Supplementary Tables

Table S1. Families of highly differentially expressed genes (and GO significant enrichment categories) during the acclimation response.

Target function/Pathway (GO enrichment category)	**Gene**	**Products/putative cellular functions**	**$\log_2$fold change[1]**
DOWN-REGULATED GENES			
Flagellum	*flgA*	Flagellar biosynthesis; hook-filament junction protein	-5.4
(Bacterial-type flagellum-dependent cell	*flgB*	Flagellar basal-body rod protein FlgB	-6.8
motility GO:0071973)	*flgC*	Flagellar basal-body rod protein FlgC	-7.4
	flgD	Flagellar biosynthesis	-7.2
	flgE	Flagellar hook protein FlgE	-7.3
	flgF	Flagellar basal-body rod protein FlgF	-7.2
	flgG	Flagellar basal-body rod protein FlgG	-7.3
	flgH	Flagellar L-ring protein FlgH	-6.9
	flgI	Flagellar P-ring protein FlgI	-6.3
	flgJ	Flagellar protein FlgJ	-5.7
	flgK	Flagellar biosynthesis; hook-filament junction protein	-4.8
	flgL	Flagellar biosynthesis; hook-filament junction protein	-3.9
	flgN	Flagellar biosynthesis protein FlgN	-3.8
	flhA	Flagellar biosynthesis protein FlhA	-5.6
	flhB	Flagellar biosynthesis protein FlhB	-6.2
	flhE	Flagellar protein	-6.6
Ribosomal proteins	*rplA*	50S ribosomal subunit protein L1	-4.0
(Structural constituent of ribosomes	*rplB*	50S ribosomal subunit protein L2	-4.4
GO:0003735)	*rplC*	50S ribosomal subunit protein L3	-4.8
	rplD	50S ribosomal subunit protein L4	-4.8
	rplE	50S ribosomal subunit protein L5	-3.4
	rplF	50S ribosomal subunit protein L6	-3.3
	rplI	50S ribosomal subunit protein L9	-4.8
	rplJ	50S ribosomal subunit protein L10	-3.6
	rplK	50S ribosomal subunit protein L11	-3.8
	rplL	50S ribosomal subunit protein L12	-3.6
	rplM	50S ribosomal subunit protein L13	-3.4

rplN	50S ribosomal subunit protein L14	-2.8
rplO	50S ribosomal subunit protein L15	-2.9
rplP	50S ribosomal subunit protein L16	-3.5
rplQ	50S ribosomal subunit protein L17	-3.4
rplR	50S ribosomal subunit protein L18	-3.2
rplS	50S ribosomal subunit protein L19	-3.4
rplT	50S ribosomal subunit protein L20	-3.2
rplU	50S ribosomal subunit protein L21	-2.6
rplV	50S ribosomal subunit protein L22	-4.0
rplW	50S ribosomal subunit protein L23	-4.0
rplX	50S ribosomal subunit protein L24	-3.4
rplY	50S ribosomal subunit protein L25	-2.9
rpmA	50S ribosomal subunit protein L27	-2.6
rpmB	50S ribosomal subunit protein L28	-3.2
rpmC	50S ribosomal subunit protein L29	-2.9
rpmD	50S ribosomal subunit protein L30	-3.2
rpmE	50S ribosomal subunit protein L31	-3.2
rpmF	50S ribosomal subunit protein L32	-2.3
rpmG	50S ribosomal subunit protein L33	-2.9
rpmH	50S ribosomal subunit protein L34	-3.5
rpmI	50S ribosomal subunit protein L35	-1.5
rpmJ	50S ribosomal subunit protein L36	-2.9
rpsA	30S ribosomal subunit protein S1	-2.8
rpsB	30S ribosomal subunit protein S2	-2.5
rpsC	30S ribosomal subunit protein S3	-3.7
rpsD	30S ribosomal subunit protein S4	-2.9
rpsE	30S ribosomal subunit protein S5	-2.7
rpsF	30S ribosomal subunit protein S6	-4.0
rpsG	30S ribosomal subunit protein S7	-3.0
rpsH	30S ribosomal subunit protein S8	-2.9
rpsI	30S ribosomal subunit protein S9	-4.3
rpsJ	30S ribosomal subunit protein S10	-4.2
rpsK	30S ribosomal subunit protein S11	-3.7
rpsL	30S ribosomal subunit protein S12	-3.7
rpsM	30S ribosomal subunit protein S13	-2.6
rpsN	30S ribosomal subunit protein S14	-2.6
rpsO	30S ribosomal subunit protein S15	-2.4

	rpsP	30S ribosomal subunit protein S16	-3.1
	rpsQ	30S ribosomal subunit protein S17	-3.1
	rpsR	30S ribosomal subunit protein S18	-4.2
	rpsS	30S ribosomal subunit protein S19	-3.7
	rpsT	30S ribosomal subunit protein S20	-3.0
	rpsU	30S ribosomal subunit protein S21	-3.4
Methionine biosynthesis (Sulfur amino acid biosynthetic process GO:0000097)	*metA*	homoserine O-succinyltransferase	-3.4
	metB	O-succinylhomoserine lyase	-0.9
	metC	Cystathionine β-lyase	-1.8
	metE	Cobalamin-independent homocysteine transnethylase	-5.3
	metF	5,10-methylenetetrahydrofolate reductase	-3.5
	metG	methionyl-tRNA synthetase	-2.0
	metH	cobalamin-dependent methionine synthetase	-1.1
	metI	L/D-methionine ABC transporter membrane subunit	-2.2
	metJ	MetJ transcriptional repressor	-1.2
	metK	S-adenosylmethionine synthetase	-3.9
	metL	Aspartate kinase	-1.8
	metN	L/D-methionine ABC transporter ATP binding subunit	-3.3
	metQ	L/D-methionine ABC transporter periplasmic binding subunit	-2.6
	metR	MetR DNA-binding transcriptional dual regulator	-2.0
Pathway for *de novo* biosynthesis of purine nucleosides (purine nucleotide biosynthetic process; GO:0006164; P=2.68*10^{-9})	*purB*	Adenylosuccinate lyase	-2.3
	purC	PurC	-3.0
	purD	Phosphoribosylamine-glycine ligase	-2.6
	purE	PurE	-3.5
	purF	PurF	-2.5
	purH	AICAR transformylase/ IMP cyclohydrolase	-3.1
	purK	N5-carboxyaminoimidazole ribonucleotide synthetase monomer	-3.0
	purL	Phosphoribosylformylglycinamide synthetase	-2.7
	purM	PurM	-3.5
	purN	Phosphoribosylglycinamide formyltransferase 1	-2.6
	purR	PurR	-2.8
	purT	Phosphoribosylglycinamide formyltransferase 2	-2.9
Pathway for *de novo* biosynthesis of pyrimidine nucleosides (pyrimidine nucleotide biosynthetic process; GO:0006221; P=2.08*10^{-2})	*pyrB*	Aspartate carbamoyltransferase, catalytic subunit	-3.1
	pyrC	PyrC	-1.9
	pyrD	Dihydroorotate dehydrogenase, type 2	-3.1
	pyrE	Orotate phosphoribosyltransferase (PyrE)	-3.3
	pyrF	Orotidine-5'-phosphate-decarboxylase (PyrF)	-3.5

	pyrI	Aspartate carbamoyltransferase, PyrI subunit	-2.7
Up-regulated genes			
Arginine degradation pathway (Arginine catabolic process; GO:0006527)	*astA*	Arginine succinyltransferase (AstA)	4.8
	astB	Succinylarginine dihydrolase (AstB)	4.3
	astC	Succinylornithine transaminase (AstC)	4.7
	astD	Succinylglutamate semialdehyde dehydrogenase	4.2
	astE	Succinylglutamate desuccinylase	4.6
Alcohol catabolic process (GO:0046164)	*mhpA*	3-(3-hydroxyphenyl)propionate 2-hydroxylase	1.2
	mhpB	2-hydroxy-6-ketonona-2,4-dienedioate hydrolase	1.2
	glcD	glycolate oxidase, predicted FAD-linked subunit	1.1
	glcE	glycolate oxidase, predicted FAD-binding subunit	1.4
	glcF	glycolate oxidase, predicted iron-sulfur subunit	2.1
	glxK	tartronate semialdehyde reductase 2	1.8
	eutC	ethanolamine ammonia-lyase, β subunit	1.0
	eutD	phosphate acetyltransferase monomer	1.4
	eutP	conserved protein with nucleoside triphosphate hydrolase domain	2.3
	eutQ	conserved protein	1.4
	eutT	predicted cobalamine adenosyltransferase in ethanolamine utilization	1.6
Glycerol catabolic process (GO:0019563; $P=5.19*10^{-2}$)	*glpA*	anaerobic glycerol-3-phosphate dehydrogenase subunit A	3.0
	glpB	anaerobic glycerol-3-phosphate dehydrogenase subunit B	3.4
	glpC	anaerobic glycerol-3-phosphate dehydrogenase subunit C	3.3
	glpD	glycerol-3-phosphate dehydrogenase, aerobic	2.1
	glpK	glycerol kinase	1.7
	glpQ	glycerophosphoryl diester phosphodiesterase, periplasmic	2.7
	glpT	glycerol-3-phosphate:phosphate antiporter	2.9
	glpX	fructose 1,6-bisphosphatase II	1.1
Monosaccharide transport (GO:0015749)	*araF*	arabinose ABC transporter - periplasmic binding protein	4.2
	araG	arabinose ABC transporter - ATP binding subunit	2.8
	araH	arabinose ABC transporter - membrane subunit	2.0
	xylF	xylose ABC transporter - periplasmic binding protein	4.1
	xylH	xylose ABC transporter - membrane subunit	1.3

[1] The logarithm (to basis 2) of the fold change

Table S2. List of i) previously reported up-regulate genes during the heat stress response, ii) previously reported up-regulated genes during the general stress response and, iii) RNAP genes, with their associated gene expression changes observed in our study.

Gene	**Protein/Product (Function)**	**Counts 37ºC** [1]	**Counts 42ºC** [1]	**$\log_2$fold change** [2]	***q*** [3]
Heat shock genes [4]					
rpoH	RpoH σ32 Sigma factor controlling the heat shock response	16804	15018	-0.2	0.563
clpA	ClpA	10115	25502	1.3	1.59E-10
clpB (htpM)	ClpB (Hsp100)	29641	16864	-0.8	0.003
clpP	ClpP (protease)	15448	13925	-0.1	0.497
clpX	ClpX (protease)	42812	29679	-0.5	0.014
creB	CreB (catabolic response regulator)	2802	2362	-0.2	0.367
cspD	CspD (cold shock protein)	10123	68262	2.8	2.83E-07
dnaJ	DnaJ (Hsp40)	26836	4420	-2.6	2.71E-11
dnaK	DnaK (Hsp70)	139053	27492	-2.3	2.17E-08
fkpA	FkpA (heat shock peptidyl-prolyl isomerase)	60517	21956	-1.5	1.17E-11
fxsA (yjeG)	FxsA overproduction inhibits F exclusion of bacteriophage T7	5116	3624	-0.5	0.113
gapA	GapA, glyceraldehyde-3-phosphate dehydrogenase	485230	63805	-2.9	1.17E-33
groEL	GroEL (Hsp60)	288824	128432	-1.2	3.44E-04
groES	GroES (Hsp10)	58890	32344	-0.9	0.006
grpE	GrpE	26524	8890	-1.6	7.96E-07
hflB (ftsh)	HflB (ATP-dependent protease)	125903	45546	-1.5	5.75E-10
hflX	HflX (protease)	24434	31466	0.4	0.146
hslO (yrfI)	Hsp33	10684	4626	-1.2	7.60E-06
hslR (yrfH)	YrfH (hsp15)	3374	1949	-0.8	0.006
hslU	HslU (protease)	33809	10710	-1.7	1.02E-05
hslV	HslV (protease)	7534	2391	-1.7	1.33E-05
htpG	HtpG (Hsp90)	56543	8294	-2.8	2.20E-12
htpX	HtpX (membrane protein)	31843	12157	-1.4	1.62E-08
ibpA (hslT)	IbpA (sHsp)	2234	781	-1.5	4.93E-04
ibpB (hslS, htpE)	IbpB (sHsp)	354	797	1.2	9.57E-06
lnt (cutE)	CutE (catalyses the last step in lipoprotein maturation)	6132	2496	-1.3	1.78E-04
lon	Lon (protease)	2256	1763	-0.4	0.159
macB (ybjZ)	YbjZ (putative component transport system)	4918	4028	-0.3	0.293
miaA (trpX)	MiaA (tRNA-transferase)	29510	32309	0.1	0.737

mutM	MutM (DNA glycosylase)	2028	511	-2.0	9.53E-15
narP	NarP (nitrate response regulator)	1330	1335	0.0	1.000
phoB	PhoB (DNA-binding response regulator)	1281	2129	0.7	0.002
phoR	PhoR (histidine kinase)	1045	1520	0.5	0.023
prlC (opdA)	PrlC (peptidase)	19266	16612	-0.2	0.343
rpoD	RpoD σ70	43196	34605	-0.3	0.120
rrmJ (ftsJ)	FtsJ (cell division)	17556	13517	-0.4	0.101
sdaC	SdaC (serine transporter)	8079	1651	-2.3	1.46E-11
topA	TopA (topoisomerase)	65053	35112	-0.9	1.02E-04
yafD	YafD	5302	4670	-0.2	0.539
yafE	YafE (putative biotin synthesis)	701	857	0.3	0.238
ybbN	YbbN (putative thioredoxin like)	13229	5801	-1.2	1.96E-07
ybeY	YbeY (metal binding, required for translation at 42ºC)	5423	4127	-0.4	0.098
ybeZ	YbeZ (pho regulon protein)	13009	12443	-0.1	0.727
yccV (hspQ)	HspQ (Hsp, hemimethylated DNA-binding protein)	5380	3375	-0.7	0.007
ycjF	YcjF (putative membrane protein)	2863	1442	-1.0	5.93E-04
yhdN	YhdN (DUF1992 family protein)	992	879	-0.2	0.587
yheL	YheL (required for tRNA synthesis)	1914	1271	-0.6	0.033
yhgH	YhgH (protein required for utilization of DNA as carbon source)	2677	1323	-1.0	2.08E-05
yrfG	YrfG (phosphatase)	3796	3472	-0.1	0.651
General stress genes [5]					
rpoS	RpoS σS Master regulator of the general stress response	17282	43645	1.3	0.010
aidB	Putative acyl-CoA dehydrogenase (flavoprotein)	702	5542	3.0	1.31E-11
artM	Arginine transport protein (ABC superfamily, membrane)	5208	5280	0.0	0.833
artP	Arginine transport protein (ABC superfamily, ATP-binding subunit)	4890	9687	1.0	1.50E-06
blc	Outer membrane lipoprotein (lipocalin)	542	3226	2.6	3.54E-13
bolA	Transcriptional activator of morphogenic pathway (BolA family)	4725	16371	1.8	1.25E-04
cbpA	Curved DNA-binding protein, cochaperone of DnaK (Hsp40 family)	1886	5203	1.5	1.84E-11
csiD	Conserved protein with clavaminate synthase-like domain	1403	19731	3.8	3.27E-06
dps	Stress response DNA-binding protein with ferritin-like domain	3598	30771	3.1	1.02E-16
fbaB	Frusctose-bisphosphate aldolase class I	1200	10070	3.1	1.65E-47
fic	Stationary-phase protein	3121	1386	-1.2	5.19E-06
gabD	Succinate-semialdehyde dehydrogenase NADP dependent	1892	17543	3.2	1.13E-19
gabP	Gamma-aminobutyrate transport protein	549	4498	3.0	9.23E-35
gadA	Glutamate decarboxylase, isozyme A	193	489	1.3	2.67E-06
gadB	Glutamate decarboxylase, isozyme B	227	934	2.0	5.85E-15
gadX	GadX DNA-binding transcriptional dual regulator	1262	10798	3.1	1.05E-16

hdeA	HdeA dimer, inactive form of acid-resistance protein	228	474	1.1	3.34E-04
hdeB	Acid stress chaperon	51	167	1.7	9.85E-05
hnr (rssB)	Response regulator involved in protein turnover	2187	6389	1.5	3.29E-13
katE	Catalase; hydroperoxidase HPII (III), RpoS dependent	1704	11430	2.7	3.36E-40
ldcC	Lysine decarboxylase 2, constitutive	1739	6305	1.9	4.67E-08
mscL	Mechanosensitive channel	3183	6212	1.0	0.060
msyB	Acidic protein suppresses mutants lacking function of protein export	1286	29382	4.5	2.10E-21
narU	Nitrate extrusion protein	215	325	0.6	0.140
osmB	Lipoprotein osmotically inducible	384	4641	3.6	1.13E-58
osmC	Resistance protein, osmotically inducible	980	8881	3.2	1.49E-50
osmY	Hyperosmotically inducible periplasmic protein	1281	5475	2.1	2.84E-22
otsA	Trehalose-6-phosphate synthase	1737	9214	2.4	1.87E-30
otsB	Trehalose-6-phosphate phosphatase, biosynthetic	614	5057	3.0	1.99E-33
pdhR	Transcriptional repressor for pyruvate dehydrogenase complex	6187	2713	-1.2	3.31E-05
poxB	Pyruvate dehydrogenase/oxidase: FAD and thiamine PPi -binding	1664	13702	3.0	4.54E-21
rpsV (sra)	30S ribosomal subunit protein S22	640	18433	4.8	8.55E-103
rssA (ychK)	Putative transmembrane protein	3027	5405	0.8	9.25E-05
talA	Transaldolase A	1723	8375	2.3	3.25E-27
tam	Trans-aconitate methyltransferase	399	2251	2.5	3.37E-27
tktB	Transketolase 2, thiamon binding, isozyme	2890	16746	2.5	1.21E-34
treA	Trehalase, periplasmic	771	10638	3.8	1.35E-58
treF	Trehalase, cytoplasmic	886	2712	1.6	1.75E-12
wrbA	Flavodoxin-like protein, trp repressor-binding protein	1801	21322	3.6	1.50E-54
xasA (gadC)	glutamic acid:4-aminobutyrate antiporter	534	1604	1.6	1.20E-11
ybiO	Putative transport protein, integral membrane location	514	1148	1.2	3.18E-06
ycgB	Conserved protein	1177	12009	3.4	8.52E-14
ycgZ	Predicted protein	173	1567	3.2	7.76E-38
yciF	Putative structural protein	74	1494	4.3	4.07E-43
yciG	Predicted protein	54	1158	4.4	1.84E-24
ydaM	diguanylate cyclase	1184	4562	1.9	1.26E-19
ydcS	Putative transport protein (ABC superfamily, periplasmic)	1042	22275	4.4	9.74E-08
ydgA	Conserved protein	20388	18034	-0.2	0.298
yedU (hchA)	glyoxalase III, Hsp31 molecular chaperone	169	187	0.2	0.513
ygaF	Enxyme: L-2-hydroxylutarate oxidase	1095	12110	3.5	7.71E-14
ygaM	Predicted protein	1016	9599	3.2	4.19E-52
ygaU	Predicted proten	1277	15746	3.6	3.96E-21
yggE	Conserved protein	3234	17585	2.4	6.11E-32

ygjG	Putrescine aminotransferase	1657	17038	3.4	1.03E-52
yhbo	Protein involved in stress response	196	567	1.5	7.55E-08
yhiD	Predicted Mg(2+) transport ATPase	47	51	0.1	0.838
yhiE (gadE)	Transcrptional regulator for gasABC operon	80	2963	5.2	2.74E-95
yhiO (uspB)	Universal stress protein B	627	3684	2.6	2.64E-06
yhiU	MdtEF-TolC multidrug efflux transport system	201	781	2.0	2.24E-13
yhiW (gadW)	GadW DNA-binding transcriptional dual regulator	273	1923	2.8	1.87E-27
yhjG	Predicted outer membrane biogenesis protein	1059	7251	2.8	4.31E-08
yiaG	Putative transcriptional regulator with DNA-binding domain	517	3103	2.6	4.35E-08
yjbJ	Predicted stress response protein	558	5164	3.2	2.38E-48
yjgB	Ahr aldehyde reductase, NADPH-dependent	440	3052	2.8	1.41E-17
ymgA	Protein involved in biofilm formation	58	1030	4.1	5.19E-49
ynhG	L,D-transpeptidase YnhG	1448	5360	1.9	1.30E-05
RNA polymerase genes and sigma factors					
rpoA	α subunit of the RNA polymerase	558792	56852	-3.3	2.56E-32
rpoB	ß subunit of the RNA polymerase	241709	71355	-1.8	8.21E-15
rpoC	ß' subunit of the RNA polymerase	255389	66283	-1.9	1.83E-17
rpoZ	ω subunit of the RNA polymerase	11084	4299	-1.4	3.73E-09
rpoD	RNA polymerase σ70	43196	34605	-0.3	0.120
rpoE	RNA polymerase σE	30888	23118	-0.4	0.095
rpoN	RNA polymerase σ54	21812	24851	0.2	0.540

[1] Mean normalized counts (sequence reads) from the indicated temperature

[2] The logarithm (to basis 2) of the fold change

[3] *q* value (adjusted by the Benjamini-Hochberg procedure)

[4] Heat shock genes (and synonyms) expressed in *E.coli* during transient up-shift in temperature (Riehle et al. 2003, Nonaka et al. 2006, Gunasekera et al. 2008).

[5] General stress response genes induced by σ^S (Weber et al. 2005).

Table S3. Estimation of the lag phase by interpolation of the growth rate for the first-step mutants and the ancestor.

Strain	**τ[1] 37ºC**	**τ[1] 42ºC**	***P*[2]**
Ancestor	0.0	1.0	0.247
SE	(0.0)	(0.6)	
I572F	0.2	2.4	**0.009**
SE	(0.2)	(0.4)	
P[3]	0.423	0.147	
I572L	0.0	2.5	0.072
SE	(0.03)	(0.7)	
P[3]	0.423	0.188	
I572N	0.5	2.5	0.136
SE	(0.4)	(0.9)	
P[3]	0.300	0.253	

[1] Duration of the effective lag phase with standard error in parenthesis.

[2] Significance value from a two-sample t-test. The null hypothesis is that the mean at 37ºC and the mean at 42ºC are equal.

[3] Significance value from a two-sample t-test. The null hypothesis is that the mean of the ancestor and the mean of the mutant are equal. Numbers in bold correspond to *P*-values <0.05.

Table S4. Criteria used to classify the genes differentially expressed during the acclimation and/or adaptive response.

Category	**Anc42 *vs* Anc37**	**Mut42 *vs* Anc42**	**Mut42 *vs* Anc37**
1) Restored	Significant ↑	Significant ↓	-
	Significant ↓	Significant ↑	-
2) Reinforced	Significant ↑	Significant ↑	Significant ↑
	Significant ↓	Significant ↓	Significant ↓
3) Unrestored	Significant ↑	Non-Significant	-
	Significant ↓	Non-Significant	-
4) Novel	Non-Significant	Significant ↑	Significant ↑
	Non-Significant	Significant ↓	Significant ↓

↑ Up-regulated genes

↓ Down-regulated genes

Significant: significantly differentially expressed gene ($q < 0.001$)

Non-Significant: not significantly differentially expressed gene ($q > 0.001$)

Table S5. Mutations and gene expression changes of the high temperature adapted clone 27.

Mutational event	**Gene**	**Product**	**Position**[1]	**Genetic effect**	**log2fold change**[2]		
					A37 vs A42	**A42 vs *I572L***	**A42 vs c27**
Point mutation *I250N*	*secF*	Polypeptide SecF involved in translocation (inner membrane)	398676	Amino acid substitution	**-1.5** ***	**1.2** ***	**1.4** ***
2,896 bp Large deletion From position 475,290 to position 478,186.	*ybaL*	Poplypeptide YbaL CPA2 transporter	473,629 - 475,305	Inactivation	-0.2	-0.0	**-3.0** ***
	fsr	Transporter: fosmidomycin efflux transporter	475,543 - 476,763	Deletion	**-1.2** ***	0.1	**NA** ***
	ushA	Enzyme: 5’ -deoxyribonucleotidase	476,981 - 478,633	Inactivation	-0.1	0.0	**-5.4** ***
71,416 bp Large deletion From position 547,700 to position 619,116.	*ybcR*	Polypeptide: DLP12 prophage	548,867 - 549,082	Deletion	**1.9** ***	**-1.8** ***	**NA** ***
	ybcS	Enzyme: DLP12 prophage; lysozyme	549,082 - 549,579	Deletion	**1.2** ***	-1.0 **	**NA** ***
	ybcT	Polypeptide: DLP12 prophage; predicted murein endopeptidase	549,576 - 550,037	Deletion	**-2.6** ***	**1.9** ***	**NA** ***
	ybcU	Polypeptide: putative lipoprotein	550,069 - 550,362	Deletion	**-4.0** ***	**2.3** ***	**NA** ***
	ECB_00510	Polypeptide: hypothetical protein	550,723 - 550,917	Deletion	0.3	-0.3	**NA** ***
	nohB	Polypeptide: DLP12 prophage; DNA packaging protein	551,306 - 551,881	Deletion	0.4	-0.3	**NA** ***
	ECB_00512	Polypeptide: putative tail component of prophage	551,841 - 554,657	Deletion	0.6 **	-0.5	**NA** ***
	ECB_00513	Polypeptide: orf	554,716 – 557,061	Deletion	0.4	0.0	**NA** ***
	ECB_00514	Polypeptide: orf	557,058 – 557,339	Deletion	0.8	0.0	**NA** ***
	ECB_00515	Polypeptide: orf	557,349 – 558,053	Deletion	1.1 **	-0.7	**NA** ***
	ECB_00516	Polypeptide: orf	558,064 – 558,357	Deletion	1.5 **	-0.5	**NA** ***
	ECB_00517	Polypeptide: orf	558,112 –	Deletion	1.3	-1.0	**NA**

		558,447		*		***
appY	Polypeptide: APPY DNA-binding transcriptional activator	559,033 – 559,782	Deletion	-0.5	1.2	**NA** ***
ompT	Enzyme: outer membrane protease VII	560,031 – 560,984	Deletion	**-1.6** ***	1.4 ***	**NA** ***
envY	Polypeptide: EnvY DNA-binding transcriptional activator	561,498 – 562,259	Deletion	0.5 *	-0.2	**NA** ***
ybcH	Polypeptide: predicted protein	562,442 – 563,332	Deletion	0.7 **	-0.4	**NA** ***
nfrA	Polypeptide: bacteriophage N4 receptor, outer membrane protein	563,333 – 566,305	Deletion	-0.6 **	-0.4	**NA** ***
yhhI-2	Enzyme: putative transposase	568,800 – 569,936	Deletion	0.1	1.0	**NA** ***
ECB_00526	Polypeptide: orf	570,615 – 570,776	Deletion	-0.0	1.1	**NA** ***
ECB_00527	Polypeptide: orf	570,901 – 571,191	Deletion	-0.3	1.1	**NA** ***
ECB_00528	Polypeptide: orf	571,188 – 571,448	Deletion	1.0	-0.1	**NA** ***
ECB_00529	Polypeptide: orf	571,481 – 572,038	Deletion	0.5	0.2	**NA** ***
ECB_00530	Polypeptide: orf	571,890 – 573,488	Deletion	-0.0	0.9	**NA** ***
cusS	Polypeptide: CusS sensory histidine kinase	574,225 – 575,673	Deletion	0.5 *	0.0	**NA** ***
cusR	Polypeptide: CusR	575,663 – 576,346	Deletion	0.5 **	-0.4	**NA** ***
cusC	Polypeptide: copper / silver efflux transport system – outer membrane porin	576,503 – 577,876	Deletion	0.7 ***	-0.4	**NA** ***
ylcC	Polypeptide: copper / silver efflux transport system – periplasmic binding protein and metallochaperone	578,034 – 578,366	Deletion	0.6 **	0.0	**NA** ***
cusB	Polypeptide: copper / silver efflux system – membrane fusion protein	578,382 – 579,605	Deletion	0.6 ***	-0.2	**NA** ***
cusA	Polypeptide: copper / silver efflux transport system – membrane subunit	579,617 – 582,760	Deletion	0.4 *	-0.1	**NA** ***

	pheP	Transporter: phenylalanine: H+ symporter PheP	582,862 – 584,238	Deletion	**-0.9** ***	0.0	**NA** ***
	ybdG	Polypeptide: mechanosensitive channel of miniconductance YbdG monomer	584,306 – 585,553	Deletion	**-1.4** ***	0.5	**NA** ***
	nfnB	Enzyme: NfsB monomer	585,661 – 586,314	Deletion	**-2.0** ***	0.8 *	**NA** ***
	ybdF	Polypeptide: conserved protein	586,408 – 586,776	Deletion	0.2	0.0	**NA** ***
	ybdJ	Polypeptide: predicted inner membrane protein	586,841 – 587,089	Deletion	0.2	-0.3	**NA** ***
	ybdK	Polypeptide: carboxylate-amine ligase	587,155 – 588,273	Deletion	**3.7** ***	**-1.5** ***	**NA** ***
	insB-7	Polypeptide: IS1 protein InsB	589,570 – 590,073	Deletion	0.4	0.2	**NA** ***
	insA-7	Polypeptide: IS1 protein InsA	589,992 – 590,267	Deletion	0.1	0.0	**NA** ***
	hokE	Polypeptide: HokE	590,543 – 590,695	Deletion	1.1 *	-0.5	**NA** ***
	insL-2	Polypeptide: IS186/IS421 transposase	590,772 – 591,884	Deletion	-0.1	0.8	**NA** ***
	entD	Enzyme: phosphopantetheinyl transferase	592,166 – 592,795	Deletion	-0.2	0.6	**NA** ***
	fepA	Polypeptide: ferric enterobactin / colicin B / colicin D outer membrane porin FepA	592,961 – 595,201	Deletion	**-0.8** ***	1.0 *	**NA** ***
	fes	Enzyme: enterochelin esterase	595,523 – 596,647	Deletion	0.9	0.0	**NA** ***
	ybdZ	Polypeptide: MbtH-like protein that enhances the catalytic function of EntF	596,650 – 596,868	Deletion	0.8	0.2	**NA** ***
	entF	Polypeptide: apo-serine activating enzyme	596,865 – 600,746	Deletion	0.7 *	0.1	**NA** ***
	fepE	Polypeptide: ferric enterobactin (enterochelin) transport	600,962 – 602,095	Deletion	0.3	-0.2	**NA** ***
	fepC	Polypeptide: ferric enterobactin ABC transporter – ATP binding subunit	602,092 – 602,907	Deletion	**-0.7** ***	0.5	**NA** ***
	fepG	Polypeptide: ferric enterobactin ABC transporter – membrane subunit	602,904 – 603,896	Deletion	**-0.7** ***	0.3	**NA** ***

	fepD	Polypeptide: ferric enterobactin ABC transporter – membrane subunit	603,893 – 604,897	Deletion	-0.7 **	0.2	**NA** ***
	ybdA	Transporter: enterobactin efflux transporter EntS	605,008 – 606,258	Deletion	**-2.0** ***	**1.3** ***	**NA** ***
	fepB	Polypeptide: ferric enterobactin ABC transporter – periplasmic binding protein	606,262 – 607,218	Deletion	-0.5	0.7	**NA** ***
	entC	Enzyme: isochorismate synthase 1	607,593 – 608,768	Deletion	0.0	1.1	**NA** ***
	entE	Enzyme: EntE	608,778 – 610,388	Deletion	0.4	0.8	**NA** ***
	entB	Polypeptide: EntB monomer	610,402 – 611,259	Deletion	0.6 *	0.7	**NA** ***
	entA	Enzyme: EntA	611,259 – 612,005	Deletion	0.7 **	0.6	**NA** ***
	ybdB	Enzyme: proofreading thioesterase in enterobactin biosynthesis	612,008 – 612,421	Deletion	0.5	0.5	**NA** ***
	ybdD	Polypeptide: conserved protein	614,879 – 615,076	Deletion	**1.6** ***	-1.0	**NA** ***
	ybdH	Polypeptide: predicted oxidoreductase	615,086 – 616,174	Deletion	**-0.8** ***	-0.6	**NA** ***
	ybdL	Enzyme: methionine-oxo-acid transaminase, PLP-dependent	616,283 – 617,443	Deletion	**-2.1** ***	0.3	**NA** ***
	ybdM	Polypeptide: conserved protein	617,444 – 618,073	Deletion	-0.4	-0.1	**NA** ***
	ybdN	Polypeptide: orf	618,046 – 619,107	Deletion	-0.1	0.1	**NA** ***
Point mutation *E271G*	*mrdB*	Polypeptide: rod shape-determining membrane protein	648086	Amino acid substitution	-0.7 **	0.3	0.9 **
Point mutation *H30R*	*ydaE*	Polypeptide: Rac prophage; zinc-binding protein	1,415,090	Amino acid substitution	-0.0	1.3	**NA** ***
Point mutation *A35E*	*ECB_02828*	Transporter: Polysialic acid transporter protein kpsM	3,028,366	Amino acid substitution	**-1.4** ***	0.6	0.9 **
Point mutation *D2E*	*glpE*	Enzyme: thiosulfate sulfurtransferase	3,490,427	Amino acid substitution	**-0.7** ***	0.7	**1.8** ***
Point mutation *I572L*	*rpoB*	Enzyme: DNA-directed RNA polymerase subunit beta	4,162,571	Amino acid substitution	**-1.5** ***	0.4	**1.4** ***

[1] Position relative to the genome of *E.coli* B REL606

[2] The logarithm (to basis 2) of the fold change. The asterisks represent the significance (*q*), with one asterisk denoting significance at $q < 0.05$, two asterisks denoting significance at $0.001 < q < 0.01$, and three asterisks denoting significance at $q < 0.001$. Bold values represent a significant differentiation of genes with red values representing up-regulation of genes and blue values representing down-regulation of genes.

Table S6. Mutations and gene expression changes of the high temperature adapted clone 97.

Mutational event	**Gene**	**Product**	**Position**[1]	**Genetic effect**	**log2fold change**[2] **A37 vs A42**	 **A42 vs *I572L***	 **A42 vs c97**
IS Insertion IS1	*ykgE*	Polypeptide: predicted oxidoreductase	294,445	Inactivation?	0.7 *	**-1.4** ***	**-1.1** ***
Point mutation *Q526P*	*dnaX*	Enzyme: DNA polymerase III subunits gamma and tau	465,735	Amino acid substitution	**-1.1** ***	**1.1** ***	**1.3** ***
Point mutation *V461G*	*ybaL*	Polypeptide: YbaL CPA2 transporter	473,924	Amino acid substitution	-0.2	-0.1	0.2
Point mutation *R69H*	*mrdB*	Polypeptide: rod shape-determining membrane protein	648,692	Amino acid substitution	-0.7 **	0.6	0.8 **
Point mutation *D96N*	*dinG*	Polypeptide: ATP-dependent helicase	825,980	Amino acid substitution	0.2	-0.2	-0.1
Deletion of 4 bp	*glpT*	Transporter: glycerol-3-phosphate antiporter	2,298,766	Inactivation?	**1.4** ***	-1.6 **	**-3.0** ***
Point mutation *L30Q*	*ECB_02828*	Transporter: Polysialic acid transport protein kpsM	3,028,381	Amino acid substitution	**-1.4** ***	**2.2** ***	**1.0** ***
Point mutation *I572L*	*rpoB*	Enzyme: DNA-directed RNA polymerase subunit beta	4,162,571	Amino acid substitution	**-1.5** ***	0.9	**1.5** ***
Point mutation *A45V*	*iclR*	Polypeptide: IclR transcriptional repressor	4,202,435	Amino acid substitution	-0.3	0.1	**3.3** ***

[1] Position relative to the genome of *E.coli* B REL606

[2] The logarithm (to basis 2) of the fold change. The asterisks represent the significance (*q*), with one asterisk denoting significance at $q < 0.05$, two asterisks denoting significance at $0.001 < q < 0.01$, and three asterisks denoting significance at $q < 0.001$. Bold values represent a significant differentiation of genes with red values representing up-regulation of genes and blue values representing down-regulation of genes.

Table S7. Estimation of the lag phase by interpolating the growth rate for the high-temperature adapted clones 27 and 97 at 42ºC compared to the mutant *I572L* and the ancestor at 37ºC and 42ºC (values in table 1).

Strain	τ [1]
27	0.7 (0.2)
97	1.1 (0.3)
Comparison	**Significance** [2]
27 *vs* ancestor at 42ºC	0.632
97 *vs* ancestor at 42ºC	0.949
27 *vs I572L* at 42ºC	0.109
97 *vs I572L* at 42ºC	0.165
27 *vs* ancestor at 37ºC	0.093
97 *vs* ancestor at 37ºC	0.083

[1] Duration of the effective lag phase with standard error in parenthesis.

[2] Significance value from a two-sample t-test. The null hypothesis is that the means are equal.

Table S8. Gene expression changes of the flagellum genes.

Gene	**Products/putative cellular functions**	**log$_2$fold change Anc@42 *vs* Anc@37**[1]	**log$_2$fold change *I572L*@42 *vs* Anc@42**	**log$_2$fold change c97@42 *vs* Anc@42**
flgA	Flagellar biosynthesis; hook-filament junction protein	-5.4	3.3	-0.8
flgB	Flagellar basal-body rod protein FlgB	-6.8	4.5	-0.9
flgC	Flagellar basal-body rod protein FlgC	-7.4	4.7	-0.9
flgD	Flagellar biosynthesis	-7.2	4.3	-1.0
flgE	Flagellar hook protein FlgE	-7.3	4.0	-1.1
flgF	Flagellar basal-body rod protein FlgF	-7.2	3.6	-1.2
flgG	Flagellar basal-body rod protein FlgG	-7.3	3.5	-1.4
flgH	Flagellar L-ring protein FlgH	-6.9	3.3	-1.5
flgI	Flagellar P-ring protein FlgI	-6.3	2.5	-1.3
flgJ	Flagellar protein FlgJ	-5.7	2.1	-1.3
flgK	Flagellar biosynthesis; hook-filament junction protein	-4.8	1.1	-1.0
flgL	Flagellar biosynthesis; hook-filament junction protein	-3.9	0.3	-1.0
flgN	Flagellar biosynthesis protein FlgN	-3.8	1.8	-0.6

flhA	Flagellar biosynthesis protein FlhA	-5.6	2.6	-0.9
flhB	Flagellar biosynthesis protein FlhB	-6.2	3.4	-1.1
flhE	Flagellar protein	-6.6	3.2	-1.1

Table S9. RNAseq libraries' read counts.

Strain	**Library**	**Total Reads**	**Unique Map Reads**	**To coding DNA sequences (CDS)**
Ancestor at 37ºC	Anc37_1	61,887,699	58,604,582 (95%)	51,434,341 (88%)
	Anc37_2	58,303,507	54,387,815 (93%)	47,723,224 (88%)
	Anc37_3	55,074,809	52,130,655 (95%)	45,809,395 (88%)
Ancestor at 42ºC	Anc42_1	69,151,100	59,635,514 (86%)	49,234,852 (83%)
	Anc42_2	69,013,539	25,551,283 (37%)	20,379,870 (80%)
I572F	I572F_1	46,551,706	44,869,857 (96%)	39,268,167 (86%)
	I572F_2	44,953,035	42,756,199 (95%)	37,243,643 (87%)
I572L	I572L_1	58,193,448	55,309,904 (95%)	48,045,329 (87%)
	I572L_2	29,751,293	27,853,863 (94%)	23,711602 (85%)
I572N	I572N_1	17,203,921	16,373,928 (95%)	14,110,467 (86%)
	I572N_2	19,905,024	18,403,051 (92%)	15,872,019 (86%)
I966S	I966S_1	46,037,970	44,039,452 (96%)	38,586,863 (88%)
	I966S_2	43,613,720	41,586,753 (95%)	36,333,792 (87%)
27	27_1	33,865,380	27,564,479 (81%)	26,970,464 (98%)
	27_2	28,775,192	26,937,439 (94%)	23,722,802 (88%)
97	97_1	46,683,449	44,373,456 (95%)	38,866,962 (88%)
	97_2	28,525,253	26,787,880 (94%)	23,179,619 (87%)

Table S10. Primers used in this study.

Name	**Function**	**Sequence**
ARV19	Amplify the CAT:tetR:YFP genomic cassette (forward primer)	5’- CGAAACATCCGGCAATAGAT -3’
ARV20	Amplify the CAT:tetR:YFP genomic cassette (reverse primer)	5’- CCAAAAGCGACTTTTTCAGC -3’
ARV34	External verification primer (forward primer)	5’- AACGCAACTGGAAACAGAGG -3’
ARV35	External verification primer (reverse primer)	5’- TGCCGGTAATACCCTGAAAC -3’
ARV48	Amplify the 117 bp fragment of the *gst* reference gene (forward primer)	5’- CTGAAGGATGAGCACTGGATC -3’
ARV49	Amplify the 117 bp fragment of the *gst* reference gene (reverse primer)	5’- AATGTGCTCTAACCCTTCCAG -3’
ARV50	Amplify the 120 bp fragment of the YFP target gene (forward primer)	5’- TGTGCTTTGCTAGATACCCAG -3’
ARV51	Amplify the 120 bp fragment of the YFP target gene (reverse primer)	5’- GTGTCTTGTAGTTCCCGTCATC -3’