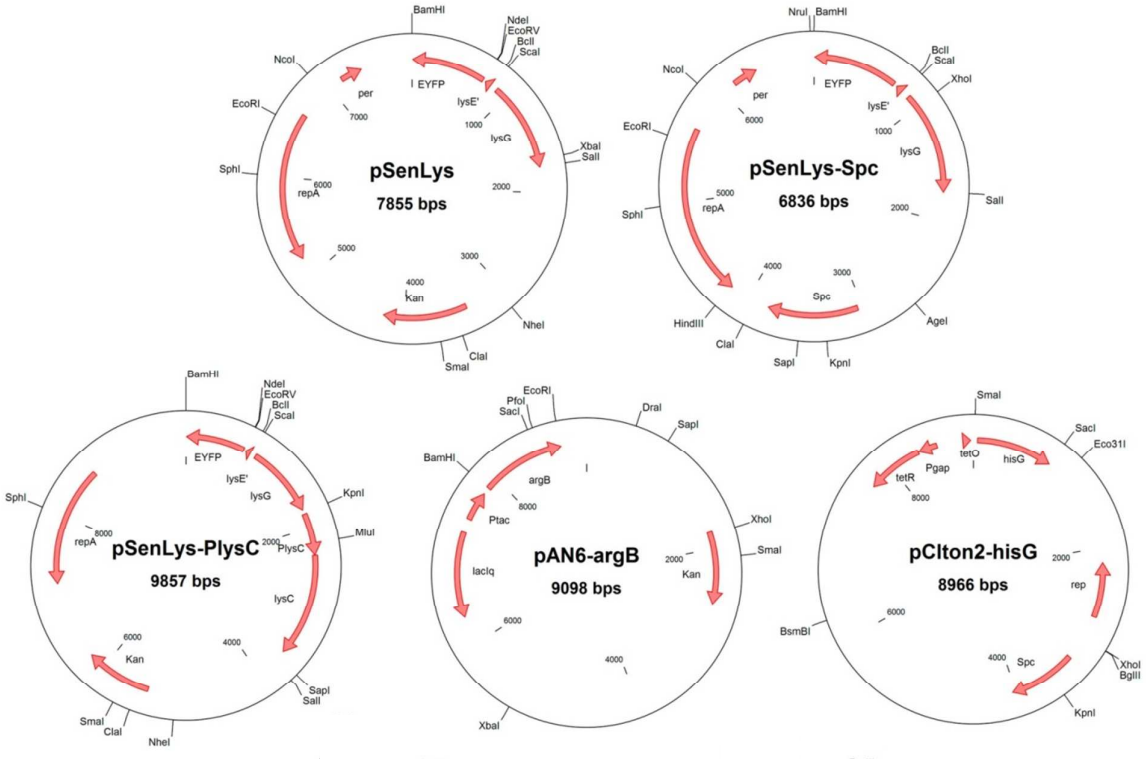
Supplementary Figure S2:

The microfluidic single cell chemostat used in this study is intended for long-term growth coupled metabolic studies. The device contains several hundred (1 µm x 1 µm x 20 µm) growth channels that allow for high-throughput single cell cultivation under environmental constant conditions. Using time-lapse-microscopy, growth coupled fluorescence studies on single cell level can be performed. In comparison to cultivation boxes, where only 8 to 9 generations can be followed (Grünberger *et al*., 2013), here growth can be studied over many generations (here over 30) at constant conditions. During the seeding phase (Figure 1A), single cells are trapped within the channels. After a sufficient amount of cells is trapped, continuous growth is initiated by constant supply of medium of interest (Figure 1B). During the experiment cells are growing and excess cells are pushed out of the channel system which are removed with the medium flow. This procedure can be maintained for up to several days, depending on organism and experimental aim.



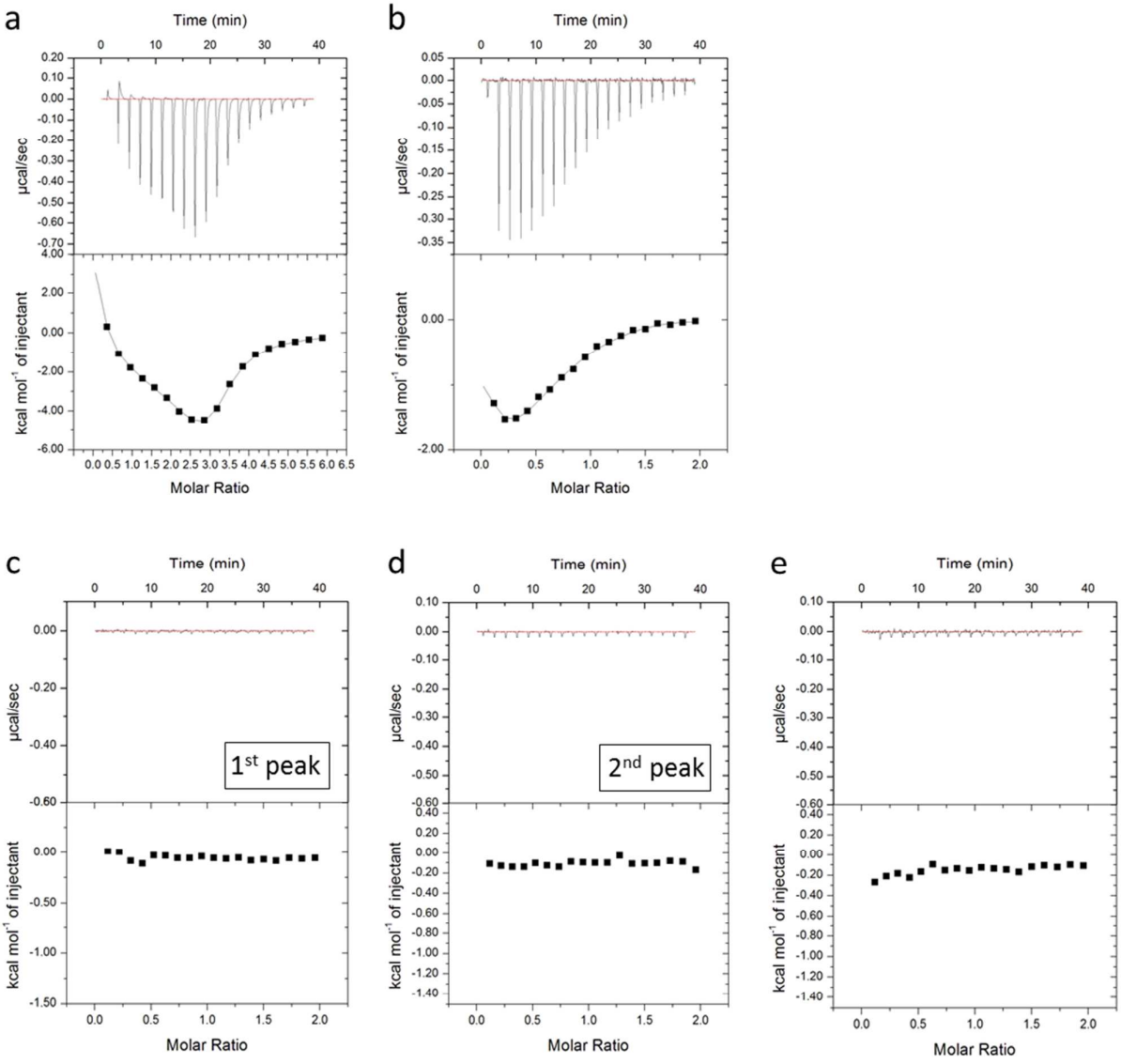
Supplementary Figure S3:

Plasmids constructed and used in this study.



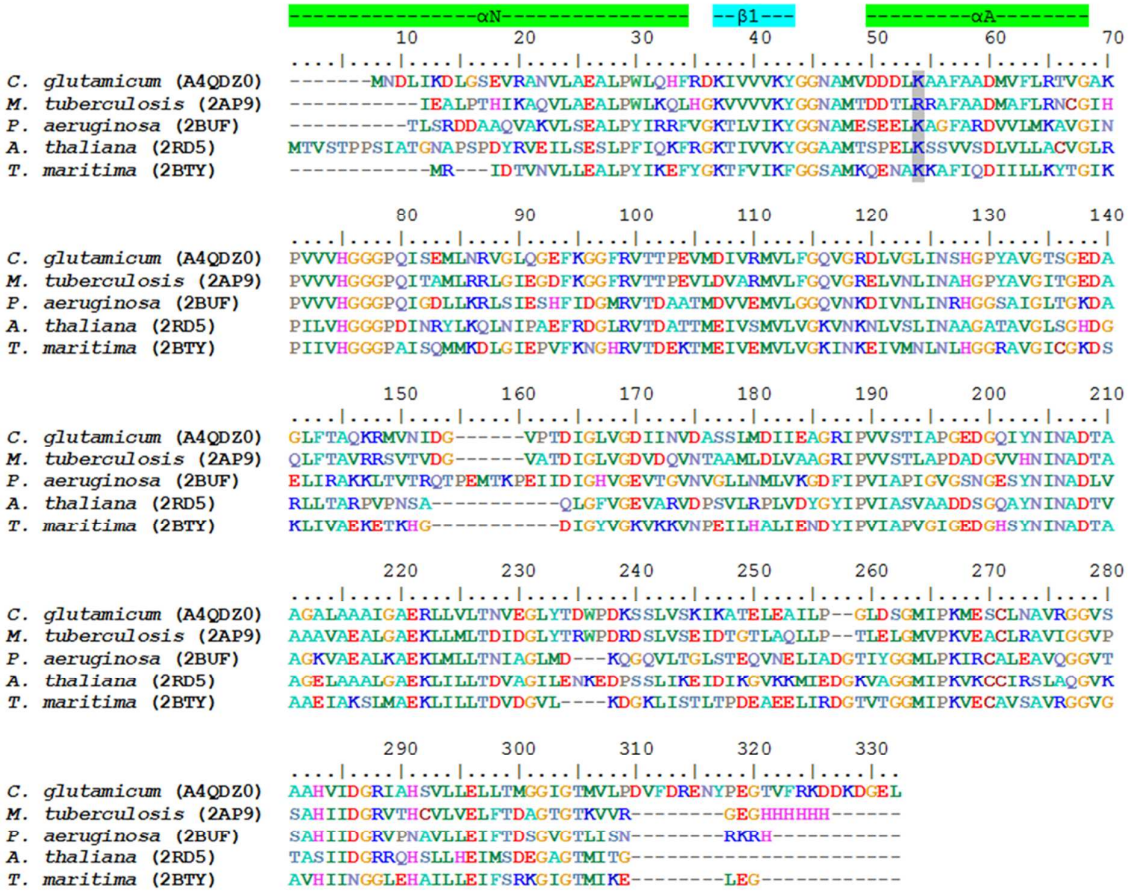
Supplementary Figure S4:

Results from isothermal titration calorimetry experiments with different ArgB proteins and L-arginine. (a) Wild-type ArgB; (b) ArgB-K47H; (c) ArgB-K47H-V65A, first peak from size exclusion chromatography (see Figure 5); (d) ArgB-K47H-V65A, second peak from size exclusion chromatography; (e) ArgB-K47H-G122T-T180P. Best fitting results for (a) and (b) were achieved with a sequential binding model and six binding sites using Origin 7G software. In case of (c), (d) and (e), no heat exchange was detectable.



Supplementary Figure S5:

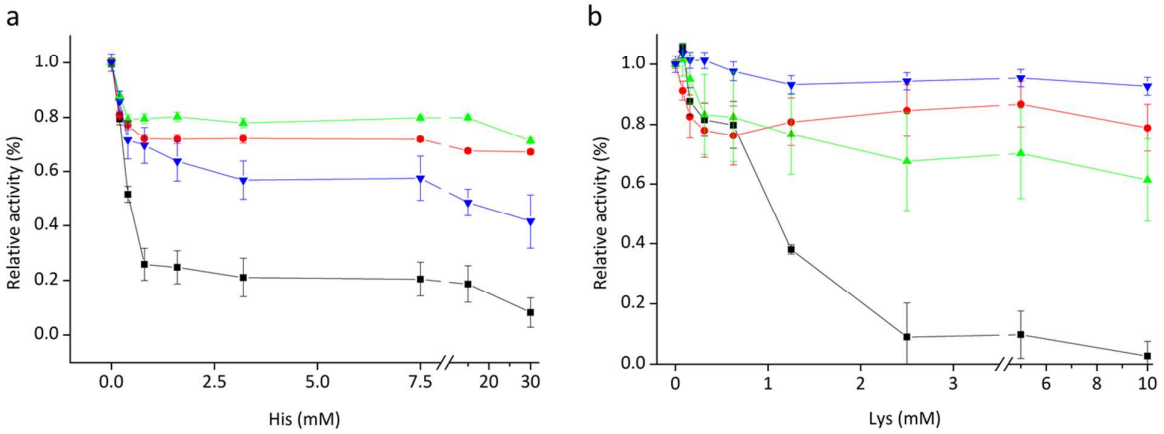
ClustalW alignment of ArgB from *C. glutamicum* (UniProt ID: A4QDZ0) with homologs of known structure such as ArgB from *M. tuberculosis* (PDB ID: 2AP9), *P. aeruginosa* (2BUF), *A. thaliana* (2RD5), and *T. maritima* (2BTY). Residues aligning with K47 from *C. glutamicum* are shaded in gray and are shown with highlighted helices αN and αA (green) as well as the β1 strand (cyan).



Supplementary Figure S6:

Inhibition kinetics of selected HisG and LysC muteins, obtained from activity assays with isolated protein. (a) Relative activity of HisG muteins in presence of L-histidine: black squares, wild-type enzyme; blue triangles, HisG-GT233HQ; red circles, HisG-N216I; green triangles, HisG-N216R. (b) Relative activity of LysC muteins in presence of L-lysine (plus 10 mM L-threonine): black squares, wild-type enzyme; green triangles, LysC-c88; red circles, LysC-c77; blue triangles, LysC-c90.

The sp A for the respective enzyme without allosteric regulator was as follows (in µmol min^{-1} mg^{-1}): HisG-WT=1.59±0.04; HisG-GT233HQ=0.61±0.03; HisG-N216I=1.68±0.04; HisG-N216R=1.56±0.04; LysC-WT=1.17±0.03; LysC-c88=1.40±0.03; LysC-c77=1.09±0.03; LysC-c90=2.29±0.13.



Supplementary Table S1:

Nucleotide and amino acids exchanges in the isolated ArgB muteins.

ArgB 14	ArgB 88	ArgB 79	ArgB 37	ArgB 39	ArgB 18	ArgB 59	Nucleotide exchange	Wild-type codon	Mutant codon	Mutation
x						x	a139g	aag	gag	K47E
					x		c176a	acc	aac	T59N
x							t194c	gtg	gcg	V65A
				x			a280t	acc	tcc	T94S
					x		a301g	att	gtt	I101V
	x	x					t329c	gtc	gcc	V110A
	x						g365c	ggc	gcc	G122A
				x			a430g	aac	gac	N144D
			x			x	a538g	acg	gcg	T180A
			x			x	t543c	att	atc	silent
	x						t566c	att	act	I189T
		x				x	t569g	tac	tgc	Y190C
			x			x	a591g	gca	gcg	silent
			x		x		c663t	acc	act	silent
					x		a674g	gat	ggt	D225G
x	x		x				c712t	ctg	ttg	silent
						x	t875a	gtg	gag	V292E

Supplementary Table S2:

Primer sets for site-specific saturation of *argB*.

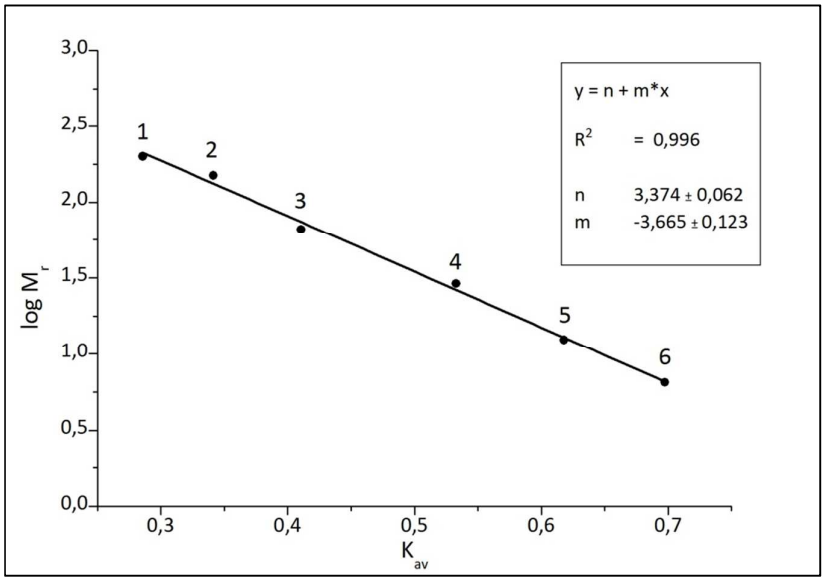
Residue	Saturated codon	Primer	Sequence
K47	AAG139NNK	fw	CAT GGT GGA TGA TGA TCT CNN KGC TGT TTT TGC TGC CGA C
		rv	GTC GGC AGC AAA AAC AGC MNN GAG ATC ATC ATC CAC CAT G
V65	GTG193NNK	fw	GGG CGC AAA ACC ANN KGT GGT GCA CGG TGG
		rv	CCA CCG TGC ACC ACM NNT GGT TTT GCG CCC
V110	GTC328NNK	fw	GGT GCT CTT TGG TCA GNN KGG TCG CGA TTT AGT TG
		rv	CAA CTA AAT CGC GAC CMN NCT GAC CAA AGA GCA CC
G122	GGC364NNK	fw	GTT TGA TCA ACT CTC ATN NKC CTT ACG CTG TGG GAA C
		rv	GTT CCC ACA GCG TAA GGM NNA TGA GAG TTG ATC AAA C
T180	ACG538NNK	fw	CAT TCC TGT GGT CTC TNN KAT TGC TCC AGG CGA AG
		rv	CTT CGC CTG GAG CAA TMN NAG AGA CCA CAG GAA TG
I189	ATT565NNK	fw	CAG GCG AAG ACG GCC AGN NKT ACA ACA TTA ACG CCG
		rv	CGG CGT TAA TGT TGT AMN NCT GGC CGT CTT CGC CTG
Y190	TAC568NNK	fw	GCG AAG ACG GCC AGA TTN NKA ACA TTA ACG CCG ATA C
		rv	GTA TCG GCG TTA ATG TTM NNA ATC TGG CCG TCT TCG C

Supplementary Table S3:
Mutations and L-arginine accumulation in culture supernatants for clones isolated from saturation libraries. From each library, 20 cells were sorted and 4 of them were selected for detailed analysis. In the case where only one allele for one position is given all 4 selected clones were identical.

Clone	Wildtype Codon	Mutant Codon	Mutation	Arg (mM)	+/-
argB47-b5	aag	gag	K47E	1.2	0.02
argB47-c4	aag	acg	K47T	3.5	0.19
argB47-c8	aag	cat	K47H	5.5	0.24
argB65-a8	gtg	gca	V65A	4.8	0.05
argB110-e4	gtc	cgt	V110R	1.4	0.20
argB122-a5	ggc	acg	G122T	11.5	0.25
argB180-a4	acg	cct	T180P	2.1	0.08
argB189-c11	att	nd	nd	0.0	0.00
argB190-g10	tac	nd	nd	0.0	0.00

Supplementary Table S4:
Elution volume of ArgB proteins separated by a Superdex 200 10/300 GL column (GE Healthcare) and calculated molecular masses.

ArgB protein	± 10 mM L-Arginine	Elution volume (ml)	Calculated molecular masses (kDa)
WT	-	12.26	231.52
WT	+	12.30	226.75
K47H	-	12.00	265.10
K47H	+	12.52	202.20
K47H-V65A	-	11.97; 7.59	269.28; ND
K47A-V65A	+	13.35; 7.56	131.22; ND
K47H-G122T-T180P	-	7.84	ND
K47H-G122T-T180P	+	12.34	222.07
K47H-V65A-T180P	-	11.95	272.10
K47H-V65A-T180P	+	12.17	242.64



Calibration of Superdex 10/300 GL column. Semilogarithmic plot of molecular weight (M_r) against K_{av} (V_0=7.8 ml; V_t=24 ml). Protein standards: 1, 200 kDa; 2, 150 kDa; 3, 66 kDa; 4, 29 kDa; 5, 12.4 kDa; 6, 6.5 kDa.

Supplementary Table S5:

Primer sets for site-specific saturation of *hisG*.

Residue in *M. tuber.*	**Equivalent residue in *C. glut.***	**Saturated codons in Cg-HisG sequence**	**Primer**	**Sequence**
D218	N216	AAC(643)NNK	fw	CTT CCT CAT GCT GGA TTA CNN KGT CGA CCG CGA CAA CCT G
			rv	CAG GTT GTC GCG GTC GAC MNN GTA ATC CAG CAT GAG GAA G
LE234	LS231	TTATCC(691)NNKNNK	fw	CCA CTG CAG TAA CCC CAG GCN NKN NKG GCC CAA CGG TAT CCC CAC
			rv	GTG GGG ATA CCG TTG GGC CMN NMN NGC CTG GGG TTA CTG CAG TGG
SPT236	GPT233	GGCCCAACG(697)NNKCCANNK	fw	GTA ACC CCA GGC TTA TCC NNK CCA NNK GTA TCC CCA CTG GCA CG
			rv	CGT GCC AGT GGG GAT ACM NNT GGM NNG GAT AAG CCT GGG GTT AC
L253	M250	ATG(748)NNK	fw	GTT GCT GTA CGC GCC NNK GTG CCA CGC AGG TCA G
			rv	CTG ACC TGC GTG GCA CMN NGG CGC GTA CAG CAA C
A273	A270	GCC(808)NNK	fw	CTG GAC TCG GCG CTG AAN NKA TCC TGG CTT CTG AAA TC
			rv	GAT TTC AGA AGC CAG GAT MNN TTC AGC GCC GAG TCC AG

Supplementary Table S6:

Nucleotide and amino acid exchanges in isolated *lysC* alleles.

Position	Mutation	Nucleotide exchange	Wild-type codon	Mutant codon
20	R20S	a60c	aga	agc
21	N21D	a61g	aac	gac
24	E24G	a71g	gaa	gga
69	L69F	c205t	ctc	ttc
78	N78S	a233g	aac	agc
123	E123E	a369g	gaa	gag
124	A124P	g370c	gca	cca
125	L125L	c375a	ctc	cta
141	K141K	a423g	aaa	aag
159	V159G	t476g	gtt	ggt
186	I186V	a556g	atc	gtc
211	I211V	a631g	att	gtt
227	L227P	t680c	ctt	cct
235	N235D	a703g	aat	gat
278	E278G	a833g	gag	ggg
280	A280V	c839t	gcg	gtg
311	T311I	c932t	acc	atc
328	K328E	a982g	aag	gag
337	N337S	a1010g	aat	agt
341	D341N	g1021a	gac	aac
350	V350A	t1049c	gtg	gcg
365	M365K	t1094a	atg	aag
391	E391G	a1172g	gaa	gga
394	L394L	c1180t	ctg	ttg
399	R399L	g1196t	cgt	ctt

Supplementary Table S7:

Nucleotide and amino acid exchanges in isolated *hisG* alleles.

Mutein	Mutation	Nucleotide exchange	Mutation	Nucleotide exchange
HisG-WT				
HisG-GT233-3	G233H	ggc742cat	T235Q	acg748cag
HisG-GT233-8	G233S	ggc742agt	T235R	acg748agg
HisG-GT233-4	G233V	ggc742gtg	T235R	acg748cgt
HisG-GT233-5	G233I	ggc742att	T235V	acg748gtt
HisG-GT233-1	G233R	ggc742cgg	T235R	acg748agg
HisG-GT233-10	G233L	ggc742ctt		
HisG-GT233-6	G233H	ggc742cat	T235F	acg748ttt
HisG-GT233-11	G233P	ggc742cct	T235L	acg748ctg
HisG-GT233-7	G233R	ggc742cgt		
HisG-GT233-2	G233S	ggc742tcg	T235H	acg748agg
HisG-N216-1	N216I	aac688att		
HisG-N216-3	N216R	aac688cgt		