Supplementary material

Chance and necessity in the evolution of minimal metabolic networks

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Supplementary Methods

I. Finding homologues across species.

Homologues of *E. coli* genes present in the metabolic network were identified for 140 completely sequenced bacterial species using STRING[1]. Lifestyle information was compiled from http://www.genomenewsnetwork.org/resources/sequenced_genomes/genome_guide_index.shtml and from the corresponding genome articles (Supplementary Table 11). For detailed comparisons of model predictions with gene content of endosymbiotic genomes, we identified orthologues by reciprocal best Blast hits between each endosymbiont and *E. coli*. This resulted in orthology assignments to all but a handful of endosymbiont genes. Of the genes without orthology assignment, most had no gene with significant sequence similarity in *E. coli K-12* (mostly hypothetical proteins), while a few were apparent duplicates (Supplementary Table 12). The presence of reactions in endosymbionts is inferred from gene orthology in combination with known enzymatic functions in *E.coli*. Detailed results of all simulations are in Supplementary Table 13.

II. Identification of horizontally transferred genes

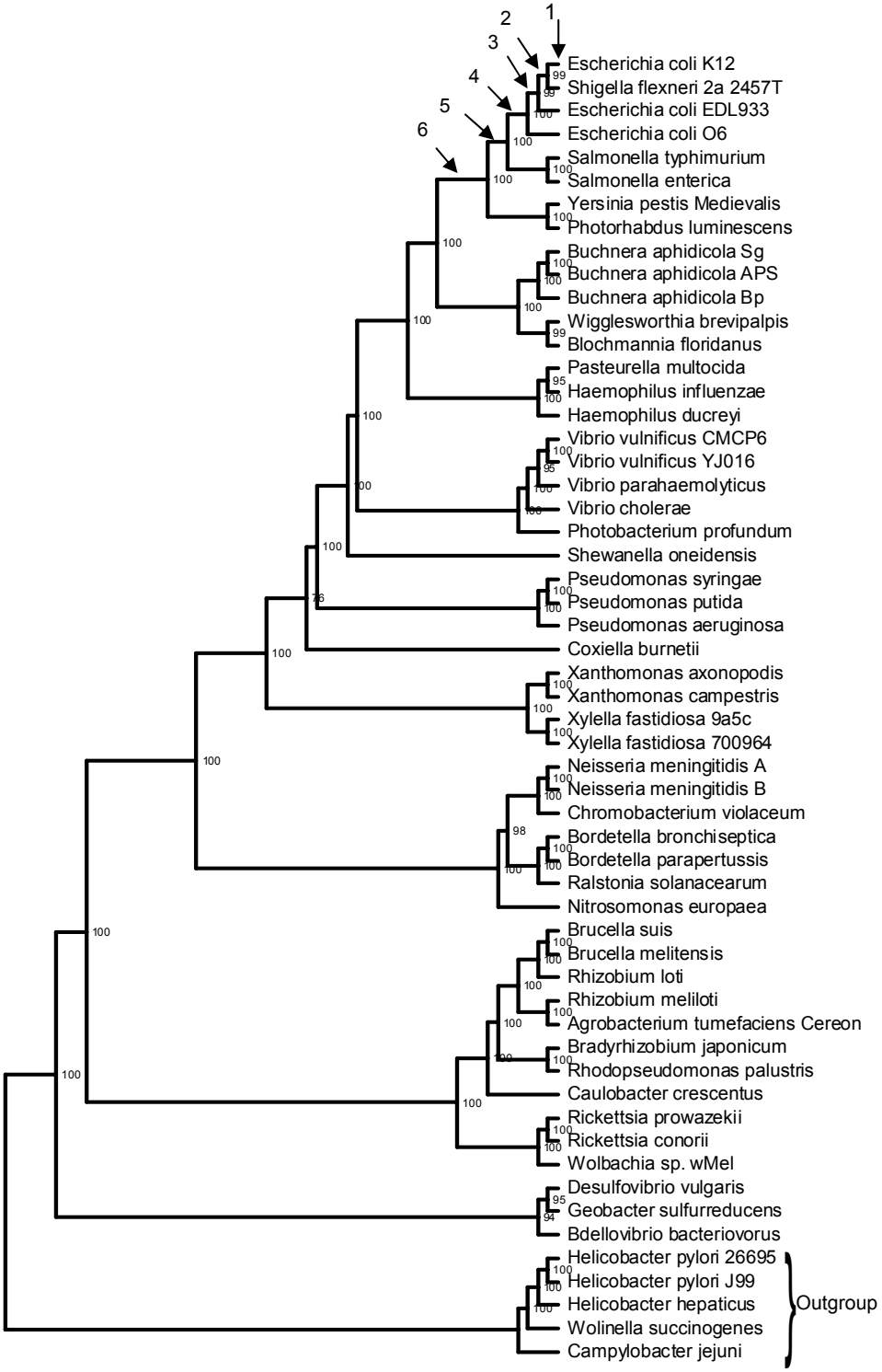
Here we briefly describe how horizontally transferred genes were identified. For more details, see [2].

Inference of phylogenetic genome tree

Putative orthologous as well as paralogous proteins were identified for 56 completely sequenced proteobacteria species (Supplementary figure 1) using STRING[3], a database that integrates and extends the widely used clusters of orthologous groups (COG)[4]. To exclude paralogues, only genes present as a single copy in all investigated genomes were retained. Each of the remaining 47 protein families was aligned using MUSCLE[5] with default settings. Highly variable parts were excluded from the alignments through GBLOCKS[6]. We constructed maximum-likelihood phylogenies from the concatenated protein sequences with PHYML[7] under an empirical substitution matrix[8] and with a Γ-model to account for among-site variation in evolutionary rates. Comparison with independent phylogenetic studies[9-12] confirmed the branching order of all previously investigated species sets. Below, we investigate 51 enterobacteria species, using the *Helicobacter/Wolinella/Campylobacter* clade as outgroups (see Supplementary Figure 1).

Parsimony analysis of gene acquisition and loss

Presence and absence calls for gene families across all 51 proteobacterial species were obtained from STRING[3]. Using the protocol of Bousseau and colleagues[9], the most parsimonious scenarios for gene-loss and horizontal transfer events (gene gains) on the rooted phylogeny were reconstructed from these data using generalized parsimony. We estimated gains and losses under both ACCTRAN (accelerated transformation) and DELTRAN (delayed transformation) algorithms as implemented in PAUP* (version 4.0). All results are obtained using relative penalties for horizontal gene transfer and deletions of 2:1 and the DELTRAN algorithm. The relative penalty value of 2:1 was previously shown to be biologically realistic[13]. We found 101 metabolic genes that were putatively transferred since the separation of the two lineages. Only 2 of them have a significant fitness contribution under the investigated conditions, confirming the reliability of the method. The transferred genes were excluded from the statistical analyses as indicated in the main text and in Supplementary Tables 3. Because all *Buchnera* genes have unquestionable *E. coli* orthologues, this reduced network represents a reconstruction of that part of the ancestral metabolic network that is relevant to the evolution of *Buchnera* lineages.



Supplementary Figure 1. Phylogenetic tree of 56 proteobacterial species

Numbers with arrows indicate the branches on which horizontal gene transfer events leading to *E. coli K12* were considered in the paper. Numbers to the right of internal branches give bootstrap proportions supporting each branch.

III. Impact of block deletions on minimal reaction sets

Deletions may frequently affect genomic regions larger than single genes (especially in the initial stage of genome reduction)[14]. To systematically explore the impact of block deletions on minimal reaction sets, we further developed the basic evolutionary model described in the main text. Specifically, we examined the effect of block deletions on: i) the size of minimal reaction sets and ii) the similarity in reaction content across simulations. The annotated *E. coli K12* genome was used as a starting point[15] (we did not attempt to reconstruct the gene order of ancestral species). The modified algorithm is as follows:

1) We remove a randomly chosen block of contiguous genes in the genome. Under the reasonable assumption that deletion size follows an exponential distribution, the probability of deleting a block with n genes is $P(n) = q^n$, where q $(0<q<1)$ specifies the exact shape of the distribution.
2) We calculate the impact of deleting these genes on the production rate of major biosynthetic components. Deletion of genes not present in the reconstructed *E. coli* metabolic network[16] was assumed to have no effect on growth. While this is clearly not the case, this method allows us not only to ignore unknown genetic interactions among non-metabolic genes, but (more importantly) it overestimates the possible sizes of deleted blocks.
3) If growth rate is nearly unaffected (in this case less than 1% effect), the deletion is assumed to be viable, and the genes are considered to be permanently lost (see Methods). Otherwise, the genes are restored to the genome. This procedure is repeated until no further enzymes in the metabolic network can be deleted; i.e. all remaining genes are essential for survival of the cell.
4) This simulation is repeated 500 times, with each run providing an independent evolutionary outcome.

The results of simulations are summarized in Supplementary Table 4.

IV. Receiver Operating Characteristic Curve (ROC) method

Two values were assigned to each *E. coli* metabolic gene: a binary variable representing presence or absence of the gene in a given *Buchnera* genome, and the number of occurrences of the gene in 500 simulated reduced genomes. For each cut-off (*c*) between 1 and 500, we took those genes that are present in at least *c* of the simulated genomes as those predicted to be also present in each of the *Buchnera* strains. Figure 2a plots the number of true-positive predictions against the number of false-positive predictions for each cut-off. The empirical area under this ROC curve was then calculated as in ref [17]. The area under the curve gives the probability that a reaction preserved in *Buchnera* is present in more simulated reduced networks than a reaction lost since divergence from *E. coli.* As this statistic is equivalent to the quantity calculated in Mann-Whitney statistical tests[17], we used the Mann-Whitney U test for the null hypothesis that the area under the ROC curve is 0.5 (i.e. the method has no discriminatory value).

V. Systematic identification of cofactors synthesized by *Wigglesworthia*

Although physiological studies indicate that *Wigglesworthia* most likely provides thiamine, pantothenic acid, pyridoxine, biotin and folic acid to its host[18], genomic data suggest that it has also retained the ability to synthesize some additional cofactors (e.g. protohaeme[19, 20], ubiquinone[20]). To explore systematically the set of cofactors most likely synthesized by *Wigglesworthia,* given its gene content, we sought the biomass composition that maximised the accuracy of the evolutionary simulation.

First, we compiled a list of potentially relevant metabolites from the 'Cofactor and Prosthetic Group Biosynthesis' sub-system of the metabolic network[16] (see table below). Note that compounds already included in the biomass equation of *E. coli* (NAD, FAD) and the five cofactors known to be provided to the host[18] were treated as fixed components of the biomass equation and not listed here. Next, we added each of these candidate metabolites to the biomass equation one at a time and repeated the evolutionary simulations. The metabolite resulting in the best prediction was kept and a new round of simulations was started, adding again each of the remaining compounds to the biomass one at a time (greedy algorithm). This iterative procedure resulted in a considerable increase of prediction accuracy from 0.758 to 0.844 (as measured by the area under the ROC curve) by complementing the biomass with haeme O, ubiquinone and glutathione (see table below).

Importantly, these results, while showing partial agreement with previous suggestions, shed some new light on the metabolic capability of *Wigglesworthia*: Although synthesis of ubiquinone had been proposed previously[20], we report that synthesis of haeme O in addition to protohaeme[19, 20] substantially improves the prediction. Haeme O is a prosthetic group of cytochrome *o* terminal oxidase[21] and is synthesized by transferring of a farnesyl group to protoheme by haeme O synthase[22] (protohaeme + farnesyl diphosphate + $H_2O \rightarrow$ haeme O + pyrophosphate). Simulations run by including haeme O in the biomass equation correctly predict not only the presence of haeme O synthase in *Wigglesworthia*, but also the retention of the pathway leading to farnesyl diphosphate. Furthermore, our evolutionary simulations predict that the ability to synthesise glutathione is also retained by the symbiont. Moreover, we found that addition of cobalamin, sirohaeme or menaquinone to the biomass decreased the accuracy of prediction, suggesting that these compounds cannot be produced by *Wigglesworthia*.

Compound added to biomass	**Effect on prediction accuracy**
Menaquinone 8	−
Ubiquinone-8	+
5,6,7,8-Tetrahydrofolate	0
Protohaeme	0
Sirohaeme	−
Haeme O	+
Riboflavin	0
Cob(I)alamin	−
Flavin mononucleotide	0
Reduced glutathione	+

Modelling conditions used for simulations of minimal metabolic networks under nutrient-rich conditions

Supplementary Table 1.a List of major biosynthetic components and their contributions to the biomass of E. coli

Biochemical knowledge on the biomass composition of *E. coli* is represented in the model as a drain of precursors and building blocks into biomass, that is a biochemical equation where the stoichiometric coefficients of the metabolites reflect their cellular concentrations (mmol / g dry weight). The biomass equation of the *E. coli* model can be found in the additional data file 1 of ref [16].

contribution (mmol / g dry weight)	component
0.05	5-Methyltetrahydrofolate
$5x10^{-5}$	Acetyl-CoA
0.488	L-Alanine
0.0010	AMP
0.281	L-Arginine
0.229	L-Asparagine
0.229	L-Aspartate
45.73	ATP
$1.29x10^{-4}$	Cardiolipin
$6.0x10^{-6}$	Coenzyme A
0.154	Glycogen
0.203	GTP
45.56	H_2O
0.09	L-Histidine
0.276	L-Isoleucine
0.428	L-Leucine
0.0084	Lipopolysaccharide
0.326	L-Lysine
0.146	L-Methionine
0.00215	NAD
0.205	L-Serine
0.0070	Spermidine
$3.0x10^{-6}$	Succinyl-CoA
0.241	L-Threonine
0.402	L-Valine

contribution (mmol / g dry weight)	component
0.126	CTP
0.087	L-Cysteine
0.0247	dATP
0.0254	dCTP
0.0254	dGTP
0.0247	dTTP
$1.0x10^{-5}$	FAD
0.25	L-Glutamine
0.25	L-Glutamate
0.582	Glycine
$5.0x10^{-5}$	NADH
$1.3x10^{-4}$	NADP
$4.0x10^{-4}$	NADPH
0.001935	Phosphatidylethanolamine
0.0276	Peptidoglycan
$4.64x10^{-4}$	Phospatidylglycerol
0.176	L-Phenylalanine
0.21	L-Proline
$5.2x10^{-5}$	Phosphatidylserine
0.035	Putrescine
0.054	L-Tryptophan
0.131	L-Tyrosine
0.0030	UDPglucose
0.136	UTP

Supplementary Table 1.b List of metabolites available for uptake

The specific uptake rates of individual compounds were not constrained in these simulations, but the total carbon influx was limited to 60 mmol/g/h.

component	component	component
(R)-Pantothenate	D-Mannose	L-Serine
(S)-Propane-1,2-diol	D-Mannose 6-phosphate	L-Tartrate
1,5-Diaminopentane	D-Ribose	L-Threonine
2-Dehydro-3-deoxy-D-gluconate	D-Sorbitol	L-Tryptophan
2-Oxoglutarate	D-Xylose	L-Tyrosine
3-(3-hydroxy-phenyl)propionate	Ethanol	L-Valine
3-hydroxycinnamic acid	Fe^{2+}	Maltohexaose
4-Aminobutanoate	Formate	Maltopentaose
Acetaldehyde	Fumarate	Maltose
Acetate	Galactitol	Maltotetraose
Acetoacetate	gamma-butyrobetaine	Maltotriose
Adenine	Glycerol	Melibiose
Adenosine	Glycerol 3-phosphate	Meso-2,6-Diaminoheptanedioate
Allantoin	Glycine	N-Acetyl-D-glucosamine
Ammonium ion	Glycine betaine	N-Acetyl-D-mannosamine
AMP	Glycolate	N-Acetylneuraminate
Butyrate (n-C4:0)	Guanine	Nicotinamide adenine dinucleotide
Choline	Guanosine	Nicotinate
Citrate	H^+	Nitrate
CO_2	H_2O	Nitrite
Cob(I)alamin	Hexadecanoate (n-C16:0)	NMN
Cyanate	Hypoxanthine	O_2
Cytidine	Indole	Octadecanoate (n-C18:0)
Cytosine	Inosine	Ornithine
Deoxyadenosine	K^+	Phenylpropanoate
Deoxycytidine	Lactose	Phosphate
Deoxyguanosine	L-Alanine	Putrescine
Deoxyinosine	L-Arabinose	Pyruvate
Deoxyuridine	L-Arginine	Sodium
D-Fructose	L-Asparagine	Spermidine
D-Galactarate	L-Aspartate	Succinate
D-Galactonate	L-Carnitine	Sucrose
D-Galactose	L-Cysteine	Sulfate
D-Galacturonate	L-Fucose	Taurine
D-Glucarate	L-Fucose 1-phosphate	Tetradecanoate (n-C14:0)
D-Gluconate	L-Glutamate	Thiamin
D-Glucosamine	L-Glutamine	Thiosulfate
D-Glucose	L-Histidine	Thymidine
D-Glucose 6-phosphate	L-Idonate	Trehalose
D-Glucuronate	L-Isoleucine	Trimethylamine
D-Glyceraldehyde	L-Leucine	Trimethylamine N-oxide
Dihydroxyacetone	L-Lysine	Uracil
Dimethyl sulfide	L-Methionine	Urea
Dimethyl sulfoxide	L-Phenylalanine	Uridine
D-Lactate	L-Proline	Xanthine
D-Mannitol	L-Rhamnose	Xanthosine

Modelling conditions used in the simulations of *Buchnera* gene content evolution

Biochemical knowledge on the biomass composition of *E. coli* is represented in the model as a drain of precursors and building blocks into biomass, that is a biochemical equation where the stoichiometric coefficients of the metabolites reflect their cellular concentrations (mmol / g dry weight). The biomass equation of the *E. coli* model (as can be found in additional data file 1 of ref[16]) was complemented with riboflavin, as physiological evidence indicates that *Buchnera* provides riboflavin[23] in addition to essential amino acids[24-28] to the host aphid (the amount of riboflavin was set to the arbitrary value of 0.01). Although the table below presents the biomass composition of *E. coli*, we also repeated the evolutionary simulations with a biomass equation containing two-fold higher levels of essential amino acids, reflecting the fact that these metabolites are also supplied to the host. Importantly, this modification had virtually no effect on the accuracy of reaction content predictions (for example in the case of *Buchnera aphidicola bp* the area under the ROC curve only changed from 0.8016 to 0.8013).

Supplementary Table 2.List of major biosynthetic components and their contributions to the biomass of Buchnera

contribution (mmol / g dry weight)	component	contribution (mmol / g dry weight)	component
0.05	5-Methyltetrahydrofolate	0.126	CTP
$5x10^{-5}$	Acetyl-CoA	0.087	L-Cysteine
0.488	L-Alanine	0.0247	dATP
0.0010	AMP	0.0254	dCTP
0.281	L-Arginine	0.0254	dGTP
0.229	L-Asparagine	0.0247	dTTP
0.229	L-Aspartate	$1.0x10^{-5}$	FAD
45.73	ATP	0.25	L-Glutamine
$1.29x10^{-4}$	Cardiolipin	0.25	L-Glutamate
$6.0x10^{-6}$	Coenzyme A	0.582	Glycine
0.154	Glycogen	$5.0x10^{-5}$	NADH
0.203	GTP	$1.3x10^{-4}$	NADP
45.56	H_2O	$4.0x10^{-4}$	NADPH
0.09	L-Histidine	0.001935	Phosphatidylethanolamine
0.276	L-Isoleucine	0.0276	Peptidoglycan
0.428	L-Leucine	$4.64x10^{-4}$	Phospatidylglycerol
0.0084	Lipopolysaccharide	0.176	L-Phenylalanine
0.326	L-Lysine	0.21	L-Proline
0.146	L-Methionine	$5.2x10^{-5}$	Phosphatidylserine
0.00215	NAD	0.035	Putrescine
0.205	L-Serine	0.054	L-Tryptophan
0.0070	Spermidine	0.131	L-Tyrosine
$3.0x10^{-6}$	Succinyl-CoA	0.0030	UDPglucose
0.241	L-Threonine	0.136	UTP
0.402	L-Valine	0.01	Riboflavin

Metabolites available for uptake

Based on biochemical evidence[26] glucose and glutamate were allowed to enter the system (the total flux of carbon uptake was limited to 60 mmol/g/h and the ratio of glucose to glutamate was not specified). In addition, unconstrained uptake of the following compounds was allowed:
CO_2, Fe^{2+}, H^+, H_2O, K^+, Na^+, NH_4, O_2, phosphate, SO_4^{2-}.

Modelling conditions used in the simulations of *Wigglesworthia* gene content evolution

It has been reported that *Wigglesworthia* provides thiamine, pantothenic acid, pyridoxine, biotin and folic acid to its host[18]. Thus, the biomass equation of *E. coli* stoichiometric model[16] was complemented with the above metabolites. We slightly modified the previously compiled *E. coli* network to enable the synthesis of thiamine and biotin. More specifically, based on the available biochemical evidence[29], we enabled the secretion of 4-Hydroxybenzyl alcohol and S-Adenosyl-4-Methylthio-2-Oxobutanoate. The early steps of biotin synthesis pathway is unknown. To circumvent this problem, we enabled the uptake of the first known intermediary product, Pimeloyl-CoA.

Supplementary Table 3a.List of major biosynthetic components and their contributions to the biomass of Wigglesworthia

contribution (mmol / g dry weight)	component	contribution (mmol / g dry weight)	component
0.05	5-Methyltetrahydrofolate	0.0247	dATP
$5x10^{-5}$	Acetyl-CoA	0.0254	dCTP
0.488	L-Alanine	0.0254	dGTP
0.0010	AMP	0.0247	dTTP
0.281	L-Arginine	$1.0x10^{-5}$	FAD
0.229	L-Asparagine	0.25	L-Glutamine
0.229	L-Aspartate	0.25	L-Glutamate
45.73	ATP	0.582	Glycine
$1.29x10^{-4}$	Cardiolipin	$5.0x10^{-5}$	NADH
$6.0x10^{-6}$	Coenzyme A	$1.3x10^{-4}$	NADP
0.154	Glycogen	$4.0x10^{-4}$	NADPH
0.203	GTP	0.001935	Phosphatidylethanolamine
45.56	H_2O	0.0276	Peptidoglycan
0.09	L-Histidine	$4.64x10^{-4}$	Phospatidylglycerol
0.276	L-Isoleucine	0.176	L-Phenylalanine
0.428	L-Leucine	0.21	L-Proline
0.0084	Lipopolysaccharide	$5.2x10^{-5}$	Phosphatidylserine
0.326	L-Lysine	0.035	Putrescine
0.146	L-Methionine	0.054	L-Tryptophan
0.00215	NAD	0.131	L-Tyrosine
0.205	L-Serine	0.0030	UDPglucose
0.0070	Spermidine	0.136	UTP
$3.0x10^{-6}$	Succinyl-CoA	0.01*	*Thiamine*
0.241	L-Threonine	0.01*	*(R)-Pantothenate*
0.402	L-Valine	0.01*	*Pyridoxine*
0.126	CTP	0.01*	*Biotin*
0.087	L-Cysteine	0.01*	*7,8-Dihydrofolate*

* The amount of these cofactors was set to the arbitrary value of 0.01, which is higher than those cofactors already present in the *E. coli* biomass, reflecting the fact that these metabolites are provided for the host. We note that setting the contribution of these compounds to an alternative value of 0.1 had virtually no effect on the accuracy of predictions (data not shown).

Due to lack of physiological or biochemical information on input metabolites consumed by *Wigglesworthia glossinidia*, we attempted to compile a list of compounds that are most likely present inside an insect cell and for which transport reactions are also annotated in the *E. coli* model[16]. As an approximation, we obtained the metabolite list of *Drosophila melanogaster* from the literature curated MetaCyc database[30] (it is the only annotated insect genome in MetaCyc). As we have no systematic data on the availability of these compounds on a quantitative scale, we did not constrain their specific uptake rates, only limited the total carbon influx to 60 mmol/g/h.

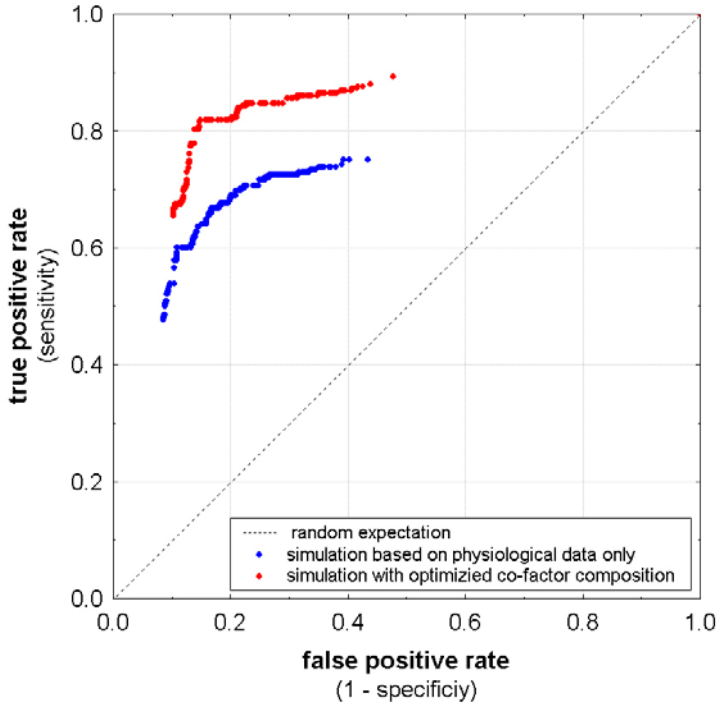
Despite the wide range of compounds allowed to enter the simulated network at the outset of the simulations, we observe that the simulations predict the evolutionary retention of transport reactions with reasonable accuracy (area under the ROC curve for transport processes is 0.738). For example, a large fraction (>80%) of simulated minimal metabolic networks import leucine, valine and isoleucine, which is in agreement with the presence of a branched-chain amino acid transport system in *Wigglesworthia*[19]. Moreover, we also run simulations where nutrient uptake was fixed throughout the simulations: only those nutrients were allowed for which the appropriate transport genes are retained in *Wigglesworthia.* Very similar results were obtained with this modification, even after excluding transport genes from analysis (area under ROC curve 0.81). This suggests that the model is robust to modifications of input conditions.

Supplementary Table 3b List of metabolites available for uptake

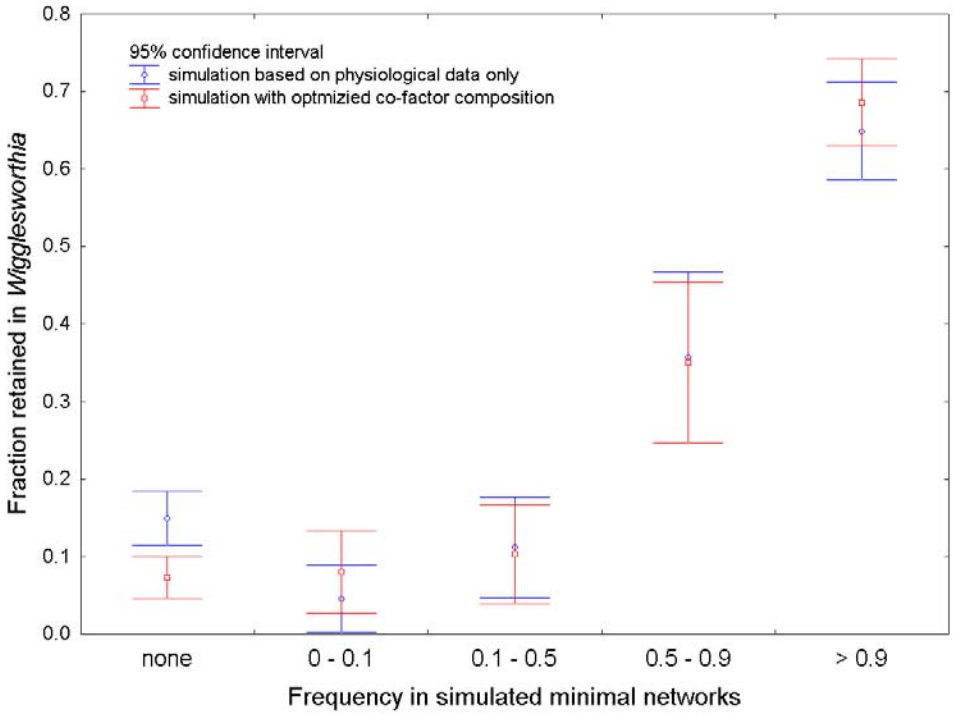
component	component
2-Oxoglutarate	L-Alanine
4-Aminobutanoate	L-Arginine
Acetaldehyde	L-Asparagine
Acetate	L-Aspartate
Acetoacetate	L-Cysteine
Adenine	L-Fucose
Adenosine	L-Glutamate
Allantoin	L-Glutamine
Ammonium ion	L-Histidine
AMP	L-Isoleucine
Choline	L-Lactate
Citrate	L-Leucine
CO_2	L-Lysine
Cytidine	L-Malate
Cytosine	L-Methionine
D-Alanine	L-Phenylalanine
Deoxyadenosine	L-Proline
Deoxyinosine	L-Serine
D-Fructose	L-Threonine
D-Galactose	L-Tryptophan
D-Glucose	L-Tyrosine
D-Glucose 6-phosphate	L-Valine
D-Lactate	Maltose
D-Mannose 6-phosphate	Na^+
Ethanol	NAD
Fe^{2+}	NMN
Formate	O_2
Fumarate	Ornithine
Glycerol	Phosphate
Glycerol 3-phosphate	Pyruvate
Glycine	Succinate
Guanine	Sucrose
Guanosine	Sulfate
H^+	Thymidine
H_2O	Trehalose
Hypoxanthine	Uracil
Indole	Urea
Inosine	Uridine
K^+	Xanthine
Lactose	Xanthosine

Supplementary Figure 2. Comparison of reaction content of simulated and *Wigglesworthia glossinidia* metabolic networks.

a) *Predictive accuracy for all possible cutoffs (ROC curve). Overall accuracy and statistics (area under curve): biomass composition derived from physiological data only, AUC=0.758 (blue dots), biomass with optimised co-factor composition, AUC=0.844 (red dots). All results are highly significant, $P<10^{-25}$.*



b) *Presence/absence of reactions in* Wigglesworthia glossinidia*, averaged over genes within defined ranges of presence/absence in the simulated minimal reaction-sets. Statistics: biomass composition derived from physiological data only,* $\chi^2=222.23$, $df=4$, $P<10^{-46}$, *biomass with optimised co-factor composition,* $\chi^2=317.06$, $df=4$, $P<10^{-66}$.



Supplementary Table 4. The impact of block deletions on the properties of minimal minimal networks

The Table shows mean ± standard deviation. The fraction of shared simulation reactions across minimal reaction sets and the number of reactions across simulated sets remain unchanged, regardless of the mean size of block deletions. However, fewer simulation steps are necessary to reach minimal reactions sets if block deletions are allowed. For more details, see Supplementary Methods.

Model	Number of metabolic genes deleted at a single step	Number of successful deletion steps to reach the minimal reaction set	Fraction of shared reactions across minimal reaction sets	Number of reactions in simulated minimal reactions sets (Mean±Standard deviation)
Only single gene deletions	1±0	658.08±6.47	0.78±0.046	288.63±9.63
Block deletion (q=0.5)	1.92±0.06	546.44±0.06	0.77±0.045	288.57±9.41
Block deletion (q=0.9)	7.18±0.35	345.29±0.34	0.77±0.044	287.74±9.30

Supplementary Table 5. Accuracy of reaction content predictions

When calculating the fraction of positive hits, specificity and sensitivity, reactions absent in some, but not all simulated networks were excluded. Sensitivity is defined as the fraction of preserved reactions correctly predicted by the model and specificity is the fraction of lost reactions correctly identified by the model. Where indicated, reactions horizontally transferred into *E. coli* were excluded from the analysis. % of positive hits indicates the fraction of true positives and true negatives. Abbreviations: Bp, *Buchnera aphidicola*, endosymbiont of *Baizongia pistaciae*; APS, *Buchnera aphidicola*, endosymbiont of *Acyrthosiphon pisum*; Sg, *Buchnera aphidicola*, endosymbiont of *Schizaphis graminum*; all – reactions present in all of the three *Buchnera* genomes. Results for *Wigglesworthia* were obtained by using the biomass equation with optimised cofactor composition (see Supplementary Methods).

Growth-rate Cut-off used in the simulations	Number of reactions investigated	Species to which the simulation results are compared	Accuracy and standard error of prediction[17] (measured analogous to Fig. 2a in main text)	% of positive hits	Sensitivity (%)	Specificity (%)
0.01	All reactions* (N=873)	*Buchnera aphidicola Bp*	0.802 (0.020)	76.8	85.0	74.4
		Buchnera aphidicola Sg	0.800 (0.019)	78.1	83.0	76.4
		Buchnera aphidicola APS	0.794 (0.019)	78.4	80.7	77.5
		Reactions present in all three *Buchnera* strains	0.790 (0.022)	74.7	85.0	72.3
		Wigglesworthia glossinidia	0.844 (0.017)	84.4	86.0	83.7
	No horizontally transferred reactions to *E. coli* (N=789)	*Buchnera aphidicola Bp*	0.787 (0.021)	74.5	85.0	71.1
		Buchnera aphidicola Sg	0.786 (0.020)	76.0	83.0	73.2
		Buchnera aphidicola APS	0.780 (0.020)	76.3	80.7	74.3
		Reactions present in all three *Buchnera* strains	0.774 (0.023)	72.2	85.0	68.7
		Wigglesworthia glossinidia	0.835 (0.017)	83.1	86.0	81.5
0.1	All reactions* (N=873)	*Buchnera aphidicola Bp*	0.794 (0.021)	76.3	83.9	74.1
		Buchnera aphidicola Sg	0.794 (0.019)	77.7	81.9	76.3
		Buchnera aphidicola APS	0.788 (0.019)	78.0	79.5	77.4
		Reactions present in all three *Buchnera* strains	0.781 (0.022)	74.1	83.8	71.8
		Wigglesworthia glossinidia	0.825 (0.017)	83.7	87.0	81.8
	No horizontally transferred reactions to *E. coli* (N=789)	*Buchnera aphidicola Bp*	0.779 (0.021)	73.8	83.9	70.6
		Buchnera aphidicola Sg	0.780 (0.020)	75.4	81.9	72.9
		Buchnera aphidicola APS	0.774 (0.020)	75.8	79.5	74.1
		Reactions present in all three *Buchnera* strains	0.764 (0.023)	71.4	83.8	68.1
		Wigglesworthia glossinidia	0.814 (0.018)	82.1	87.0	78.6

* Reactions without annotated genes are not included in the analyses.

Supplementary Table 6. Accuracy of reaction content prediction in different metabolic subsystems

(a) The area under the ROC curve and its standard error[17] was calculated for each metabolic subsystem where at least one reaction was preserved in all *Buchnera* strains and one reaction was lost in at least one *Buchnera* strain. **(b)** The area under the ROC curve and its standard error[17] was calculated for each metabolic subsystem of *Wigglesworthia* (prediction is from simulations run with the optimised biomass composition, Supplementary Methods).

Table 6a *Buchnera*

Metabolic subsystem	Fractions of reactions retained in *Buchnera* genomes	Number of reactions	Area under the ROC curve	Standard error
Threonine and Lysine Metabolism	0.923	13	1	0
Pyruvate Metabolism	0.285	7	1	0
Anaplerotic Reactions	0.142	7	1	0
Pentose Phosphate Cycle	0.777	9	1	0
Oxidative phosphorylation	0.125	40	0.971	0.053
Glycine and Serine Metabolism	0.25	8	0.833	0.199
Folate Metabolism	0.5	6	0.833	0.184
Transport, Extracellular	0.027	146	0.83	0.127
Glycolysis/Gluconeogenesis	0.555	18	0.825	0.1
Alternate Carbon Metabolism	0.031	127	0.81	0.132
Arginine and Proline Metabolism	0.151	33	0.757	0.133
Citrate Cycle (TCA)	0.076	13	0.75	0.299
Cell Envelope Biosynthesis	0.121	74	0.72	0.101
Cofactor and Prosthetic Group Biosynthesis	0.196	122	0.669	0.066
Membrane Lipid Metabolism	0.16	25	0.667	0.161
Methionine Metabolism	0.285	7	0.6	0.259
Cysteine Metabolism	0.142	7	0.583	0.339
Tyrosine, Tryptophan, and Phenylalanine Metabolism	0.7	20	0.583	0.139
Nucleotide Salvage Pathways	0.364	85	0.564	0.066
Valine, leucine, and isoleucine metabolism	0.4	15	0.5	0.157
Methylglyoxal Metabolism	0.333	3	0.5	0.408
Purine and Pyrimidine Biosynthesis	0.333	24	0.492	0.127

Table 6b *Wigglesworthia*

Metabolic subsystem	Fraction of reactions reatained in *Wigglesworthia* genome	Number of reactions investigated	Area under the ROC curve	Standard error
Threonine and Lysine Metabolism	0.692	13	1	0
Pentose Phosphate Cycle	0.555	9	1	0
Histidine Metabolism	0.1	10	1	0
Alternate Carbon Metabolism	0.031	127	0.958	0.07
Methionine Metabolism	0.285	7	0.9	0.164
Cofactor and Prosthetic Group Biosynthesis	0.622	122	0.859	0.033
Membrane Lipid Metabolism	0.6	25	0.85	0.077
Tyrosine, Tryptophan, and Phenylalanine Metabolism	0.2	20	0.844	0.131
Glycine and Serine Metabolism	0.25	8	0.833	0.199
Cell Envelope Biosynthesis	0.364	74	0.827	0.054
Pyruvate Metabolism	0.285	7	0.8	0.217
Glycolysis/Gluconeogenesis	0.555	18	0.756	0.115
Glutamate Metabolism	0.2	5	0.75	0.327
Transport, Extracellular	0.047	146	0.748	0.109
Purine and Pyrimidine Biosynthesis	0.916	24	0.739	0.159
Nucleotide Salvage Pathways	0.317	85	0.731	0.062
Oxidative Phosphorylation	0.15	40	0.64	0.131
Alanine and Aspartate Metabolism	0.444	9	0.6	0.201
Anaplerotic Reactions	0.285	7	0.6	0.259
Folate Metabolism	0.5	6	0.556	0.253
Methylglyoxal Metabolism	0.333	3	0.5	0.408
Citrate Cycle (TCA)	0.461	13	0.452	0.165
Arginine and Proline Metabolism	0.212	33	0.398	0.117

Metabolic pathways showing variability in retention across simulations

To quantify metabolic flexibility, pairwise similarities of reaction contents of 500 simulated minimal networks were calculated for each functional subsystem of the metabolism (defined as in ref [16]). Subsystems where all reactions are lost in either *Buchnera* or in the simulated genomes were not considered here. Many subsystems show little or no variability among simulated genomes (e.g. amino acid synthesis groups, membrane and cofactor metabolism). Most of the variability among genomes lie within transport processes, alternate carbon metabolism, anapleurotic reactions, pyruvate metabolism and in nucleotide salvage pathways. The simulations shown result from boundary conditions that mimic the evolution of *Buchnera* (Supplementary Table 2).

Supplementary Table 7.

Metabolic subsystem (as in ref [16])	Average pairwise similarity of minimal networks (Jaccard index)
Alternate Carbon Metabolism (N=127)	0.52
Transport, Extracellular (N=146)	0.57
Anaplerotic Reactions (N=7)	0.58
Pyruvate Metabolism (N=7)	0.72
Nucleotide Salvage Pathways (N=85)	0.76
Folate Metabolism (N=6)	0.87
Arginine and Proline Metabolism (N=33)	0.89
Oxidative Phosphorylation (N=40)	0.9
Glycine and Serine Metabolism (N=8)	0.91
Threonine and Lysine Metabolism (N=13)	0.92
Glycolysis/Gluconeogenesis (N=18)	0.92
Cysteine Metabolism (N=7)	0.94
Citrate Cycle (TCA) (N=13)	0.95
Membrane Lipid Metabolism (N=25)	0.95
Purine and Pyrimidine Biosynthesis (N=24)	0.96
Tyrosine, Tryptophan, and Phenylalanine Metabolism (N=20)	0.98
Cofactor and Prosthetic Group Biosynthesis (N=122)	0.99
Pentose Phosphate Cycle (N=9)	0.99
Cell Envelope Biosynthesis (N=74)	1
Valine, Leucine, and Isoleucine Metabolism (N=15)	1
Histidine Metabolism (N=10)	1
Methionine Metabolism (N=7)	1

Supplementary Table 8. Examples of alternative metabolic solutions during reductive evolution of *Buchnera*

The Table provides examples of alternative solutions (pathways) to three metabolic problems. Simulated metabolic networks contain one or other of the two alternative solutions, but never both (data not shown). Importantly, *Buchnera* strains also retained only one of these pathways (proteins encoded by *Buchnera* genomes are underlined in the table). Descriptions are from Ecocyc[31].

Metabolic solution #1 (enzymes and reactions retained by *Buchnera*)	**Metabolic solution #2 (enzymes and reactions lost by *Buchnera*)**	**Description**
Ribonucleoside-diphosphate reductase (NrdA & NrdB) NDP + reduced thioredoxin → dNDP + H_2O + oxidized thioredoxin	Ribonucleoside-triphosphate reductase (NrdD) NTP + reduced thioredoxin → dNTP + H_2O + oxidized thioredoxin	Deoxyribonucleotides are synthetised from ribonucleotides. It can be achieved by two ways in *E. coli*: either NDP is reduced to dNDP (ribonucleoside-diphosphate reductase), or NTP is reduced to dNTP. This latter reaction is catalysed by an oxygen sensitive enzyme, thus *E. coli* uses it under anaerobic conditions. *Buchnera* strains retained the oxygen requiring ribonucleoside-diphosphate reductase.
Phosphate H^+ symporter (PitA or PitB) H^+_{ex} + Pi_{ex} ↔ H^+_{in} + Pi_{in}	Phosphate ABC transporter complex (pstA & pstB & pstC & pstS) ATP_{in} + H_2O_{in} + Pi_{ex} → ADP_{in} + H^+_{in} + 2 Pi_{in}	Phosphate can be imported by *E. coli* either by a low affinity transporter PitA (or PitB) driven by proton motive force, or by a high affinity ABC transporter system (pstABCS), which is ATP-dependent. *Buchnera* strains retained the low affinity PitA transporter.
Step 1: Acetate kinase (AckA or TdcD or PurT) acetate + ATP ↔ acetyl-P + ADP Step 2: Phosphotransacetylase (Pta or EutD) acetyl-P + Coa ↔ acetyl-CoA + Pi	Acetyl-CoA synthetase (Acs) acetate + ATP + CoA → acetyl-CoA + AMP + PPi	There are two distinct pathways by which *E. coli* activates acetate to acetyl-CoA. Acetyl-CoA synthetase (ACS) catalyzes one of them. It is thought that this ACS pathway functions in a mainly anabolic role, scavenging acetate present in the extracellular medium. *Buchnera* strains retained the acetate kinase - phosphotransacetylase pathway (proteins AckA and Pta).

(ex = extracellular metabolite, in = intracellular metabolite)

Supplementary Table 9. Cross comparison of *Buchnera* and *Wigglesworthia* reaction content predictions

Although the metabolic functions of the endosymbionts *Buchnera* and *Wigglesworthia* differ significantly, their reaction contents show considerable overlap (0.38 – 0.40). Even a model that predicts gene content evolution in *Buchnera* with 100% accuracy would explain a large fraction of the gene content evolution in *Wigglesworthia* as genes lost in these lineages show significant overlap. Therefore, it is important to investigate whether the differences in the boundary conditions between *Buchnera* and *Wigglesworthia* simulations are substantial enough to specifically predict the reaction content of *Buchnera* and *Wigglesworthia*, respectively.

Our cross-comparisons show that simulations constructed to reflect the lifestyle of *Buchnera* predict the reaction content of *Buchnera* strains with higher accuracy than those of *Wigglesworthia*. Similarly, simulations set up for *Wigglesworthia* predict the reaction content of *Wigglesworthia* more successfully than those of *Buchnera* strains (a biomass equation with optimised cofactor composition was used for *Wigglesworthia* simulations, see Supplementary Methods).

Simulation	**Genome**	**Accuracy of reaction content prediction** (area under the ROC curve and its standard error)
Buchnera	*Buchnera aphidicola Bp*	0.802 (0.020)
	Buchnera aphidicola Sg	0.800 (0.019)
	Buchnera aphidicola APS	0.794 (0.019)
	Wigglesworthia glossinidia	0.708 (0.021)
Wigglesworthia	*Buchnera aphidicola Bp*	0.764 (0.022)
	Buchnera aphidicola Sg	0.775 (0.020)
	Buchnera aphidicola APS	0.756 (0.020)
	Wigglesworthia glossinidia	0.844 (0.017)

Supplementary Table 10. Physiologically coupled reactions are frequently lost together in endosymbionts

To identify physiologically coupled enzyme sets in the *E.coli* network, we followed a previously described protocol[31].We considered a condition where all external nutrients are available; this scenario gives a condition-insensitive underestimate of physiologically coupled reactions. Briefly, the analysis is based on fixing the flux through one reaction, and then maximising and minimizing the flux through all other reactions in turn. We concentrated on fully coupled reaction pairs. Assuming that endosymbiont networks are close to minimal, one would expect that such reactions are retained or lost together. We identified 619 fully coupled reaction pairs by flux coupling analysis[32] in *E. coli*. Fully coupled reactions have fixed flux ratios and are always used together in steady state flux distributions of the network (e.g. steps of a linear pathway). We found 619 fully coupled reaction pairs. Having identified coupled reactions, we counted the number of cases when both members of a coupled reaction pair are either lost or retained in a given endosymbiont. We found that 74-84% of coupled pairs are lost or retained together, which is significantly higher than 50-55% expected by chance ($P < 10^{-5}$ for all species, calculated by randomising pairings of coupled reactions). This conclusion remains when horizontally transferred genes are excluded from the analysis (data not shown).

Note however, that as most reactions have more than one fully coupled reaction partner, this analysis is not suitable for measuring the fraction of compulsory reactions in endosymbionts. One could get a better view of this process by analysing physiologically coupled reaction sets. In these sets, all reaction pairs are fully coupled to each other. The 619 fully coupled reaction pairs in E.coli comprise 100 independent, fully coupled reactions sets. As these sets can only fulfil their function if all of their constituent reactions are present, one would expect to find either all or none of the coupled reactions in endosymbionts. Reactions present in a set missing some of their reactions may be considered superfluous. 13-20% of the reactions in endosymbionts fulfil this requirement (data not shown). This figure is still likely to be an overestimate, however, if endosymbionts (but not E.coli) can uptake some of the intermediate metabolites.

Species	% of coupled reaction pairs where both members are either lost or retained	% of reaction pairs expected by chance
Buchnera aphidicola Bp	83.8	55.1
Buchnera aphidicola Sg	73.8	52.6

Buchnera aphidicola APS	79.3	51.2
Wigglesworthia glossinidia	76.5	50.4

Supplementary Table 11. List of 140 bacterial species investigated and their lifestyles

Species	lifestyle
Buchnera aphidicola APS	endosymbiont
Buchnera aphidicola Bp	endosymbiont
Buchnera aphidicola Sg	endosymbiont
Blochmannia floridanus	endosymbiont
Wigglesworthia brevipalpis	endosymbiont
Aeropyrum pernix	free living
Aquifex aeolicus	free living
Bacillus halodurans	free living
Deinococcus radiodurans	free living
Halobacterium sp. NRC-1	free living
Methanobacterium thermautotrophicum	free living
Methanococcus jannaschii	free living
Methanococcus maripaludis	free living
Methanopyrus kandleri	free living
Oceanobacillus iheyensis	free living
Photobacterium profundum	free living
Pyrobaculum aerophilum	free living
Pyrococcus abyssi	free living
Pyrococcus furiosus	free living
Pyrococcus horikoshii	free living
Sulfolobus solfataricus	free living
Sulfolobus tokodaii	free living
Thermoanaerobacter tengcongensis	free living
Thermoplasma acidophilum	free living
Thermoplasma volcanium	free living
Thermotoga maritima	free living
Thermus thermophilus	free living
Nostoc sp. PCC 7120	free living
Bacillus subtilis	free living
Caulobacter crescentus	free living
Chlorobium tepidum	free living
Chromobacterium violaceum	free living
Clostridium acetobutylicum	free living
Corynebacterium efficiens	free living
Corynebacterium glutamicum	free living
Corynebacterium glutamicum 13032	free living
Desulfovibrio vulgaris	free living
Escherichia coli K12	free living
Geobacter sulfurreducens	free living
Gloeobacter violaceus	free living
Lactococcus lactis	free living
Listeria innocua	free living
Methanosarcina acetivorans	free living
Methanosarcina mazei	free living
Pasteurella multocida	pathogen
Photorhabdus luminescens	pathogen
Ureaplasma parvum	intracellular pathogen

Species	lifestyle
Nitrosomonas europaea	free living
Prochlorococcus marinus SS120	free living
Prochlorococcus marinus MIT9313	free living
Prochlorococcus marinus CCMP1378	free living
Pseudomonas putida	free living
Rhodopirellula baltica	free living
Rodopseudomonas palustris	free living
Shewanella oneidensis	free living
Streptomyces avermitilis	free living
Streptomyces coelicolor	free living
Synechococcus elongatus	free living
Synechococcus sp. WH8102	free living
Synechocystis sp. PCC6803	free living
Streptococcus mutans	pathogen
Agrobacterium tumefaciens Cereon	pathogen
Agrobacterium tumefaciens WashU	pathogen
Bacillus anthracis	pathogen
Bacillus cereus ATCC 10987	pathogen
Bacillus cereus ATCC 14579	pathogen
Bordetella bronchiseptica	pathogen
Bordetella parapertussis	pathogen
Bordetella pertussis	pathogen
Borrelia burgdorferi	pathogen
Brucella melitensis	pathogen
Brucella suis	pathogen
Campylobacter jejuni	pathogen
Clostridium perfringens	pathogen
Clostridium tetani	pathogen
Corynebacterium diphtheriae	pathogen
Enterococcus faecalis	pathogen
Escherichia coli O157:H7	pathogen
Escherichia coli EDL933	pathogen
Escherichia coli O6	pathogen
Fusobacterium nucleatum	pathogen
Haemophilus ducreyi	pathogen
Haemophilus influenzae	pathogen
Helicobacter hepaticus	pathogen
Helicobacter pylori 26695	pathogen
Helicobacter pylori J99	pathogen
Leptospira interrogans 56601	pathogen
Leptospira interrogans L1-130	pathogen
Listeria monocytogenes EGD	pathogen
Listeria monocytogenes F2365	pathogen
Mycobacterium bovis	pathogen
Neisseria meningitidis A	pathogen
Neisseria meningitidis B	pathogen

Supplementary Table 11. List of 140 bacterial species investigated and their lifestyles (continued).

Species	lifestyle
Porphyromonas gingivalis	pathogen
Pseudomonas aeruginosa	pathogen
Pseudomonas syringae	pathogen
Ralstonia solanacearum	pathogen
Salmonella enterica	pathogen
Salmonella typhi	pathogen
Salmonella typhimurium	pathogen
Shigella flexneri 2a 301	pathogen
Shigella flexneri 2a 2457T	pathogen
Staphylococcus aureus MW2	pathogen
Staphylococcus aureus Mu50	pathogen
Staphylococcus aureus N315	pathogen
Streptococcus agalactiae III	pathogen
Streptococcus agalactiae V	pathogen
Streptococcus pneumoniae TIGR4	pathogen
Streptococcus pneumoniae R6	pathogen
Streptococcus pyogenes M1	pathogen
Streptococcus pyogenes MGAS8232	pathogen
Streptococcus pyogenes MGAS315	pathogen
Streptococcus pyogenes SSI-1	pathogen
Treponema denticola	pathogen
Treponema pallidum	pathogen
Vibrio cholerae	pathogen
Vibrio parahaemolyticus	pathogen
Vibrio vulnificus YJ016	pathogen
Xanthomonas axonopodis	pathogen
Xanthomonas campestris	pathogen
Xylella fastidiosa 9a5c	pathogen
Xylella fastidiosa 700964	pathogen
Yersinia pestis CO92	pathogen
Yersinia pestis KIM	pathogen
Chlamydia muridarum	obligate intracellular
Chlamydia pneumoniae AR39	obligate intracellular
Chlamydia pneumoniae CWL029	obligate intracellular
Chlamydia pneumoniae J138	obligate intracellular
Chlamydia trachomatis	obligate intracellular
Chlamydophila caviae	obligate intracellular
Chlamydophila pneumoniae TW183	obligate intracellular
Coxiella burnetii	obligate intracellular
Mycobacterium leprae	obligate intracellular
Phytoplasma Onion yellows	obligate intracellular
Rickettsia conorii	obligate intracellular
Rickettsia prowazekii	obligate intracellular
Wolbachia sp. wMel	obligate intracellular
Mycoplasma genitalium	obligate intracellular
Mycoplasma penetrans	obligate intracellular
Mycoplasma pneumoniae	obligate intracellular

Supplementary Table 12. Endosymbiont genes without orthologues in *E. coli* K-12

Supplementary table 12.a *Buchnera aphidicola* genes with no orthologs in *E. coli*

Gi number	Gene	Annotation	Blast to *E. coli K-12*	Best hits from Blast to all of Genbank exc. *Buchnera*
27904528	yidD	hypothetical protein	no significant hit	hypothetical protein (many species) / alpha-hemolysin (*Bacillus*)
27904577	fliJ	flagellar FliJ protein	no significant hit	flagellar protein FliJ (*Shewanella*)
27904578	fliK	flagellar FliK protein	no significant hit	rpoB (*Plasmodium*)
27904675	grpE	GrpE protein 2	best hit has other ortholog (16130533)	GrpE protein/HSP-70 cofactor/heat shock protein (many species)
27904706	ftsL	cell division protein FtsL homolog	best hit is cell division protein (16128076, e=0.002)	cell division protein FtsL (*Haemophilus*)
27904850	aroQ	3-dehydroquinate dehydratase	no significant hit	3-dehydroquinate dehydratase (*Erwinia*)
27904996	trpG	anthranilate synthase component II	best hit has other ortholog (16129224)	anthranilate synthase component II (*Shigella*)
27905000	yba3	hypothetical protein	no significant hit	no significant hit outside *Buchnera*
28191368	repA2	replication associated protein	no significant hit	repA1 (*Sodalis*)
28191366	repA1	replication associated protein	no significant hit	repA (*Sodalis*)

Supplementary Table 12b. *Buchnera aphidicola sg* genes with no orthologs in *E. coli*

Gi number	Gene	Annotation	Blast to *E. coli K-12*	Best hits from Blast to all of Genbank exc. *Buchnera*
21672309	yidD	hypothetical protein	no significant hit	hypothetical protein (many species) | alpha-hemolysin (*Bacillus*)
21672465	grpE	GrpE	best hit has other ortholog (16130533)	GrpE protein/HSP-70 cofactor (*Photorhabdus*)
21672467	smpA	small protein A	no significant hit	no significant hit anywhere
21672642	secG	protein-export membrane protein SecG	no significant hit	no significant hit outside *Buchnera*
21672710	ribD1	riboflavin biosynthesis protein RibD	best hit has other ortholog (16128399)	bifunctional: diaminohydroxyphosphoribosylaminopyrimidine deaminase + uracil reductase (*Escherichia coli K-12*)
21672822	Yba4	hypothetical protein	no significant hit	paramyosin (*Boophilus*)
10954447	repA1	replication associated protein	no significant hit	repA1 (*Sodalis*)
16129145	repA2	replication associated protein	no significant hit	repA1 (*Sodalis*)

Supplementary Table 12c. *Buchnera aphidicola sp* genes with no orthologs in *E. coli*

Gi number	Gene	Annotation	Blast to *E. coli K-12*	Best hits from Blast to all of Genbank exc. *Buchnera*
10957102	trpG2	anthranilate synthase small subunit	best hit has other ortholog (16129224)	anthranilate synthase component II (*Erwinia*)
15617003	aroD	type II 3-dehydroquinase	no significant hit	3-dehydroquinate dehydratase (*Erwinia*)
15617059	ribD1	riboflavin deaminase	best hit has other ortholog (16128399)	bifunctional: diaminohydroxyphosphoribosylaminopyrimidine deaminase + uracil reductase (*Escherichia coli K-12*)
10957104	repA1	replication-associated protein RepA1	no significant hit	repA1 protein (*Sodalis*)
10957106	repA2	replication-associated protein RepA2	no significant hit	repA protein (*Sodalis*)
15616700	yba1	hypothetical protein	no significant hit	no significant hit outside *Buchnera*
15616863	grpE1	heat shock protein GrpE1	best hit has other ortholog (16130533)	grpE (*Wigglesworthia*)
15616941	flgN	flagella synthesis protein FlgN	no significant hit	no significant hit outside *Buchnera*
15616658	cof	Cof protein	best hit has other ortholog (49176424)	haloacid dehalogenase-like hydrolase-like protein (*Leishmania*)
15616984	secG	protein-export membrane protein SecG	no significant hit	biotin carboxylase subunit of acetyl CoA carboxylase, putative (*Plasmodium*)
15617175	yba4	hypothetical protein	no significant hit	erythrocyte membrane protein pfemp3 (*Plasmodium*)
10957101	trpG	anthranilate synthase small subunit	best hit has other ortholog (16129224)	anthranilate synthase component II (*Erwinia*)
15616800	yba2	hypothetical protein	no significant hit	hypothetical protein (many species)

Supplementary Table 12d. *Wigglesworthia glossinidia* genes with no orthologs in *E. coli*

Gi number	Gene	Annotation	Blast to E. coli K-12	Best hits from Blast to all of Genbank
50470479	-	conjugative transfer surface exclusion lipoprotein	no significant hit	TraT+ conjugative transfer: surface exclusion (*Salmonella*)
32490800	fliO	hypothetical protein	no significant hit	no significant hit anywhere
32491016	recD	hypothetical protein	no significant hit	exodeoxyribonuclease V alpha chain *(Buchera Bp*) ; no other hits
32490785	flgN	hypothetical protein	no significant hit	flagella synthesis protein (*Erwinia*; e=2e-4); no other hits
32491035	wg003	hypothetical protein	no significant hit	hypothetical protein (*Photorhabdus*) | Ccm2 + Ccm1 (*Proteus*) | putative membrane protein (*Yersinia*)
32491341	wg001	hypothetical protein	no significant hit	probable transmembrane protein (*Blochmania*) | putative membrane protein (*Erwinia*)
50470477	repA	replication protein A	no significant hit	replication protein RepA + replication initiator and transcription repressor (*Erwinia*)

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Supplementary Table 13. Metabolic gene content of endosymbionts and simulated minimal genomes

Presence (+) or absence (-) in endosymbiontic genomes of 904 genes annotated in the stoichiometric model of E. coli.
Figure 1c in the main text is based on results of the general simulations (column 7).
Four genes (b1416, b1417, b3767, b3768) are not annotated appropriatelyin the NCBI genome database, and therefore these genes were excluded from all analysis.

		Presence/Absence of the ortholog in endosymbiont genomes				Fraction of simulated minimal gene sets with the presence of the gene		
Blattner names (*E.coli*)	Gene name (*E.coli*)	*Buchnera aphidicola*	*Buchnera aphidicola Sg*	*Buchnera sp*	*Wigglesworthia brevipalpis*	General (nutrient rich) conditions	*Wigglesworthia* conditions	*Buchnera* conditions
b0002	thrA	+	+	+	+	0.34	0.36	0.498
b0003	thrB	+	+	+	-	0.342	0.092	0.606
b0004	thrC	+	+	+	-	0.342	0.092	0.606
b0007	yaaJ	-	-	-	-	0.01	0.008	0
b0008	talB	-	-	-	-	0.494	0.444	0.512
b0019	nhaA	-	-	-	+	0.268	0.004	0
b0025	ribF	-	+	+	+	1	1	1
b0029	lytB	+	+	+	+	0	1	0
b0031	dapB	+	+	+	+	0.704	1	1
b0032	carA	+	+	+	+	0	0	0.012
b0033	carB	+	+	+	+	0	0	0.012
b0036	caiD	-	-	-	-	0	0	0
b0038	caiB	-	-	-	-	0	0	0
b0040	caiT	-	-	-	-	0	0	0
b0048	folA	+	+	+	+	1	1	1
b0049	apaH	+	+	+	+	0	0	0
b0052	pdxA	-	-	-	+	0	1	0
b0061	araD	-	-	-	-	0	0	0
b0062	araA	-	-	-	-	0	0	0
b0063	araB	-	-	-	-	0	0	0
b0066	sfuC	-	-	-	-	0	0	0
b0067	sfuB	-	-	-	-	0	0	0
b0068	sfuA	-	-	-	-	0	0	0
b0071	leuD	+	-	+	-	0.802	0.744	1
b0072	leuC	+	-	+	-	0.802	0.744	1
b0073	leuB	+	-	+	-	0.802	0.744	1
b0074	leuA	+	-	+	-	0.802	0.744	1
b0077	ilvI	+	+	+	-	0.368	0.406	0.414

b0078	ilvH	+	+	+	-	0.368	0.406	0.414
b0085	murE	+	-	+	+	1	1	1
b0086	murF	+	-	+	+	1	1	1
b0087	mraY	+	-	+	+	1	1	1
b0088	murD	+	+	+	+	1	1	1
b0090	murG	+	+	+	+	1	1	1
b0091	murC	+	-	+	+	1	1	1
b0092	ddlB	+	+	-	-	0.53	0.492	0.51
b0096	lpxC	-	-	-	+	1	1	1
b0099	mutT	-	+	+	-	0.488	0.498	0.48
b0104	guaC	+	+	+	-	0	0	0
b0109	nadC	-	-	-	+	0.198	0.188	1
b0112	aroP	-	-	-	-	0.636	0.7	0
b0114	aceE	+	+	+	+	0	0.002	1
b0115	aceF	+	+	+	+	0	0.002	1
b0116	lpdA	+	+	+	+	1	0.934	1
b0118	acnB	-	-	-	-	0.478	0.458	0.484
b0120	speD	-	+	+	-	0.184	1	1
b0121	speE	-	+	+	-	0.186	1	1
b0124	gcd	-	-	-	-	0	0	0
b0125	hpt	-	+	+	-	0.03	0.088	0.054
b0126	yadF	-	-	-	-	0.514	0.506	0.536
b0131	panD	-	-	-	-	0.27	1	1
b0133	panC	-	+	+	+	0.27	1	1
b0134	panB	-	+	+	+	0.27	1	1
b0142	folK	-	-	-	+	1	1	1
b0154	hemL	-	-	-	+	0	1	0
b0158	yadT	-	-	-	-	0	0	0
b0159	mtn	+	+	+	+	0.184	1	1
b0160	dgt	-	-	-	-	0	0	0
b0166	dapD	+	+	+	+	0.704	1	1
b0171	pyrH	+	+	+	+	0.668	0.6	0.618
b0173	dxr	-	+	+	+	0	1	0
b0174	uppS	+	+	+	+	0	0	0
b0175	cdsA	-	-	-	+	1	1	1
b0179	lpxD	-	-	-	+	1	1	1
b0180	fabZ	+	+	-	-	1	1	1
b0181	lpxA	-	-	-	+	1	1	1
b0182	lpxB	-	-	-	+	1	1	1
b0185	accA	-	-	-	+	1	1	1
b0186	ldcC	-	-	-	-	0	0	0

b0197	yaeC	-	-	-	-	0.858	0.808	0
b0198	yaeE	-	-	-	-	0.858	0.808	0
b0199	abc	-	-	-	-	0.858	0.808	0
b0200	yaeD	-	-	-	-	1	1	1
b0207	yafB	-	-	-	-	0	0	0
b0212	gloB	+	+	+	+	0	0	0
b0221	fadF	-	-	-	-	0	0	0
b0222	gmhA	-	+	+	-	1	1	1
b0238	gpt	+	+	+	-	0.032	0.104	0.046
b0242	proB	-	-	-	-	0.766	0.728	0.388
b0243	proA	-	-	-	-	0.766	0.728	0.388
b0273	argF	+	+	+	-	0.4	0.388	0.502
b0312	betB	-	-	-	-	0	0	0
b0314	betT	-	-	-	-	0	0	0
b0323	yahI	-	-	-	-	0.314	0.378	0.302
b0331	prpB	-	-	-	-	0	0	0.002
b0333	prpC	-	-	-	-	0	0	0.002
b0334	prpD	-	-	-	-	0	0	0.002
b0335	prpE	-	-	-	-	0	0.04	0.014
b0336	codB	-	-	-	-	0.08	0.088	0
b0337	codA	-	-	-	-	0.094	0.122	0.122
b0339	cynT	-	-	-	-	0.486	0.494	0.464
b0340	cynS	-	-	-	-	0	0	0
b0341	cynX	-	-	-	-	0	0	0
b0343	lacY	-	-	-	-	0	0	0
b0344	lacZ	-	-	-	-	0	0	0
b0347	mhpA	-	-	-	-	0	0	0
b0348	mhpB	-	-	-	-	0	0	0
b0349	mhpC	-	-	-	-	0	0	0
b0350	mhpD	-	-	-	-	0	0	0
b0351	mhpF	-	-	-	-	0.02	0	0.044
b0352	mhpE	-	-	-	-	0	0	0
b0353	mhpT	-	-	-	-	0	0	0
b0356	adhC	-	-	-	-	0.01	0.016	0.008
b0365	tauA	-	-	-	-	0	0	0
b0366	tauB	-	-	-	-	0	0	0
b0367	tauC	-	-	-	-	0	0	0
b0368	tauD	-	-	-	-	0	0	0
b0369	hemB	-	-	-	+	0	1	0
b0381	ddlA	-	-	-	+	0.47	0.508	0.49
b0386	proC	-	-	-	+	0.912	0.892	1

b0388	aroL	-	-	-	-	0.49	0.514	0.52
b0401	brnQ	-	-	-	+	0.84	0.856	0
b0403	malZ	-	-	-	-	0.004	0	0
b0414	ribD	+	+	+	+	1	1	1
b0415	ribH	+	+	+	+	1	1	1
b0417	thiL	-	+	+	+	0	0	0
b0418	pgpA	-	-	-	+	0.524	0.49	0.46
b0420	dxs	-	+	+	+	0	1	0
b0421	ispA	-	+	+	+	0	1	0
b0423	thiI	-	-	-	-	0	1	0
b0425	panE	-	-	-	+	0.002	0.03	0
b0428	cyoE	+	+	+	+	0	1	0
b0429	cyoD	+	+	+	+	1	1	1
b0430	cyoC	+	+	+	+	1	1	1
b0431	cyoB	+	+	+	+	1	1	1
b0432	cyoA	+	+	+	+	1	1	1
b0451	amtB	-	-	-	-	1	1	1
b0469	apt	-	-	-	-	0.036	0.186	0.126
b0474	adk	+	+	+	+	1	1	1
b0475	hemH	-	-	-	+	0	1	0
b0477	gsk	-	-	-	-	0.314	0.224	0
b0480	ushA	-	-	-	-	1	1	1
b0485	ybaS	-	-	-	-	0	0	0
b0505	allA	-	-	-	-	0	0	0
b0507	gcl	-	-	-	-	0	0.012	0.014
b0508	hyi	-	-	-	-	0	0	0.004
b0509	glxR	-	-	-	-	0	0.006	0.008
b0511	allP	-	-	-	-	0	0	0
b0512	allB	-	-	-	-	0	0	0
b0514	glxK	-	-	-	-	0	0.01	0.012
b0516	allC	-	-	-	-	0	0	0
b0521	arcC	-	-	-	-	0.35	0.272	0.354
b0522	purK	-	-	-	+	0.72	0.846	1
b0523	purE	-	-	-	+	0.72	0.846	1
b0529	folD	+	+	+	+	1	1	1
b0576	pheP	-	-	-	-	0.038	0.04	0
b0583	entD	-	-	-	-	0	0	0
b0586	entF	-	-	-	-	0	0	0
b0593	entC	-	-	-	-	0	0	0
b0594	entE	-	-	-	-	0	0	0
b0595	entB	-	-	-	-	0	0	0

b0596	entA	-	-	-	-	0	0	0
b0612	citT	-	-	-	-	0	0	0
b0615	citF	-	-	-	-	0	0	0
b0616	citE	-	-	-	-	0	0	0
b0617	citD	-	-	-	-	0	0	0
b0621	dcuC	-	-	-	-	0	0	0
b0638	phpB	-	-	-	-	0	0	0
b0639	nadD	-	+	+	+	1	1	1
b0652	gltL	-	-	-	+	0	0	0
b0653	gltK	-	-	-	+	0	0	0
b0654	gltJ	-	-	-	+	0	0	0
b0655	gltI	-	-	-	-	0	0	0
b0662	ubiF	-	-	-	+	0	1	0
b0674	asnB	-	-	-	+	0.066	0.086	0.306
b0677	nagA	-	-	-	-	0.028	0	0
b0678	nagB	-	-	+	-	0	0	0
b0679	nagE	-	-	-	-	0.02	0	0
b0688	pgm	-	-	-	+	0.504	0.548	0.476
b0692	potE	-	-	-	-	0.686	0.372	0
b0693	speF	-	-	-	-	0.014	0.45	0.45
b0696	kdpC	-	-	-	-	0	0	0
b0697	kdpB	-	-	-	-	0	0	0
b0698	kdpA	-	-	-	-	0	0	0
b0720	gltA	-	-	-	-	1	1	1
b0721	sdhC	-	-	-	+	1	1	1
b0722	sdhD	-	-	-	+	1	1	1
b0723	sdhA	-	-	-	+	1	1	1
b0724	sdhB	-	-	-	+	1	1	1
b0726	sucA	+	+	+	+	1	0.932	1
b0727	sucB	+	+	+	+	1	0.932	1
b0728	sucC	-	-	-	+	0.956	0.908	0.692
b0729	sucD	-	-	-	+	0.956	0.908	0.692
b0733	cydA	-	-	-	-	0	0	0
b0734	cydB	-	-	-	-	0	0	0
b0750	nadA	-	-	-	+	0.198	0.188	1
b0751	pnuC	-	-	-	-	0.546	0.67	0
b0754	aroG	-	-	-	-	0.312	0.304	0.326
b0755	gpmA	+	+	+	+	0.34	0.362	0.32
b0757	galK	-	-	-	-	0	0	0
b0758	galT	-	-	-	-	0.002	0.002	0
b0759	galE	-	-	-	-	0.002	0.002	0

b0774	bioA	+	+	+	+	0	1	0
b0775	bioB	+	+	+	+	0	1	0
b0776	bioF	+	-	-	+	0	1	0
b0778	bioD	+	-	+	+	0	1	0
b0809	glnQ	-	-	-	-	0	0	0
b0810	glnP	-	-	-	-	0	0	0
b0811	glnH	-	-	-	-	0	0	0
b0825	fsa	-	-	-	-	0.248	0.212	0.138
b0828	ybiK	-	-	-	-	0	0	0
b0854	potF	-	-	-	-	0.01	0	0
b0855	potG	-	-	-	-	0.01	0	0
b0856	potH	-	-	-	-	0.01	0	0
b0857	potI	-	-	-	-	0.01	0	0
b0860	artJ	-	-	-	-	0.024	0.02	0
b0861	artM	-	-	-	-	0.024	0.02	0
b0862	artQ	-	-	-	-	0.024	0.02	0
b0864	artP	-	-	-	-	0.024	0.02	0
b0870	ltaA	-	-	-	-	0	0	0
b0871	poxB	-	-	-	-	0	0	0
b0888	trxB	+	+	+	+	1	1	1
b0894	dmsA	-	-	-	-	0	0	0
b0895	dmsB	-	-	-	-	0	0	0
b0896	dmsC	-	-	-	-	0	0	0
b0902	pflA	-	-	-	-	0	0	0.064
b0903	pflB	-	-	-	-	0	0	0.064
b0904	focA	-	-	-	-	0.008	0.012	0.08
b0907	serC	+	+	+	+	1	1	1
b0908	aroA	+	+	+	-	1	1	1
b0910	cmk	+	-	-	+	0.968	0.942	0.792
b0915	lpxK	-	-	-	+	1	1	1
b0918	kdsB	-	-	-	+	1	1	1
b0928	aspC	-	-	-	+	1	1	1
b0931	pncB	-	+	+	-	0.256	0.142	0
b0945	pyrD	-	+	+	+	0.892	0.82	1
b0954	fabA	-	-	-	+	1	1	1
b0963	mgsA	-	-	-	-	0	0	0
b0972	hyaA	-	-	-	-	0	0	0
b0973	hyaB	-	-	-	-	0	0	0
b0974	hyaC	-	-	-	-	0	0	0
b0996	torC	-	-	-	-	0	0	0
b0997	torA	-	-	-	-	0	0	0

b1002	agp	-	-	-	-	0	0	0
b1006	ycdG	-	-	-	-	0.016	0.052	0
b1014	putA	-	-	-	+	0	0.002	0
b1015	putP	-	-	-	-	0.036	0.034	0
b1033	ycdW	-	-	-	-	0	0	0.002
b1054	lpxL	-	-	-	-	1	1	1
b1062	pyrC	-	+	+	+	0.892	0.82	1
b1091	fabH	-	-	-	-	0.904	0.888	0.558
b1092	fabD	+	+	-	+	1	1	1
b1093	fabG	+	+	+	+	1	1	1
b1095	fabF	-	-	-	-	1	1	1
b1096	pabC	-	-	-	-	1	1	1
b1098	tmk	+	+	+	+	1	1	1
b1101	ptsG	+	+	+	-	0.002	0	0.374
b1109	ndh	-	-	-	+	0	0	0
b1123	potD	-	-	-	-	0.366	0	0
b1124	potC	-	-	-	-	0.366	0	0
b1125	potB	-	-	-	-	0.366	0	0
b1126	potA	-	-	-	-	0.366	0	0
b1131	purB	+	+	+	+	0.794	0.888	1
b1136	icdA	-	-	-	-	1	1	1
b1186	nhaB	-	-	-	-	0.204	0.006	0.002
b1189	dadA	-	-	-	-	0	0.002	0
b1190	dadX	-	-	-	+	0.506	0.474	0.528
b1197	treA	-	-	-	-	0	0	0
b1198	dhaH	-	-	-	-	0.01	0.012	0.106
b1199	dhaK2	-	-	-	-	0.01	0.012	0.106
b1200	dhaK1	-	-	-	-	0.01	0.012	0.106
b1207	prsA	+	+	+	+	1	1	1
b1208	ispE	-	+	+	+	0	1	0
b1210	hemA	-	-	-	+	0	1	0
b1215	kdsA	-	-	-	+	1	1	1
b1216	chaA	-	-	-	-	0.27	0.024	0.004
b1223	narK	-	-	-	-	0	0	0.002
b1224	narG	-	-	-	-	0	0	0
b1225	narH	-	-	-	-	0	0	0
b1226	narJ	-	-	-	-	0	0	0
b1227	narI	-	-	-	-	0	0	0
b1232	purU	-	-	-	+	0.85	0.86	0.494
b1236	galU	-	-	-	+	0.55	0.464	0.526
b1238	tdk	-	-	-	-	0.212	0.286	0.13

b1241	adhE	-	-	-	-	1	1	0
b1249	cls	+	+	+	+	1	1	1
b1260	trpA	+	+	+	-	0.176	0.176	1
b1261	trpB	+	+	+	-	0.176	0.176	1
b1262	trpC	+	+	+	-	0.026	0.042	1
b1263	trpD	+	+	+	-	0.026	0.042	1
b1264	trpE	+	-	+	-	0.026	0.042	1
b1270	btuR	-	-	-	-	0	0	0
b1276	acnA	-	-	-	-	0.522	0.542	0.516
b1277	ribA	+	+	+	+	1	1	1
b1278	pgpB	-	-	-	-	0.476	0.51	0.54
b1281	pyrF	+	+	+	+	0.892	0.82	1
b1288	fabI	+	+	+	+	1	1	1
b1297	ycjK	-	-	-	-	0.528	0.532	0.51
b1300	aldH	-	-	-	-	0.002	0	0.002
b1302	goaG	-	-	-	-	0.112	0.062	0.058
b1363	trkG	-	-	-	-	0	0	0
b1380	ldhA	-	-	-	-	0	0	0
b1385	feaB	-	-	-	-	0	0	0
b1386	tynA	-	-	-	-	0	0	0
b1398	paaK	-	-	-	-	0	0	0
b1415	aldA	-	-	-	-	1	1	1
b1416	gapC_2	?	?	?	?	0.348	0.302	0.298
b1417	gapC_1	?	?	?	?	0.348	0.302	0.298
b1440	ydcS	-	-	-	-	0.45	0	0
b1441	ydcT	-	-	-	-	0.45	0	0
b1442	ydcU	-	-	-	-	0.45	0	0
b1443	ydcV	-	-	-	-	0.45	0	0
b1469	narU	-	-	-	-	0	0	0
b1474	fdnG	-	-	-	-	0.158	0.186	0.082
b1475	fdnH	-	-	-	-	0.158	0.186	0.082
b1476	fdnI	-	-	-	-	0.158	0.186	0.082
b1479	sfcA	-	-	-	-	0.014	0.014	0.044
b1492	xasA	-	-	-	-	0	0	0.006
b1493	gadB	-	-	-	-	0.02	0.066	0.056
b1519	tam	-	-	-	-	0	0	0
b1521	uxaB	-	-	-	-	0	0	0
b1524	yneH	-	-	-	-	0	0	0
b1584	speG	-	-	-	+	0	0	0
b1602	pntB	-	-	-	-	0.068	0.122	0.03
b1603	pntA	-	-	-	-	0.068	0.122	0.03

b1605	arcD	-	-	-	-	0.114	0.176	0
b1611	fumC	-	-	-	+	0.354	0.354	0.338
b1612	fumA	-	-	-	-	0.3	0.362	0.342
b1613	manA	-	-	-	-	0	0	0
b1621	malX	-	-	-	-	0	0.004	0.328
b1622	malY	-	-	-	-	0.056	0.094	0.49
b1623	add	-	-	-	-	0	0.002	0
b1636	pdxY	-	-	-	-	0	0	0
b1638	pdxH	-	-	-	+	0	0	0
b1646	sodC	-	-	-	-	0	0	0
b1651	gloA	-	-	-	-	0	0	0
b1656	sodB	-	-	-	-	0	0	0
b1662	ribE	+	+	+	+	1	1	1
b1676	pykF	-	-	-	-	0.426	0.528	0.362
b1692	ydiB	-	-	-	-	0.526	0.542	0.466
b1693	aroD	-	-	-	-	1	1	1
b1702	pps	-	-	-	-	0	0	0
b1704	aroH	+	+	+	-	0.336	0.334	0.34
b1709	btuD	-	-	-	-	0	0	0
b1711	btuC	-	-	-	-	0	0	0
b1723	pfkB	-	-	-	-	0	0	0.44
b1732	katE	-	-	-	-	0	0	0
b1740	nadE	-	+	+	+	0.454	0.33	1
b1744	astE	-	-	-	-	0	0	0
b1745	astB	-	-	-	-	0	0	0
b1746	astD	-	-	-	-	0	0	0
b1747	astA	-	-	-	-	0	0	0
b1748	astC	-	-	-	-	0	0	0
b1761	gdhA	-	-	-	-	1	1	1
b1764	selD	-	-	-	-	0	0	0
b1767	ansA	-	-	-	-	0	0	0
b1768	pncA	-	-	-	-	0	0.142	0
b1773	b1773	-	-	-	-	0.296	0.274	0.348
b1779	gapA	+	+	+	+	0.652	0.698	0.702
b1801	yeaV	-	-	-	-	0.014	0.004	0.012
b1805	fadD	-	-	-	-	0	0	0
b1812	pabB	-	-	-	-	1	1	1
b1814	sdaA	-	-	-	-	0.064	0.038	0.258
b1817	manX	-	-	-	-	0.052	0	0.298
b1818	manY	-	-	-	-	0.052	0	0.298
b1819	manZ	-	-	-	-	0.052	0	0.298

b1849	purT	-	-	-	+	0.342	0.382	0.568
b1850	eda	-	-	-	-	0	0	0
b1851	edd	-	-	-	-	0	0	0
b1852	zwf	+	+	+	-	0.018	0.142	1
b1854	pykA	+	+	+	+	0.554	0.44	0.42
b1855	msbB	-	-	-	-	1	1	1
b1865	ntpA	-	-	-	-	0.512	0.502	0.52
b1872	torZ	-	-	-	-	0	0	0
b1873	torY	-	-	-	-	0	0	0
b1896	otsA	-	-	-	-	0	0	0
b1897	otsB	-	-	-	-	0	0	0
b1898	araH_2	-	-	-	-	0	0	0
b1899	araH_1	-	-	-	-	0	0	0
b1900	araG	-	-	-	-	0	0	0
b1901	araF	-	-	-	-	0	0	0
b1907	tyrP	-	-	-	-	0.008	0.006	0
b1912	pgsA	-	-	-	+	1	1	1
b1982	amn	-	-	-	-	0	0	0.002
b1991	cobT	-	-	-	-	0	0	0
b1992	cobS	-	-	-	-	0	0	0
b1993	cobU	-	-	-	-	0	0	0
b2019	hisG	+	+	+	-	0.292	0.228	1
b2020	hisD	+	+	+	-	0.292	0.228	1
b2021	hisC	+	+	+	-	0.292	0.228	1
b2022	hisB	+	+	+	-	0.292	0.228	1
b2023	hisH	+	+	+	-	0.292	0.228	1
b2024	hisA	+	+	+	-	0.292	0.228	1
b2025	hisF	+	+	+	-	0.292	0.228	1
b2026	hisI	+	+	+	-	0.292	0.228	1
b2028	ugd	-	-	-	+	0	0	0
b2029	gnd	+	+	+	-	0.018	0.142	1
b2036	glf	-	-	-	-	0	0	0
b2038	rfbC	-	-	-	-	0	0	0
b2039	rfbA	-	-	-	-	0	0	0
b2040	rfbD	-	-	-	-	0	0	0
b2041	rfbB	-	-	-	-	0	0	0
b2042	galF	-	-	-	-	0.45	0.536	0.474
b2045	wcaK	-	-	-	-	0.002	0.002	0
b2048	cpsG	-	-	-	-	0	0	0
b2049	manC	-	-	-	-	0	0	0
b2052	fcl	-	-	-	-	0	0	0

b2053	gmd	-	-	-	-	0	0	0
b2065	dcd	+	+	+	+	0	0	0
b2066	udk	-	-	-	-	0.156	0.264	0.142
b2091	gatD	-	-	-	-	0	0	0
b2092	gatC	-	-	-	-	0	0	0
b2093	gatB	-	-	-	-	0	0	0
b2094	gatA	-	-	-	-	0	0	0
b2095	gatZ	-	-	-	-	0	0	0
b2096	gatY	-	-	-	-	0	0	0
b2097	fbaB	-	-	-	-	0.264	0.28	0.322
b2103	thiD	-	-	-	+	0	1	0
b2104	thiM	-	-	-	-	0	0	0
b2128	yehW	-	-	-	-	0	0	0
b2129	yehX	-	-	-	-	0	0	0
b2130	yehY	-	-	-	-	0	0	0
b2131	yehZ	-	-	-	-	0	0	0
b2132	bglX	-	-	-	-	0	0	0
b2133	dld	-	-	-	-	0	0	0
b2143	cdd	-	-	-	-	0.042	0.06	0.172
b2148	mglC	-	-	-	-	0	0	0
b2149	mglA	-	-	-	-	0	0	0
b2150	mglB	-	-	-	-	0	0	0
b2153	folE	-	+	-	+	1	1	1
b2156	lysP	-	-	-	-	0.388	0.356	0
b2167	fruA	-	-	-	-	0	0	0
b2168	fruK	-	-	-	-	0	0	0
b2169	fruB	-	-	-	-	0	0	0
b2210	mqo	-	-	-	+	0	0	0
b2221	atoD	-	-	-	-	0	0	0
b2222	atoA	-	-	-	-	0	0	0
b2223	atoE	-	-	-	-	0	0	0
b2224	atoB	-	-	-	-	0	0	0
b2232	ubiG	-	-	-	+	0	1	0
b2234	nrdA	+	+	+	-	0.366	0.364	0.312
b2235	nrdB	+	+	+	-	0.366	0.364	0.312
b2239	glpQ	-	-	-	-	0	0	0.006
b2240	glpT	-	-	-	-	1	1	0
b2241	glpA	-	-	-	-	0	0.002	0
b2242	glpB	-	-	-	-	0	0.002	0
b2243	glpC	-	-	-	-	0	0.002	0
b2260	menE	-	-	-	-	0	0	0

b2261	menC	-	-	-	-	0	0	0
b2262	menB	-	-	-	-	0	0	0
b2264	menD	-	-	-	-	0	0	0
b2265	menF	-	-	-	-	0	0	0
b2276	nuoN	+	+	+	-	1	1	1
b2277	nuoM	+	+	+	-	1	1	1
b2278	nuoL	+	+	+	-	1	1	1
b2279	nuoK	+	+	+	-	1	1	1
b2280	nuoJ	+	+	+	-	1	1	1
b2281	nuoI	+	+	+	-	1	1	1
b2282	nuoH	+	+	+	-	1	1	1
b2283	nuoG	+	+	+	-	1	1	1
b2284	nuoF	+	+	+	-	1	1	1
b2285	nuoE	+	+	+	-	1	1	1
b2286	nuoC	+	+	+	-	1	1	1
b2287	nuoB	+	+	+	-	1	1	1
b2288	nuoA	+	+	+	-	1	1	1
b2296	ackA	+	+	+	+	0.294	0.256	0.16
b2297	pta	+	+	+	+	0.464	0.438	0.466
b2306	hisP	-	-	-	-	0.12	0.104	0
b2307	hisM	-	-	-	-	0.12	0.104	0
b2308	hisQ	-	-	-	-	0.12	0.104	0
b2309	hisJ	-	-	-	-	0.098	0.086	0
b2310	argT	-	-	-	-	0.028	0.03	0
b2311	ubiX	-	-	-	+	0	0.514	0
b2312	purF	-	-	-	+	0.72	1	1
b2315	folC	+	+	+	+	1	1	1
b2316	accD	-	-	-	+	1	1	1
b2320	pdxB	-	-	-	+	0	1	0
b2323	fabB	+	+	+	+	1	1	1
b2329	aroC	+	+	+	-	1	1	1
b2344	fadL	-	-	-	-	0.934	0	0
b2366	dsdA	-	-	-	-	0	0	0
b2378	lpxP	-	-	-	-	0	0	0
b2388	glk	-	-	-	-	0.008	0.006	0.026
b2393	nupC	-	-	-	-	0.046	0.102	0
b2400	gltX	+	+	+	+	0	1	0
b2406	xapB	-	-	-	-	0.062	0.088	0
b2407	xapA	-	-	-	-	0.208	0.352	0.38
b2411	lig	+	-	+	+	0	0	0
b2413	cysZ	-	-	-	-	0	0	0.348

b2414	cysK	-	+	+	-	0	0	0.514
b2415	ptsH	+	+	+	-	0.084	0.016	1
b2416	ptsI	+	+	+	-	0.084	0.016	1
b2417	crr	+	+	+	-	0.002	0.004	0.702
b2418	pdxK	-	-	-	-	0	0	0
b2421	cysM	-	-	-	-	0	0	0.486
b2422	cysA	-	-	-	-	0	0	1
b2423	cysW	-	-	-	-	0	0	1
b2424	cysU	-	-	-	-	0	0	1
b2425	cysP	-	-	-	-	0	0	0.652
b2429	yfeV	-	-	-	-	0	0	0
b2436	hemF	-	-	-	+	0	1	0
b2440	eutC	-	-	-	-	0	0	0.006
b2441	eutB	-	-	-	-	0	0	0.006
b2458	eutD	-	-	-	-	0.426	0.452	0.422
b2463	maeB	-	-	-	-	0.054	0.09	0.094
b2464	talA	+	+	+	+	0.504	0.5	0.482
b2465	tktB	+	-	-	-	0.502	0.542	0.53
b2472	dapE	+	+	+	+	0.704	1	1
b2476	purC	-	-	-	+	0.72	0.846	1
b2478	dapA	+	+	+	+	0.704	1	1
b2492	focB	-	-	-	-	0.018	0.018	0.072
b2497	uraA	-	-	-	-	0.012	0.042	0
b2498	upp	-	-	-	-	0.018	0.038	0.118
b2499	purM	-	-	-	+	0.72	1	1
b2500	purN	-	-	-	-	0.668	0.846	0.736
b2507	guaA	-	-	-	+	0.674	0.758	1
b2508	guaB	-	-	-	+	0.674	0.758	1
b2515	gcpE	-	+	+	+	0	1	0
b2518	ndk	-	-	-	+	1	1	1
b2530	iscS	+	+	+	+	0	1	0
b2533	suhB	+	+	+	+	0	0	0
b2536	hcaT	-	-	-	-	0	0	0
b2538	hcaE	-	-	-	-	0	0	0
b2539	hcaF	-	-	-	-	0	0	0
b2540	hcaC	-	-	-	-	0	0	0
b2541	hcaB	-	-	-	-	0	0	0
b2542	hcaD	-	-	-	-	0	0	0
b2551	glyA	+	+	+	+	1	1	1
b2557	purL	-	-	-	+	0.72	1	1
b2563	acpS	+	+	+	+	0	0	0

b2564	pdxJ	-	-	-	+	0	1	0
b2574	nadB	-	-	-	-	0.198	0.188	1
b2585	pssA	-	-	-	+	1	1	1
b2587	kgtP	-	-	-	-	0	0	0
b2599	pheA	+	+	+	-	0.906	0.872	1
b2600	tyrA	-	-	-	-	0.94	0.92	1
b2601	aroF	-	-	-	+	0.352	0.362	0.334
b2615	yfjB	+	+	+	+	1	1	1
b2661	gabD	-	-	-	-	0.196	0.116	0.064
b2662	gabT	-	-	-	-	0.11	0.056	0.052
b2663	gabP	-	-	-	-	0	0	0.006
b2675	nrdE	-	-	-	+	0	0	0
b2676	nrdF	-	-	-	+	0	0	0
b2677	proV	-	-	-	-	0	0.002	0
b2678	proW	-	-	-	-	0	0.002	0
b2679	proX	-	-	-	-	0	0.002	0
b2687	luxS	-	-	-	-	0	0	0
b2688	gshA	+	+	+	+	0	1	0
b2690	yqaB	-	-	-	-	0.494	0.45	0.524
b2702	srlA	-	-	-	-	0	0	0
b2703	srlE	-	-	-	-	0	0	0
b2704	srlB	-	-	-	-	0	0	0
b2705	srlD	-	-	-	-	0	0	0
b2719	hycG	-	-	-	-	0	0	0
b2720	hycF	-	-	-	-	0	0	0
b2721	hycE	-	-	-	-	0	0	0
b2722	hycD	-	-	-	-	0	0	0
b2723	hycC	-	-	-	-	0	0	0
b2724	hycB	-	-	-	-	0	0	0
b2738	ygbL	-	-	-	-	0	0	0
b2746	ispF	-	+	+	+	0	1	0
b2747	ispD	-	+	+	+	0	1	0
b2750	cysC	-	+	+	-	0	0	1
b2751	cysN	-	-	+	-	0	0	1
b2752	cysD	-	-	+	-	0	0	1
b2762	cysH	-	-	+	-	0	0	1
b2763	cysI	-	-	+	-	0	0	1
b2764	cysJ	-	+	+	-	0	0	1
b2779	eno	+	+	+	+	0.984	0.978	1
b2780	pyrG	+	+	+	+	0.946	0.918	1
b2781	mazG	-	-	-	-	0	0	0

b2787	gudD	-	-	-	-	0	0	0
b2788	ygcY	-	-	-	-	0	0	0
b2789	gudP	-	-	-	-	0	0	0
b2796	sdaC	-	-	-	+	0	0	0
b2797	sdaB	-	-	-	-	0.06	0.05	0.262
b2799	fucO	-	-	-	-	0	0	0
b2800	fucA	-	-	-	-	0	0	0
b2801	fucP	-	-	-	-	0	0	0
b2802	fucI	-	-	-	-	0	0	0
b2803	fucK	-	-	-	-	0	0	0
b2818	argA	-	+	+	-	0.678	0.652	1
b2827	thyA	+	+	+	+	0.874	0.784	1
b2836	aas	-	-	-	-	0.934	0	0.006
b2838	lysA	+	+	+	-	0.644	0.71	1
b2841	araE	-	-	-	-	0	0	0
b2874	yqeA	-	-	-	-	0.336	0.326	0.332
b2883	ygfP	-	-	-	-	0	0	0
b2889	idi	-	-	-	-	0	0	0
b2901	bglA	-	-	-	-	0	0	0
b2903	gcvP	-	-	-	-	0	0.004	0.11
b2904	gcvH	-	-	-	-	0	0.004	0.11
b2905	gcvT	-	-	-	-	0	0.004	0.11
b2907	ubiH	-	-	-	+	0	1	0
b2913	serA	-	-	-	-	1	1	1
b2914	rpiA	+	+	+	+	0.5	0.496	0.462
b2917	sbm	-	-	-	-	0	0	0.002
b2919	ygfG	-	-	-	-	0	0	0.002
b2920	ygfH	-	-	-	-	0	0.04	0.014
b2925	fbaA	+	+	+	+	0.31	0.336	0.304
b2926	pgk	+	+	+	+	1	1	1
b2927	epd	-	-	-	-	0	1	0
b2935	tktA	-	+	+	+	0.498	0.458	0.47
b2937	speB	-	-	-	-	0	0.018	0.098
b2938	speA	-	-	-	-	0	0.006	0.064
b2942	metK	-	+	+	+	0.184	1	1
b2943	galP	-	-	-	-	0.004	0.006	0.026
b2947	gshB	+	+	+	+	0	1	0
b2957	ansB	-	-	-	-	0	0	0
b2964	nupG	-	-	-	-	0.074	0.1	0
b2965	speC	-	-	-	-	0.014	0.532	0.452
b2975	glcA	-	-	-	-	0.08	0.088	0.276

b2976	glcB	-	-	-	-	0.452	0.484	0.354
b2978	glcF	-	-	-	-	0.82	0.804	0.412
b2979	glcD	-	-	-	-	0.82	0.804	0.412
b2987	pitB	-	-	-	-	0.5	0.486	0.494
b2988	gsp	-	-	-	-	0	0	0
b2994	hybC	-	-	-	-	0	0	0
b2997	hybO	-	-	-	-	0	0	0
b3008	metC	-	-	-	-	0.086	0.098	0.51
b3012	yqhE	-	-	-	-	0	0	0
b3018	plsC	-	-	-	+	1	1	1
b3041	ribB	+	+	+	+	1	1	1
b3052	rfaE	-	+	+	-	1	1	1
b3058	folB	-	-	-	+	1	1	1
b3061	ttdA	-	-	-	-	0	0	0
b3062	ttdB	-	-	-	-	0	0	0
b3063	ygjE	-	-	-	-	0	0	0
b3073	ygjG	-	-	-	-	0.002	0.004	0
b3089	sstT	-	-	-	-	0.002	0	0
b3091	uxaA	-	-	-	-	0	0	0
b3092	uxaC	-	-	-	-	0	0	0
b3093	exuT	-	-	-	-	0	0	0
b3111	tdcGa	-	-	-	-	0	0	0
b3112	tdcGb	-	-	-	-	0	0	0
b3114	tdcE	-	-	-	-	0	0.002	0.138
b3115	tdcD	-	-	-	-	0.264	0.258	0.202
b3116	tdcC	-	-	-	-	0.004	0.008	0
b3117	tdcB	-	-	-	-	0.044	0.036	0.246
b3124	garK	-	-	-	-	0	0.002	0.002
b3125	garR	-	-	-	-	0	0.006	0.002
b3126	garL	-	-	-	-	0	0	0
b3127	garP	-	-	-	-	0	0	0
b3128	garD	-	-	-	-	0	0	0
b3132	agaZ	-	-	-	-	0	0	0
b3137	agaY	-	-	-	-	0	0	0
b3161	mtr	-	-	-	-	0.87	0.842	0
b3172	argG	+	+	+	-	0.884	0.824	1
b3176	mrsA	+	-	+	+	1	1	1
b3177	folP	-	-	-	+	1	1	1
b3187	ispB	-	-	-	+	0	1	0
b3189	murA	+	+	+	+	1	1	1
b3212	gltB	-	-	-	-	0	0	0

b3213	gltD	-	-	-	-	0	0	0
b3222	nanK	-	-	-	-	0.006	0	0
b3223	nanE	-	-	-	-	0.006	0	0
b3224	nanT	-	-	-	-	0.006	0	0
b3225	nanA	-	-	-	-	0.006	0	0
b3236	mdh	-	-	-	-	1	1	1
b3255	accB	-	-	-	+	1	1	1
b3256	accC	-	-	-	+	1	1	1
b3258	panF	-	-	-	-	0.73	0	0
b3281	aroE	+	+	+	-	0.474	0.458	0.534
b3359	argD	-	+	+	-	0.668	0.644	1
b3360	pabA	-	-	-	-	1	1	1
b3365	nirB	-	-	-	-	0	0	0
b3366	nirD	-	-	-	-	0	0	0
b3367	nirC	-	-	-	-	0	0	0
b3368	cysG	-	-	+	-	0	0	0
b3380	yhfW	-	-	-	-	0.256	0.346	0.102
b3385	gph	-	-	-	-	0	0	0
b3386	rpe	+	+	+	+	0.504	0.508	0.498
b3389	aroB	+	+	+	-	1	1	1
b3390	aroK	+	+	+	+	0.51	0.486	0.48
b3403	pckA	-	-	-	+	0.016	0.032	0.03
b3409	feoB	-	-	-	-	0	1	0
b3415	gntT	-	-	-	-	0	0	0
b3416	malQ	-	-	-	-	0.006	0.002	0
b3417	malP	-	-	-	-	0.006	0.002	0
b3425	glpE	-	-	-	-	0	0	0
b3426	glpD	-	-	-	-	0	0	0
b3428	glgP	-	-	-	-	0	0	0
b3429	glgA	-	-	-	-	1	1	1
b3430	glgC	-	-	-	-	1	1	1
b3433	asd	+	+	+	+	0.864	1	1
b3437	gntK	-	-	-	-	0	0	0
b3450	ugpC	-	-	-	-	0	0.004	0
b3451	ugpE	-	-	-	-	0	0.004	0
b3452	ugpA	-	-	-	-	0	0.004	0
b3453	ugpB	-	-	-	-	0	0.004	0
b3454	livF	-	-	-	-	0.02	0.018	0
b3455	livG	-	-	-	-	0.02	0.018	0
b3456	livM	-	-	-	-	0.02	0.018	0
b3457	livH	-	-	-	-	0.02	0.018	0

b3458	livK	-	-	-	-	0.002	0	0
b3460	livJ	-	-	-	-	0.018	0.018	0
b3493	pitA	+	+	+	+	0.5	0.506	0.482
b3500	gor	-	-	-	-	0	0	0
b3517	gadA	-	-	-	-	0.024	0.052	0.054
b3519	treF	-	-	-	-	0	0	0
b3526	kdgK	-	-	-	-	0	0	0
b3528	dctA	-	-	-	-	0	0	0
b3551	bisC	-	-	-	-	0	0	0
b3553	yiaE	-	-	-	-	0	0	0.002
b3564	xylB	-	-	-	-	0	0	0
b3565	xylA	-	-	-	-	0	0	0
b3566	xylF	-	-	-	-	0	0	0
b3567	xylG	-	-	-	-	0	0	0
b3568	xylH	-	-	-	-	0	0	0
b3572	avtA	-	-	-	-	0.296	0.328	0
b3575	yiaK	-	-	-	-	0	0	0
b3579	yiaO	-	-	-	-	0	0	0
b3581	sgbH	-	-	-	-	0	0	0
b3583	sgbE	-	-	-	-	0	0	0
b3588	aldB	-	-	-	-	0	0	0
b3599	mtlA	+	+	+	-	0	0	0
b3600	mtlD	+	+	+	-	0	0	0
b3603	lldP	-	-	-	-	0.1	0.108	0.312
b3605	lldD	-	-	-	-	0	0	0
b3607	cysE	+	+	+	-	0	0	1
b3608	gpsA	-	-	-	+	1	1	1
b3612	yibO	-	-	-	-	0.31	0.284	0.32
b3616	tdh	-	-	-	-	0.01	0	0.012
b3617	kbl	-	-	-	-	0.01	0	0.012
b3619	rfaD	-	-	-	-	1	1	1
b3620	rfaF	-	-	-	-	1	1	1
b3621	rfaC	-	-	-	-	1	1	1
b3622	rfaL	-	-	-	-	1	1	1
b3626	rfaJ	-	-	-	-	1	1	1
b3627	rfaI	-	-	-	-	1	1	1
b3631	rfaG	-	-	-	-	1	1	1
b3633	kdtA	-	-	-	+	1	1	1
b3634	coaD	-	+	+	+	1	0	1
b3640	dut	+	+	+	+	0.124	0.13	0.252
b3642	pyrE	+	-	+	+	0.892	0.82	1

b3648	gmk	+	+	+	+	1	1	1
b3653	gltS	-	-	-	-	0	0	0.006
b3654	yicE	-	-	-	-	0.284	0.198	0.046
b3665	yicP	-	-	-	-	0.018	0.11	0.098
b3666	uhpT	-	-	-	-	0.056	0.032	0
b3670	ilvN	-	-	-	-	0.358	0.322	0.36
b3671	ilvB	-	-	-	-	0.358	0.322	0.36
b3691	dgoT	-	-	-	-	0	0	0
b3692	dgoA	-	-	-	-	0	0	0
b3693	dgoK	-	-	-	-	0	0	0
b3708	tnaA	-	-	-	-	0.742	0.742	0.278
b3709	tnaB	-	-	-	-	0.028	0.018	0
b3725	pstB	-	-	-	-	0	0.004	0.024
b3726	pstA	-	-	-	-	0	0.004	0.024
b3727	pstC	-	-	-	-	0	0.004	0.024
b3728	pstS	-	-	-	-	0	0.004	0.024
b3729	glmS	+	+	+	+	0.92	1	1
b3730	glmU	+	+	+	+	1	1	1
b3731	atpC	+	+	+	+	1	1	1
b3732	atpD	+	+	+	+	1	1	1
b3733	atpG	+	+	+	+	1	1	1
b3734	atpA	+	+	+	+	1	1	1
b3735	atpH	+	+	+	+	1	1	1
b3736	atpF	+	+	+	+	1	1	1
b3737	atpE	+	+	+	+	1	1	1
b3738	atpB	+	+	+	+	1	1	1
b3739	atpI	-	-	-	-	1	1	1
b3744	asnA	-	-	-	-	0.934	0.914	0.694
b3748	rbsD	-	-	-	-	0	0	0
b3749	rbsA	-	-	-	-	0	0	0
b3750	rbsC	-	-	-	-	0	0	0
b3751	rbsB	-	-	-	-	0	0	0
b3752	rbsK	-	-	-	-	0	0	0
b3767	ilvG_1	?	?	?	?	0.216	0.23	0.226
b3768	ilvG_2	?	?	?	?	0.216	0.23	0.226
b3769	ilvM	-	-	-	-	0.216	0.23	0.226
b3770	ilvE	-	-	-	-	0.65	0.67	1
b3771	ilvD	+	+	+	-	0.942	0.958	1
b3772	ilvA	-	-	-	-	0.04	0.036	0.234
b3774	ilvC	+	+	+	-	0.944	0.97	1
b3784	wecA	-	-	-	-	0	0	0

b3786	wecB	-	-	-	-	0	0	0
b3787	wecC	-	-	-	-	0	0	0
b3788	rffG	-	-	-	-	0	0	0
b3789	rffH	-	-	-	-	0	0	0
b3790	wecD	-	-	-	-	0	0	0
b3791	wecE	-	-	-	+	0	0	0
b3793	wecF	-	-	-	-	0	0	0
b3794	wecG	-	-	-	-	0	0	0
b3803	hemX	-	-	-	-	0	0	0
b3804	hemD	-	-	-	+	0	1	0
b3805	hemC	-	-	+	+	0	1	0
b3806	cyaA	-	-	-	-	0	0	0
b3809	dapF	+	+	+	+	0.704	1	1
b3821	pldA	-	-	-	-	0	0	0.006
b3825	pldB	-	-	-	-	0	0	0.006
b3829	metE	+	+	+	-	0.08	0.52	0.51
b3831	udp	-	-	-	-	0.16	0.214	0.17
b3833	ubiE	-	-	-	+	0	1	0
b3835	ubiB	-	-	-	+	0	1	0
b3843	yigC	-	-	-	+	0	0.486	0
b3845	fadA	-	-	-	-	0	0	0
b3846	fadB	-	-	-	-	0	0	0
b3849	trkH	-	-	-	+	0	0	0
b3850	hemG	-	-	-	+	0	1	0
b3870	glnA	-	-	-	+	0.472	0.468	0.49
b3892	fdoI	-	-	-	-	0.174	0.146	0.06
b3893	fdoH	-	-	-	-	0.174	0.146	0.06
b3894	fdoG	-	-	-	-	0.174	0.146	0.06
b3902	rhaD	-	-	-	-	0	0	0
b3903	rhaA	-	-	-	-	0	0	0
b3904	rhaB	-	-	-	-	0	0	0
b3907	rhaT	-	-	-	-	0	0	0
b3908	sodA	+	+	+	+	0	0	0
b3909	kdgT	-	-	-	-	0	0	0
b3916	pfkA	+	+	+	-	0	0	0.534
b3917	sbp	-	-	-	-	0	0	0.348
b3918	cdh	-	-	-	-	0	0	0
b3919	tpiA	+	+	+	+	1	1	1
b3926	glpK	-	-	-	-	0.418	0.416	0.398
b3927	glpF	-	+	+	-	0.212	0.21	0.276
b3929	menG	-	-	-	-	0	0	0

b3930	menA	-	-	-	-	0	0	0
b3939	metB	-	-	-	-	0.142	0.192	1
b3940	metL	-	-	-	-	0.37	0.386	0.502
b3941	metF	+	+	+	-	1	1	1
b3942	katG	-	-	-	-	0	0	0
b3945	gldA	-	-	-	-	0.52	0.5	0.362
b3946	talC	-	-	-	-	0.246	0.234	0.102
b3951	pflD	-	-	-	-	0	0	0.054
b3952	pflC	-	-	-	-	0	0	0.054
b3956	ppc	-	-	-	-	0.098	0.526	0.482
b3957	argE	-	+	+	-	0.678	0.652	1
b3958	argC	-	+	+	-	0.678	0.652	1
b3959	argB	-	+	+	-	0.678	0.652	1
b3960	argH	+	+	+	-	0.884	0.824	1
b3962	sthA	-	-	-	-	0.072	0.024	0.032
b3966	btuB	-	-	-	-	0	0	0
b3967	murI	+	+	+	+	1	1	1
b3972	murB	+	+	+	+	1	1	1
b3974	coaA	-	-	-	+	1	0	1
b3990	thiH	-	-	-	+	0	1	0
b3991	thiG	-	-	-	+	0	1	0
b3992	thiF	-	-	-	-	0	1	0
b3993	thiE	-	-	-	+	0	1	0
b3994	thiC	-	-	-	+	0	1	0
b3997	hemE	-	-	-	+	0	1	0
b4005	purD	-	-	-	+	0.72	1	1
b4006	purH	+	+	+	+	0.8	0.886	1
b4013	metA	-	-	-	-	0.142	0.192	1
b4014	aceB	-	-	-	-	0.546	0.488	0.28
b4015	aceA	-	-	-	-	0.978	0.95	0.544
b4019	metH	-	-	-	-	0.062	0.48	0.49
b4024	lysC	-	-	-	-	0.154	0.254	0
b4025	pgi	+	+	+	+	0.934	0.956	0.92
b4031	xylE	-	-	-	-	0	0	0
b4032	malG	-	-	-	-	0.006	0.002	0
b4033	malF	-	-	-	-	0.006	0.002	0
b4034	malE	-	-	-	-	0.006	0.002	0
b4035	malK	-	-	-	-	0.006	0.002	0
b4036	lamB	-	-	-	-	0.006	0.002	0
b4039	ubiC	-	-	-	-	0	1	0
b4040	ubiA	-	-	-	+	0	1	0

b4041	plsB	-	-	-	+	1	1	1
b4042	dgkA	-	-	-	-	1	1	1
b4053	alr	-	-	-	-	0.494	0.506	0.472
b4054	tyrB	-	-	-	-	0.224	0.23	0
b4069	acs	-	-	-	-	0.082	0.068	0.112
b4077	gltP	-	-	-	-	0	0	0.01
b4079	fdhF	-	-	-	-	0.634	0.638	0.338
b4090	rpiB	-	-	-	-	0.5	0.504	0.538
b4111	proP	-	-	-	-	0.052	0.082	0
b4117	adiA	-	-	-	-	0	0.012	0.034
b4119	melA	-	-	-	-	0	0	0
b4120	melB	-	-	-	-	0	0	0
b4122	fumB	-	-	-	-	0.346	0.284	0.32
b4123	dcuB	-	-	-	-	0	0	0
b4131	cadA	-	-	-	-	0	0	0
b4132	cadB	-	-	-	-	0	0	0
b4138	dcuA	-	-	-	-	0	0	0
b4139	aspA	-	-	-	+	0.088	0.016	0.03
b4151	frdD	-	-	-	-	0	0	0
b4152	frdC	-	-	-	-	0	0	0
b4153	frdB	-	-	-	-	0	0	0
b4154	frdA	-	-	-	-	0	0	0
b4160	psd	-	-	-	+	1	1	1
b4177	purA	+	+	+	+	0.764	0.836	1
b4196	sgaH	-	-	-	-	0	0	0
b4197	sgaU	-	-	-	-	0	0	0
b4198	sgaE	-	-	-	-	0	0	0
b4208	cycA	-	-	-	-	0.008	0.032	0.014
b4226	ppa	+	+	+	+	1	1	1
b4227	ytfQ	-	-	-	-	0	0	0
b4228	ytfR	-	-	-	-	0	0	0
b4229	ytfS	-	-	-	-	0	0	0
b4230	ytfT	-	-	-	-	0	0	0
b4231	yjfF	-	-	-	-	0	0	0
b4232	fbp	-	-	-	+	0.87	0.89	0
b4238	nrdD	-	-	-	-	0.634	0.636	0.688
b4239	treC	-	-	-	-	0	0	0
b4240	treB	-	-	-	-	0	0	0
b4244	pyrI	-	+	+	+	0.892	0.82	1
b4245	pyrB	-	+	+	+	0.892	0.82	1
b4254	argI	-	-	-	-	0.484	0.436	0.498

b4265	idnT	-	-	-	-	0	0	0
b4266	idnO	-	-	-	-	0	0	0
b4267	idnD	-	-	-	-	0.09	0.036	0.032
b4268	idnK	-	-	-	-	0	0	0
b4301	sgcE	-	-	-	-	0.494	0.482	0.486
b4321	gntP	-	-	-	-	0	0	0
b4322	uxuA	-	-	-	-	0	0	0
b4323	uxuB	-	-	-	-	0	0	0
b4381	deoC	-	-	-	-	0.006	0.006	0.55
b4382	deoA	-	-	-	-	0.006	0.004	0.082
b4383	deoB	+	+	+	-	0.252	0.302	0.6
b4384	deoD	+	+	+	-	0.254	0.34	0.352
b4388	serB	-	-	-	-	1	1	1
b4395	gpmB	-	-	-	-	0.334	0.332	0.36
b4407	thiS	-	-	-	-	0	1	0